Wednesday, 8 July 1998

Plenary Session III with best abstracts

PL-1083 DEVELOPMENT OF A TRANSGENIC MOUSE MODEL FOR COLD AGGLUTININ DISEASE (CAD) WITH ANTI-Sia-1b SPECIFICITY

S. Havouis, G. Dumas, O. Pritsch, G. Dighiero, C. Pourcel. *Immuno-Hématholgie & Immunopathologie, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France*

Cold agglutinins (CA) are autoantibodies (mostly IgM), which recognise glycolipid antigens on human erythrocytes, cause haemolytic anaemia (AIHA) and frequently evolve to a lymphoma. To develop an animal model for this disease, we have produced transgenic mice expressing the heavy and light chain of a human CA. We chose a CA displaying the rare anti-Sia-1b specificity (CA.GAS), because this CA agglutinates murine erythrocytes and is able to hemolyse them in the presence of complement. VH and VL fragments of CA.GAS were isolated by PCR, and introduced into vectors containing all the sequences necessary to express the immunoglobulin light and heavy chains. The recombinant CA has the same characteristics than the "natural" CA.GAS and, by IP injection into mice, is able to induce a typical AIHA[1]. Transgenic mice were obtained bearing either the mu or kappa gene without plasmid sequences. One strain with the mu gene (strain GAS-μ5) and one strain with the kappa gene (strain GAS-κ5) were shown by northern blot analysis and RT-PCR to express the transgene in the spleen at a high level.

Double transgenics simultaneously expressing the H and L chains from CA-GAS were obtained by breeding GAS-μ5 and GAS-κ5 mice. The serum of these mice contains CA.GAS, and, in vitro at 4°C, is able to agglutinate mouse erythrocytes at a dilution of 1/64. The induction of AIHA in mice upon exposure to cold is under study.

These results show for the first time the possibility to create a murine model of CA disease and to study tolerance to glycolipid antigens. Indeed, our preliminary data suggest that the mechanism of clonal deletion is not used in this model to eliminate the pathogenic immunoglobulin.

(1) Dumas et al. Br. J. Haematol., 98:589, 1997

PL-1084 EARLY DIAGNOSIS OF FUNGAL INFECTIONS USING MOLECULAR METHODS: RESULTS OF PROSPECTIVE STUDIES IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND IMPLICATIONS FOR ANTIFUNGAL TREATMENT STRATEGIES

H. Einsele, H. Hebart, J. Löffler, M. Hartmann, K. Quabeck, D. Schmidt, F. Serrey, U.W. Schäfer, A. Böhme, H. Martin, U. Schumacher, L. Kanz. *Department of Internal Medicin II and Department of Medical Microbiology, University, of Tübingen, and Medical Clinic III, Section of Hematology, University of Frankfurt, Germany*

Invasive fungal infections (IFI) have emerged as a major infection-related cause of death in patients with acute leukemia and recipients of an allogeneic stem cell transplant. Early diagnosis and initiation of antifungal therapy are essential to improve the outcome of pts. with this life-threatening complication. We developed a highly sensitive method for the detection of a wide range of fungal pathogens using primers for DNA amplification binding to a highly conserved region of the 18ssu RNA gene and species-specific DNA probes hybridising to highly variable internal sequences. We report on our extensive screening programme using this technology to analyse various clinical materials for the presence of fungal pathogens.

A prospective screening program of blood samples was performed between 4/1996 and 1/1998 in 87 recipients of an allogeneic SCT. Samples were routinely taken twice weekly starting during conditioning therapy until discharge. PCR was not considered for clinical decision making. Proven and clinical IFI were diagnosed according to criteria defined by the EORTC. All patients were nursed in laminar air flow units and received antifungal prophylaxis. 55/87 paients suffered from advanced hematological malignancies. 35/87 pts. developed a positive PCR until day 30 posttransplant. None of the PCR-neg. pts. developed IFI until discharge, indicating a NPV of 100%. All pts. with proven and probable IFI (Aspergillosis, n = 7; Candidemia, n = 1) early posttransplant were found PCR positive. 3 out of 7 PCR+ pts. not treated empirically with antifungals early posttransplant developed invasive Aspergillosis after day 100 during intensified immunosuppression for cGvHD. A positive PCR was found to be correlated with IFI pretransplant, severe aGvHD and transplant from an unrelated donor. When screening 507 sequential BAL samples from 134 BMT recipients detection of Aspergillus DNA in the BAL at the time of transplant (n = 7) was associated with a high risk to develop invasive Aspergillosis in the early posttransplant period. 5/7 pts. found PCR positive and finally culture positive in subsequent BAL and/or lung biopsies developed invasive pulmonary Aspergillosis thus indicating proliferation of endogenous Aspergillus to be a possible pathomechanism of this devastating infectious complication.

Thus, molecular techniques provide improved detection methods for fungal pathogens and might allow to more thoroughly study the epidemiology of invasive fungal disease.

PL-1085 INTENSIVE THERAPY IN 100 PATIENTS WITH PHI AND/OR *BCR-ABL* POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): FIRST INTERIM ANALYSIS OF THE FRENCH-BELGIAN-AUSTRALIAN LALA-94 TRIAL

H. Dombret, X. Thomas, D. Blaise, F. Huguet, J.M. Boiron, A. Buzyn, A. Stamatoulas, A. Delannoy, K. Bradstock, C. Boucheix, C. Charrin, J. Gabert, G. Lebbé, V. Lhéritier, D. Fière. *For the LALA Group, Hôpital Edouard Herriot, Lyon, France*

Four hundred nineteen patients (pts) with ALL (15–55 y) were enrolled in the ongoing multicenter LALA-94 trial between June 94 and November 97. They all received a standard 4-drug/4-week induction course with an initial DNR vs IDA randomization. One hundred of these pts (24%) were diagnosed before Day 35 to have B-lineage Ph1+/BCR-ABL+ (67%), Ph1+ (11%), or BCR-ABL+ (22%) ALL (M/F, 58/42; median age, 41 y; median WBC, 19.10^9/L; CNS+, 3 pts; m/M/mM bcr, 58/25/4). They were all eligible to receive then an intensive MTZ-IDaraC course as consolidation or salvage therapy, followed by 1–2 pre-transplant MTX-Aspa courses. Allogeneic (if available matched related or unrelated donor) or autologous stem cell transplantation (SCT) was offered to all responding pts. Complete remission (CR) rates after induction (Time 1), consolidation/salvage (Time 2), and at transplant time (Time 3) were 49%, 62%, and 53%, with mortality rates of 2%, 6%, and 8%, respectively. Among the 49 pts alive in failure at Time 1, the salvage rate at Time 2 was 37%. Among the 67 pts achieving a CR at Time 1 or 2, 14 pts (21%) were in very early relapse at Time 3. In CR pts, a molecular remission was observed in 12/31 pts tested at Time 1 and in 9/24 pts tested at Time 3. In univariate analysis, non-blastic D8 bone marrow ($p = 0.01$), WBC < 25.10^9/L, ($p = 0.01$), and karyotype features ($p = 0.01$) were prognostic factors for being in CR at Time 3. Multivariate analysis showed that the result of D8 marrow examination was the main prognostic factor in 48 evaluable pts ($p = 0.004$). SCT feasibility was evaluable in 96 pts; 58 pts (60%) including 42 CR pts were actually transplanted (29 allo/geno-id, 12 allo/pheno-id, 9 auto/PBSC, 8 auto/BM). Sixteen pts were transplanted in failure or early relapse (11 allo/geno-id, 4 allo/pheno-id, 1 auto/PBSC). With a median follow-up of 24 months, median overall survival was 13 months. Patients with WBC < 25.10^9/L had significant better survival than others ($p = 0.008$). In the 58 transplanted pts, the median post-graft survival was 14 months. Patients allografted in failure or early relapse had worse post-graft survival than those allografted in CR ($p = 0.0003$).

In conclusion: 1) Rapid BCR-ABL molecular analysis is feasible and useful in prospective multicenter ALL trials; 2) D8 marrow and WBC are the two major prognostic factors which could be use to intensify earlier the treatment in very high-risk pts.

PL-1086 X-LINKED DYSKERATOSIS CONGENITA IS CAUSED BY MUTATIONS IN A HIGHLY CONSERVED GENE ENCODING A PROTEIN WITH PUTATIVE NUCLEOLAR FUNCTIONS

N.S. Heiss[1], S.W. Knight[2], T.J. Vulliamy[3], S.M. Klauck[1], S. Wiemann[1], P.J. Mason[2], A. Poustka[1], I. Dokal[2]. [1]*Deutsches Krebsforschungszentrum,*

Department of Molecular Genome Analysis, Im Neuenheimer Feld, Heidelberg, Germany; [2]Department of Haematology, Imperial College School of Medicine and Hammersmith Hospital, London, England

X-linked recessive dyskeratosis congenita (DKC) is a severe inherited bone marrow failure syndrome linked to Xq28 (1.4 Mb interval between Xq3274 and DXS1108). Hybridisation screening with 28 positional candidate cDNAs resulted in the detection of a 3 deletion in one DKC patient with cDNA probe XAP101. Five different missense mutations in five unrelated patients were subsequently identified in the XAP101 cDNA, indicating that XAP101 is the DKC1 gene. *DKC1* is highly conserved across species barriers and is the orthologue of the rat NAP57 and *Saccharomyces cerevisiae CBF5* genes. The peptide, dyskerin, contains two TruB pseudouridine (ι) synthase motifs, multiple phosphorylation sites, and a C-terminal lysine-rich repeat domain. By analogy to the function of the known dyskerin orthologues, a putative involvement in the cell cycle and nucleolar function is predicted for the protein.

HEMATOLOGY-IN-FOCUS-SYMPOSIA

HiF9. Lymphoma

HiF-1087 MANAGEMENT OF FOLLICULAR LYMPHOMA

T.A. Lister. *ICRF Department of Medical Oncology, St. Bartholomew's Hospital, London EC1A 7BE, UK*

The strategy of the management of follicular lymphoma has until very recently by its repeated incomplete responsiveness to alkylating agent based therapy, but little evidence of curability. It is possible that this may change with the introduction of novel 'biological' approaches to treatment. Interferon a has been adopted by some as part of 'conventional therapy' on the basis of the results of randomised clinical trials: provocative results have been reported with both 'naked' antibody and targeted irradiation: vaccination is becoming a practical possibility. This, against a background of increasing experience with high dose therapy and purine analogue containing combinations may provide the opportunity to test the hypothesis that 'molecular' remission is a feasible and possibly worthwhile goal.

HiF-1088 TREATMENT OF NON-FOLLICULAR INDOLENT DISSEMINATED LYMPHOMAS

B. Coiffier. *Hospices Civils de Lyon, Lyon, France*

Indolent lymphoma is not a subgroup of the REAL or WHO classifications but an entity that regroups lymphomas with a low growing rate even if most of them are disseminated at the time of diagnosis and are not curable with standard therapies. Lymphomas regrouped under this term are small lymphocytic, lymphoplasmocytoid, marginal zone, MALT, follicular, and mantle cell lymphomas. If the treatment of follicular lymphoma or localized MALT lymphoma is more or less defined, it is not the case for the other lymphomas and very few prospective trials have been reported for them.

The percentage of patients with disseminated stage in each of these entities and their median survival is presented in the following table.

% of patients with disseminated stage			Median survival
Small lymphocytic L		>90%	5–6 y
Lymphoplasmocytoid L		~80%	4–5 y
	Marginal zone L		
	splenic	>90%	8–10 y
	nodal	~60%	4–5 y
	MALT	20–25%~5 y	
Mantle cell L		80–90%	~3 y

Adverse prognostic factors were not different from the ones reported in follicular or large cell lymphomas even if the application of the International Prognostic Index does not discriminate very well between the good and poor outcome patients.

Recommended treatments comprise chlorambucil, purine analogs, CVP, CHOP or more intensive therapies with autotransplant. They will be reviewed and the place of rituximab (anti-CD20 monoclonal antibody) will be delineated.

HiF-1089 LONG-TERM RISK OF SOLID TUMORS IN SURVIVORS OF HODGKIN'S DISEASE

F.E. Van Leeuwen, W.J. Klokman, A. Hagenbeek, M.B. Van 't Veer, J.W. Baars, B.M.P. Aleman, N.S. Russell, R. Somers. *The Netherlands Cancer Institute, Amsterdam, The Netherlands*

Background: Although many studies have assessed the overall risk of second cancer (SC) development in patients with Hodgkin's disease (HD), few reports have focussed specifically on the effect of age at diagnosis of HD disease on the long-term risk of this complication.

Methods: We assessed SC risk in 1301 survivors of HD who were less than 40 years old at diagnosis of HD and who were admitted to the Netherlands Cancer Institute (Amsterdam) or the Dr Daniel den Hoed Cancer Center (Rotterdam) between 1966 and 1986. The median follow-up duration amounted to 14 years.

Results: In all, 135 patients developed a SC (19.3 cases expected on the basis of cancer incidence in the general population; relative risk (RR): 7.1 (95% CI: 5.9–8.3). The mean 25-year actuarial risk of all SCs was 28%. The RR of all solid tumors combined rose with increasing follow-up period, with RR estimates of 3.0 (1–9 yr follow-up period), 5.7 (10–14 yr), 8.8 (15–19 yr), 8.1 (≥20 yr). In 15-year survivors, significantly increased RRs were observed for lung cancer (n = 9; RR = 9.1), breast cancer (n = 22; RR = 10.1), stomach cancer (n = 4; RR = 14.5), colorectal cancer (n = 4, RR = 4.8). The increased RR of solid tumors was related to radiotherapy (RT); 15-year survivors treated with a combination of RT and chemotherapy had similar risk as patients treated with RT alone (RR = 8.6; 95% CI: 6.1–11.8 versus RR = 9.3; 95% CI: 6.0–13.7). The RR of solid cancers increased strongly with younger age at first treatment of HD. For breast cancer, the RRs in women first treated at ages 30–39, 20–29, and before age 20 were 2.1 (95% CI: 0.8–4.6), 6.1 (95% CI: 3.0–10.8) and 26.0 (95% CI: 11.9–49.3), respectively. For all solid tumors exclusive of breast, the RRs in women first treated at ages 30–39, 20–29, and before age 20 were 4.6 (95% CI: 3.2–6.4), 7.1 (95% CI: 4.8–10.1) and 15.6 (95% CI: 8.7–25.7), respectively.

Conclusion: Solid tumor risk in survivors of HD is strongly increased, even after more than 20 years of follow-up. Age at HD diagnosis is a strong determinant of the excess risk of both breast cancer and other solid tumors.

HiF10. Hematopoietic stem cell transplantation

HiF-1090 THE ROLE OF MATCHED UNRELATED DONOR TRANSPLANTS IN THE MANAGEMENT OF MALIGNANCY

J.F. Apperley. *Imperial College School of Medicine, London, UK*

Allogeneic stem cell transplantation remains the treatment of choice for younger patients with a variety of haematological malignancies who have HLA-identical siblings. For those patients without genotypically identical donors, the optimal treatment is less clearly defined and depends on a number of factors including age, disease status, response to conventional treatment, and availability, gender and degree of compatibility of an alternative donor. In general the results using unrelated donors are inferior to those using siblings. The reasons for the decreased disease free survival (DFS) and the increased transplant related mortality (TRM) include increases in the incidences of acute and chronic GVHD, graft failure and life threatening infections, all of which presumable reflect the presence of varying degrees of HLA-disparity, and a tendency to delay the procedure. Attempts to modify the risks of graft failure and GVHD by using intensified conditioning regimens and more rigorous GVHD prophylaxis, have in turn resulted in increases in infectious complications, pneumonitis and disease recurrence. These modifications are necessary to compensate for the presence of HLA-disparity between the recipient and donor. Theoretically therefore, improvements in outcome would seem to depend on improved donor selection. Selection of unrelated donors has previously relied upon serological identification of HLA-A, B and RD alleles. Sequencing of the HLA-genes has now revealed a greater degree of polymorphism at these loci than that detected by serology. The influence of matching for Class I HLA-alleles has long been recognised. The cytotoxic T-cell precursor (CTLp) frequency assay reflects differences at the Class I loci and at least two groups have identified the presence of high frequency CTLp to be useful prognostic indicator of outcome after unrelated donor transplant. However in a recent analysis of 320 patients transplanted for CML in 1st CP from unrelated donors and reported to the EBMT, matching for HLA-DRB1 was the most important factor influencing DFS. This confirmed data previously reported by the Seattle Transplant Team. The dilemma now facing physicians is that the use of sophisticated techniques for HLA-matching is likely to render the identification of a fully matched unrelated donor impossible. The goal must now be to identify acceptable degrees of "mismatch" but with the enormous heterogeneity of the HLA locus, even this may be beyond our reach.

HiF-1091 CORD BLOOD BANKING AND TRANSPLANTATION

E. Gluckman, V. Rocha, C. Chastang. *For Eurocord; Hôpital Saint-Louis, Bone Marrow Transplant Unit, 1 avenue Claude Vellfaux, Paris, France*

The number of cord blood transplants has been increasing very quickly with more than 250 cases reported to Eurocord Registry and more than 500 cases transplanted through the New York Cord Blood Bank. Cord blood transplants have been performed either with related or unrelated cord blood. Several cord blood banks established a group called Netcord whose goal is the standardization of the procedures, the organization of internal audits for accreditation and qualification, and the communication and exchange by Internet of donor search on an international basis. More than 15000 units of frozen cord blood are currently available and this number is increasing rapidly worldwide. Analysis of the clinical results has shown that related cord blood transplants gives better results than unrelated cord blood transplants. Factors associated with better survival in related and unrelated cord blood transplants were younger age, diagnosis with better results in inborn errors and good risk children acute leukemia. Higher number of nucleated cells in the transplant and recipient negative CMV serology were also favorable risk factors for survival. Engraftment was improved with higher number of cells and HLA identity. Graft versus Host disease was reduced when compared to adult allogeneic bone marrow or peripheral blood transplants. HLA disparities did not influence GVH, the only factor associated with increased GVH was recipient positive CMV serology. This study shows that cord blood is an alternative source of hematopoietic stem cells for allogeneic transplantation in children and in some adults. HLA disparity is not a limiting factor but the number of cells infused is important, currently the use of a number of nucleated cells inferior to 1×10^7/kg is not recommended. Several questions remain including the criteria of choice of the donor, the indications in children and in adults, the comparison of cord blood transplants to other sources of hematopoietic stem cells and the role of growth factors and expansion for improving the speed of engraftment.

HiF-1092 HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR AUTOIMMUNE DISEASE (AD)

A. Fassas. *George Papanicolaou Hospital, Thessaloniki, Greece*

In animal models of AD, HSCT can induce durable remissions, even cures. Besides, resolution of autoimmunity has been observed in patients with AD who underwent HSCT for concomitant malignancies. Based on these data, HSCT has been proposed for the treatment of severe AD. HSCT could either replace the aberrant immune system (allogeneic setting) or tip the immune balance towards suppression (autologous setting). In Auto-HSCT, T cell-depletion is most probably necessary to avoid reinfusion of autoreactive cells. A few reports of AD cases without malignancies who were treated with auto-HSCT have already come out, including CREST syndrome, systemic sclerosis, ITP, Evans syndrome (umbilical cord HSCT), RA, SLE, amd multiple sclerosis (MS). Meaningful improvements have been observed in some of these patients, with reduction of disease to low and responsive levels. Relapses do occur, as well as treatment failures. Since 1995, we have conducted two consecutive, phase I/II trials of autologous blood HSCT in 24 patients with progressive MS. The protocol was same in both studies, using CY/G-CSF for stem cell (SC) mobilization, BEAM for conditioning, and ATG after SC infusion for T cell-depletion. In study #2, CD34+ cell-selection of the graft was also performed. Stabilization of disease and even durable improvements were observed in 21 patients. There was one treatment-related death (4%) and in 2 cases the disease progressed. Progression at 12 and 18 months was 5% and 7%, resp. Before concluding on the usefulness of HSCT in treatment of AD, more studies need to be conducted. Moreover, long-term outcomes will determine whether benefits can counterbalance toxicity and cost.

HiF11. Infectious diseases in hematological patients

HiF-1093 THE EUROCARE STUDY. LYON: IARC SCIENTIFIC PUBLICATION, NO 135, 1995. THE USE OF AMINOGLYCOSIDES IN FEBRILE NEUTROPENIA: WHERE DO WE STAND?

C. Cordonnier. *Service d'Hematologie Clinique, Hopital Henri Mondor, 94000 Creteil, France*

The gold standard therapy for febrile neutropenia has been established years ago as the combination of a betalactam (BL) and an aminoglycoside (AG) on the rationale of broad spectrum, synergistic effect, and low risk of selection of resistant mutants. However, the advent of new broad-spectrum and strongly bactericidal BL has led to consider the possibility of empiric monotherapy. Several clinical trials have studied the effects of BL, with or without AG, with controversial results. In the light of these trials, several questions must be discussed: (1) Should be use AG in the initial combination of empiric therapy? The clinical relevance of an AG depends on the choice of the BL combined with it. The more bactericidal is the BL, the less useful is the AG. However, from a microbiological point of view, the microbiological effect of a given BL is always better when associated with an AG, than alone. High-risk patients, especially those with Pseudomonas infections, are most likely to benefit from the combination. However, Pseudomonas infections rarely represent more than 5% of the episodes and unnecessary renal toxicity may balance the benefit of AG on a large cohort of patients. Therefor, high-risk patients should be recognised to be given the optimal treatment as soon as possible. (2) If AG are necessary, for how long should we give them? In case of fever of unknown origin, it is now recommended to stop AG after 48–72 h. In case of documented infection, most teams give AG at least for 7 days, except for pseudomonas infections which justify a longer duration of bitherapy. (3) If AG are necessary, what is the best schedule of administration? Several advantages argue for the once daily administration: reduced risk of nephrotoxicity, prolonged post-antibiotic effect, lower risk of adaptative resistance, better tissular concentrations. However, because of increased volume of distribution and plasmatic clearance in neutropenic patients, the risk is that the plasma levels stay for a long time below the minimal inhibitory concentrations. Therefore, the once daily administration is not widely used in neutropenic patients.

HiF-1094 ANTIVIRAL TREATMENT IN HEMATOLOGICAL PATIENTS

P. Ljungman. *Dept. of Hematology, Huddinge University Hospital, Huddinge, Sweden*

Viral infections are important causes of morbidity and also mortality in hematological patients and recent developments have improved the possibilities for prevention and therapy of viral infections. The most important viruses in hematological patients belong to the herpesvirus group. New drugs such as famciclovir and valaciclovir are available for herpes simplex virus and varicella zoster virus infections. In allogeneic BMT patients different strategies are available to prevent CMV disease including ganciclovir prophylaxis and preemptive therapy with ganciclovir and foscarnet. Treatment results of established CMV disease are, however, still poor. The importance of therapeutic strategies against the more recently identified herpesviruses human herpesvirus types 6 and 8 needs to be established. Respiratory syncytial virus (RSV) is being recognized as an important cause of severe respiratory disease in hematological patients in particular BMT patients. Ribavirin is the only drug currently available for RSV infection but its efficacy needs to be proven by controlled studies. New agents with efficacy against RSV are urgently needed. Several new antiviral agents are in development for influenza and hepatitis B and will hopefully be introduced into clinical practice in the next few years.

HiF-1095 TREATMENT OF FEBRILE NEUTROPENIA ON AN OUTPATIENT SCHEDULE

P. Martino. *Dipartimento di Biotecnologie Cellulari ed Ematologia, University "La Sapienza", Rome, Italy*

The standard clinical approach in severely immunocompromised cancer patients, particularly those with persistent, severe neutropenia, is that these subjects remain hospitalized until the infective complication has resolved, allowing convenient antibiotic delivery and continuous medical surveillance. However, an accurate clinical stratification of patients on the base of different medical risk could differenziate the approach to infective complications. Several large studies show that patients whose medical condition is likely to remain stable could be examined in ambulatory regimen or briefly hospitalized and safely discharged to receive antibiotic therapy at home. This clinical approach seems to be safe and effective, well accepted by patients, and cost-saving. On the contrary, patients with severe neutropenia, with serious comorbidity and/or uncontrolled malignancy are characterized by progressively falling immunologic defenses allowing much higher risk of subsequent severe infective complications. However, quality of life and cost containement could justifie home management of this category of patients. An advanced home care program with a continuous medical and nursing assistance and with a complete laboratory support could be a safe and effective alternative to hospitalization for the management of infections patients with progressive hematologic malignancies. Recent advances in antimicrobial therapy, including the availability of broad spectrum betalactam antibiotics with long half-lives and the development of oral antipseudomonal agents, could allow an efficacious and manageable home treatment.

HiF12. Hemoglobinopathy. Molecular biology and treatment

HiF-1096 MAGNESIUM ORAL SUPPLEMENTS AND HEMOGLOBINOPATHIES

L. De Franceschi, Y. Beuzard, F. Galacteros, M.D. Cappellini, D. Bachir, O. Platt, S.L. Alper, G. Graziadei, J. Tchernia, R. Corrocher, C. Brugnara. *Dept of Internal Medicine, University of Verona; Dept of Internal Medicine, University of Milan, Italy; Experimental Laboratory of Gene Therapy, Hopital St Louis, Paris; Centre de la Drepanocytose and INSERM U91, Hopital H Mondor, Creteil, Paris; Centre Hospitalier Le Kremlin Bicetre, Paris, France; Dept of Pathology, Children's Hospital and Beth Israel Hospital, Harvard Medical School, Boston, MA, USA*

Magnesium (Mg) is the second most abundant intracellular metal in cells and plays an essential role in the activity of many enzymatic pathways and membrane cation transport systems. Recently, we have reported that cell Mg content is significantly reduced in erythrocytes (RBC) of patients with sickle cell anemia and β thalassemia intermedia. We have also shown that oral supplementation with Mg pidolate can increase the red cell Mg content in these patients. Cell Mg is also involved in modulation of K-Cl cotransport which is abnormally increased in both sickle and β thal erythrocytes. In β thalassemia intermedia we have shown that oral supplementation with Mg pidolate improves some of the characteristic abnormalities of β thal erythrocytes such as increased Na-K pump, K-Cl cotransport; cell dehydration, increased osmotic resistence. In patients with sickle cell disease, a significant inhibition of RBC K-Cl cotransport and sickle dehydration can be achieved in vivo when erythrocyte Mg is increased with dietary Mg pidolate over a 4 wk period. When this treatment is maintained for a longer period of time (6 months) we have observed that the increase RBC Mg is associated with a significant reduction in the activity of the Na-Mg exchanger, the main mechanism for Mg extrusion from RBC. In addition oral administration of Mg pidolate induces a significant reduction in the number of painful crisis, indicating that cellular changes induced by Mg therapy are associated with clinically significant improvements in the painful manifestations of SS disease. These results suggest the possible therapeutic value of oral Mg pidolate in patients with sickle cell disease and β thalassemia.

HiF-1097 GENETIC VARIANTS MODULATING HAEMOGLOBIN F/F CELL LEVELS

S.L. Thein. *MRC Molecular Haematology Unit, Institute of Molecular Medicine, Oxford, UK*

In normal adults, Hb F accounts for <0.6% of the total haemoglobin, restricted to a sub-set of erythrocytes termed F cells (FC). The percentages of HbF/FC vary considerably (up to 20-fold) in normal adults, the distribution of these proportions being positively skewed. Although family studies have shown that high levels of Hb F and FC appear to be inherited, the number of genetic factors involved and the extent to which they account for the heritability of Hb F/FC remain uncertain. Among the factors known to influence Hb F/FC levels are age, sex, different sequence variants in the β globin cluster, including the T/C variation at position-158 of the $^{G}\gamma$ globin gene (*Xmn* I-$^{G}\gamma$ site) and the co-inheritance of β thalassaemia. 10–15% of the population have ≥4.5% FC corresponding to ≥0.6% Hb F. This increase in Hb F (1%–4% of the total Hb) is transmitted in the condition referred to as heterocellular HPFH (hereditary persistence of fetal haemoglobin). In heterocellular HPFH, the modestly increased levels of Hb F are distributed unevenly among the FC, no mutations are identifiable with the β globin complex, and in many families the high FC phenotype segregates independently of the β complex, implicating the presence of trans-acting factors. Two such loci have been mapped by linkage analysis, one on chromosome 6q23 and the another to the Xp22.2–p22.3 region with additional trans-acting autosomal loci implicated in other families. The talk will focus on two approaches which we have employed towards dissection of the genetic basis of FC/Hb F production: (1) Positional cloning of a major QTL on 6q; (2) Twin/sib pair studies to delineate and map the different QTLs controlling Hb F/FC production.

The mapping and characterisation of these QTLs should help our understanding of variation in the Hb F/FC levels in adults and the variable response to pharmacological reactivation of Hb F in sickle cell disease and β thalassaemia.

HiF-1098 THE DYNAMICS OF β-GLOBIN GENE SWITCHING

F.G. Grosveld, E. De Boer, T. McMorrow, J. Gribnau, E. Milot, T. Trimborn, M. Wijgerde, P. Fraser. *Dept. of Cell Biology & Genetics, Erasmus University Rotterdam, Rotterdam, The Netherlands*

The most important level of the regulation of the β-globin genes is by activation of all of the genes by the Locus Control Region (LCR) and repression of the early genes by an as yet unknown factors acting on sequences flanking the genes. Superimposed on this is a mechanism in which the early genes (ε and γ) suppress the late genes (δ and β) by competition for the interaction with the LCR. Although this extra level of gene regulation is quantitatively of less importance than the direct repression mechanism, it has important implications and has provided an excellent assay system to probe the regulation of transcription at the single cell level.

These studies indicate that the LCR interacts with individual globin genes and that LCR/gene interactions are dynamic with complexes forming and dissociating continually. The levels of expression of each of the genes appear to depend on: (1) the frequency of interaction which is itself dependent on the distance of the gene to the LCR, (2) the affinity of the LCR for the gene and (3) the stability of the LCR/gene complex. The latter two are dependent on the balance of transcription factors. We conclude that transcription only appears to take place while the LCR and gene interact and that the level of transcription is determined by the frequency and duration of such interaction rather than changes the rate of transcription of the promoters.

HiF13. Phosphatases in signalling in hematopoietic cells

HiF-1099 STRUCTURE FUNCTIONS OF PHOSPHATASES IN SIGNALING AND CELL CYCLE

J.E. Dixon. *Department of Biological Chemistry, The University of Michigan Medical School, Ann Arbor, MI 48109-0606, USA*

Protein tyrosine phosphatases (PTPases) play important roles in regulating the phosphorylation status of the cell. The PTPase family includes receptor-like proteins, intracellular catalysis and phosphatases which can catalyze the removal of both phosphotyrosine as well as phospho-serine/threonine from proteins. Structural information and biochemical characterization suggest that the PTPase family of enzymes employ a common catalytic mechanism. In addition to the phosphatases which have a specificity for removal of phosphatase from tyrosine, another group of catalysts which will dephosphorylate both Ser/Thr- and Tyr-containing residues have been described. This group of enzymes is collectively referred to as dual-specificity phosphatases. We have recently initiated studies identifying a dual-specific phosphatase in S. cerevisiae and have utilized the power of yeast genetics to explore their functions as well as to identify substrates for these phosphatases. There appears to be approximately 17 different genes encoding protein tyrosine phosphates and the dual specific phsophatases in the yeast genome. The function of these catalyst in the cell cycle and the substrate specificity of these yeast phosphatases will be discussed.

HiF-1100 ROLE OF SHP-2 IN HEMATOPOIETIC CELL SIGNALLING

B.G. Neel[1], H. Gu[1], B. Chen[1], J. Timms[1], S. Kamatkar[1], J.D. Griffin[2]. *[1]Beth Israel Deaconess Medical Center, Boston, MA; [2]Dana Farber Cancer Institute, Boston, MA, USA*

Previous studies have implicated the SH2-containing tyrosine phosphatase SHP-2 in signaling by both hematopoietic receptor tyrosine kinases, such as c-Kit and cytokine receptors. We have used reverse genetic and gene knockout models to explore SHP-2 function in hematopoiesis in more detail. Primary bone-marrow derived mast cells from heterozygotic SHP-2 knockout mice are hypo-sensitive to IL-3. Biochemical analysis indicates that MAPK induction in response to IL-3 is diminished in these mice. This appears to represent a generalized defect in signaling through the IL3 R beta chain, since bone marrow-derived macrophages from these mice are hypo-responsive to GM-CSF stimulation. Mast cells from SHP-2 +/− mice also are hypo-responsive to SCF; this defect is exacerbated in mice doubly deficient for SHP-2 and c-Kit (Wv/+: SHP-2/+ and Wv/Wv:SHP2 +/− mice, respectively). However, CSF-1R signaling does not appear to be defective in SHP2 +/− macrophages. To further elucidate the targets of SHP-2 action in signaling by IL-3 and other cytokines, we have focused on a 97kD phosphotyrosyl protein that is phosphorylated in response to stimulation by a broad range of cytokines and associates with SHP-2 via the latter's SH2 domains. Interestingly, this protein is constitutively phosphorylated and associated with SHP-2 in BCR-ABL transformed cell lines. Studies with C/S "trapping" mutants of SHP-2 suggest that p97 is an SHP-2 substrate. We therefore purified p97 to near homogeneity, obtained microsequence information and cloned its cDNA. Sequence analysis reveals p97 to be a homolog of Drosophila DOS. The role of this protein in hematopoietic cell signalling will be discussed.

HiF-1101 REGULATION OF LYMPHOCYTE ACTIVATION BY ITIM-BEARING RECEPTORS AND THEIR ACTIVATING COUNTERPARTS

Eric Vivier. *Centre d'Immunologie INSERM/CNRS de Marseille-Luminy and Institut Universitaire de France, France*

A family of cell surface receptors involved in the control of cell activation has recently emerged. These receptors are characterized by the presence of 1 to 4 intracytoplasmic Immunoreceptor Tyrosine-based Activation Motifs (ITIMs) based on the consensus (I/V/L/S/)xYxx(L/V). ITIM-bearing receptors encompass at least 15 sub-families which include more than 75 distinct cDNAs. Remarkably, ITIM-bearing receptors belong to one of two receptor families: the immunoglobulin- and the C2 lectin proteins. ITIM-bearing receptors are expressed on hematopoietic cells as well as non-hematopoietic cells (e.g. SIRP molecules are expressed on fibroblasts). Finally, ITIM-bearing receptors require co-aggregation with protein tyrosine kinase-dependent receptors (e.g. the T-cell receptor complex, the B-cell receptor complex) in order to mediate their inhibitory function. Upon engagement, the Tyrosine residue present in ITIM is phosphorylated and allows the association with SH2-containing intracytoplasmic phosphatases. Photophatases recruited *in vivo* by ITIM-bearing receptors belong to two categories: the protein tyrosine phosphatases, SHP-1/SHP-2 and the polyphosphate inositol phosphatase, SHIP. Whereas FcγRIIB is the only SHIP-associating ITIM-bearing receptors, all other ITIM-bearing molecules bind to SHP-1 and/or SHP-2. We focused our attention on Killer-cell Inhibitory Receptors (KIRs), which serve as NK and T lymphocyte receptors for MHC Class Ia and showed that they bear ITIMs. In addition, it is striking that isoforms of each ITIM-bearing receptors have been identified, and are characterized by their lack of intracytoplasmic ITIM, as well as their activating properties. We have identified KAPAP, a novel 12 kDa transmembrane polypeptide bearing an ITAM (Immunoreceptor Tyrosine-based Activation Motif) which associates with KARs (Killer-cell Activating receptors), the activating isoforms of KIRs. Genetic analysis of KAR and KARAP will undoubtedly contribute to the precize understanding of KAR function *in vivo*.

SIMULTANEOUS SESSIONS

SS19. Acute lymphoblastic leukemia

O-1102 TCRγδ⁺ T-CELL ACUTE LYMPHOBLASTIC LEUKEMIAS: Vδ1 AND NON-Vδ1 EXPRESSING CASES REPRESENT DISTINCT MATURATION STAGES

A.W. Langerak[1], I.L.M. Wolvers-Tettero[1], M.W.M. Van den Beemd[1], E.R. Van Wering[2], W.-D. Ludwig[3], K. Hählen[4], A. Necker[5], J.J.M. Van Dongen[1]. *[1]Dept. of Immunology, Erasmus University Rotterdam; [2]Dutch Childhood Leukemia Study Group, The Hague; [4]Dept. of Pediatrics/Div. Hemato-Oncology, Erasmus University Rotterdam, THe Netherlands; [3]Dept. of Hematology/Oncology,/Tumor Immunology, Humboldt University of Berlin, Germany; [5]Immunotech, Marseille, France*

A large series of 30 T cell receptor (TCR)γδ⁺ T-cell acute lymphoblastic leukemias (T-ALL) was analyzed for their immunophenotype as well as the rearrangements and junctional regions of the *TCRG* and *TCRD* genes. In addition, in 15 cases membrane expression of TCRγδ proteins was studied extensively by flow cytometry with a recent Vγ/Vδ antibody panel.

Virtually all TCRγδ⁺ T-ALLs expressed TdT, CD2, CD3, CD5, CD6, and CD7, but were heterogeneous in their CD1/CD4/CD8 immunophenotype. The majority expressed either CD4⁺/CD8⁻ or CD4⁺/CD8⁺, whereas only 7/30 TCRγδ⁺ T-ALLs lacked both antigens. Despite the heterogeneity with respect to the rearranged *TCRG* and *TCRD* genes, we found a preference for Vγ1 (21/30), Jγ2.3 (19/30) and Cγ2 (21/30) gene products in the TCRγδ⁺ T-ALL. Expressed *TCRD* genes were largely limited to Vδ1-Jδ1, but in 6 cases non-Vδ1 TCRδ chains (Vδ2-Jδ1, Vδ2-Jδ3, Vδ3-Jδ1, Vδ6-Jδ2, and two Vα-Jδ1) were found. The junctional region diversity of *TCRG* and *TCRD* genes was rather extensive due to the insertion of nucleotides (average of 8.6 and 28.7, respectively) and deletion of nucleotides (average of 9.6 and 4.9, respectively). Analysis of Vγ and Vδ protein expression with the antibody panel confirmed the predominant, but not exclusive, expression of Vγ1 and Vδ1 proteins. Importantly, not a single T-ALL expressed the common peripheral blood Vγ9⁺/Vδ2⁺ phenotype.

Comparison of non-Vδ1⁺ TCRγδ T-ALLs with the more common Vδ1⁺ type revealed several differences. First, more complete *TCRD* rearrangements were identified on the non-expressed allele in the non-Vδ1⁺ group (83% vs. 43%); second, a higher number of complete *TCRB* rearrangements was found in non-Vδ1 cases (79% vs. 50%). Together these data suggest that Vδ1 and non-Vδ1 TCRγδ T-ALL represent distinct maturation stages, with non-Vδ1 cases showing a more mature genotype.

O-1103 COMPARISON OF ALLOGENIC TRANSPLANTATION, AUTOLOGOUS TRANSPLANTATION AND CHEMOTHERAPY AS POST INDUCTION TREATMENT IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL). LONG TERM REPORT OF THE FRENCH GROUP OF TREATMENT OF ADULT ALL (LALA 87 PROTOCOL)

D. Fière, C. Sebban, J.P. Boiron, J.P. Vernant, F. Rigal Huguet, J.P. Miclea, C. Sutton, J.Y. Cahn, F. Dreyfus, E. Lepage. *Hematological Department Hôpital Edouard Herriot Lyon, France*

572 eligible adult ALL have been included in the prospective French study (LALA 87) between November 1986 and July 1991. Protocol pattern and

results have been published elsewhere (J Clin Oncol 11: 1190, 1993 - J Clin Oncol 12: 2580, 1993 - Blood 84: 1603, 1994). This report focus on very long term (median follow up 8 years) outcome of patients in complete remission (CR) included either in the allogeneic BMT trial or in the autologous BMT trial. Patients in each trial were stratified in high risk or standard risk according to Hoelzer'criteria.

All patients in CR between 15 and 40 years old with at least one sibling were included in allo BMT trial: patients with an HLA identical sibling were in the early allo BMT arm, others were in the control group.

Patients without identical sibling between 15 and 50 years still in CR during the second course of consolidation chemotherapy were randomized in autologous BMT arm or in maintenance chemotherapy arm.

436 patients (74%) achieved CR. 257 patients were included in the allo BMT trial with 116 in allo BMT arm and 141 in the control group. In the autologous BMT trial, 95 patients were randomizedd during the second consolidation in autologous arm and 96 in chemotherapy arm. Analysis was mad on an intention to treat basis. At 8 years, in BMT trial overall survival was 46 in allo arm versus 29 in control group. The difference was highly significant when comparing high risk patients. 44% allo versus 11% in control group and the result in standard risk were 49% versus 39%. In auto BMT trial, overall survival was 34% for auto arm versus 29% for chemotherapy arm ($p = 0.65$) and there were no significant difference when patients were stratified in standard or high risk: high risk 16% for auto against 11% for chemo ($p = 0.7$). Standard risk 49% for auto against 40 for chemotherapy ($p = 0.7$). These results favours allo BMT in first CR in high risk patients and shows that auto BMT is equivalent to chemotherapy with a possible superiority in term of quality of life (shorter period of treatment).

O-1104 PROGNOSTIC VALUE OF DRUG RESISTANCE PROFILES IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

M.L. Den Boer[1], R. Pieters[1], K.M. Kazemier[1], D.O. Harms[2], G.E. Janka-Schaub[2], A. Van der Does-Van den Berg[3], W.A. Kamps[3], E.R. Van Wering[3], G.J.L. Kaspers[1], A.J.P. Veerman[1]. *[1]University Hospital Vrije Universiteit, Pediatric Hematology/Oncology, Amsterdam; [3]Dutch Childhood Leukemia Study Group (DCLSG), The Hague, NL; [2]COALL study group, Hamburg, Germany*

A profile combining in vitro prednisolone, vincristine and L-asparaginase (PVA) cytotoxicity (as determined by MTT assay) was an independent prognostic factor in children with acute lymphoblastic leukemia (ALL) treated according to DCLSG protocols. [1] In the present prospective study we confirmed the prognostic value of the PVA profile in 202 children with ALL treated according to the Germany COALL-92 protocol. With a preliminary follow up of 25 months (range 1–64) the 2-yr disease free survival (DFS) was 78%, 86% and 93% in patients with a resistant, intermediate and sensitive PVA profile, respectively ($p = .04$). In the pooled group of 314 DCLSG and COALL patients, the PVA profile was the strongest and only independent prognostic factor at multivariate analysis including age, immunophenotype and white blood cell count (WBC) ($p < .001$). At a median follow up of 3 years the DFS among high risk patients only (proB or T-ALL or age $\geq$10 yr or WBC $\geq$ 25/nl) was 64%, 79% and 96% for the resistant, intermediate and sensitive group respectively ($p = .005$). The PVA profile was also of strong prognostic value among low risk patients only, i.e. 3-yr DFS of 63%, 86% and 95% for resistant, intermediate and sensitive PVA profiles, respectively ($p = .01$). In conclusion, the PVA profile indicates patients at high and at low risk of treatment failure independent of conventional risk factors and may therefore improve the stratification of patients. In the COALL-97 treatment protocol children with ALL are currently stratified using the PVA profile. Supported by the Dutch Cancer Society - grant VU 93–641.

(1) Kaspers GJL et al., 1997, Blood 90: 2723–2729.

O-1105 INVESTIGATION OF MINIMAL RESIDUAL DISEASE (MRD) IN Ph-1 POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) BY A COMBINATION OF CELL SORTING (CD34+ CELLS) AND FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

N. Cambier, C. Cossement, A. Cosson, J.L. Lai, P. Fenaux, C. Preudhomme. *CHU Lille, France*

The Ph-1 chromosome is found in about 25% of adult ALL.RT-PCR analysis of BCR-ABL fusion transcripts has demonstrated its usefulness in the diagnosis of Ph-1 ALL, especially in cases of cytogenetic failure. RT-PCR analysis is also a useful tool in the evaluation of MRD after treatment, particularly after allogeneic BMT. However, after induction chemotherapy (I.CT), most of the patients remain PCR-positive in the bone marrow. In contrast, conventional cytogenetic methods are useful for diagnosing the disease but not sensitive enough to detect low numbers of residual leukemic cells. FISH makes it possible to score a large number of cells and is a potentially more sensitive method for detecting chromosomal abnormalities. This study was designed to investigate MRD in patients with Ph-1 positive ALL in clinical and cytogenetic (ie normal caryotype in 50 mitoses examined) remission by using a combination of cell sorting (CD34 + cells) and FISH. Six patients in clinical and cytogenetic CR after I CT, consolidation CT (C CT) or allo BMT were studied (in pts n°4, 5, 6, allo BMT had been performed 1, 2 and 3 months, resp before last examination).

Results:

% CD34 cells with positive FISH

		after I.CT	after C1 CT	after C2 CT	after allo BMT
pts 1	blood/BM	8/34	5/41	39/38	
pts 2	Blood/BM	9/38	3/5	18/89	
pts 3	blood/BM	8/10			
pts 4	blood/BM		6/36		1/3
pts 5	blood/BM				2/3
pts 6	blood/BM				1/2

Two round RT-PCR was still positive after C2 CT for patients 1 and 2 and was negative after allo BMT in patients 4 to 6. Pts 1 and pts 2 relapsed a few weeks after C2CT whereas patients 3 to 6 remained in CR but only 1 month after last examination.

Conclusion: Using a combined method of cell sorting followed by FISH, we were able to detect residual leukemic cells in patients in clinical and cytogenetic CR at different time points. This combination of two methods can be used as a sensitive and quantitative method to monitor the number of residual leukemic cells following induction and consolidation chemotherapy in Ph1 ALL and possibly other leukemias.

O-1106 IG AND TCR GENE REARRANGEMENT PATTERNS IN ADULT ALL PATIENTS ARE MORE IMMATURE AS COMPARED TO CHILDHOOD ALL: IMPLICATIONS FOR SELECTION OF PCR TARGETS FOR DETECTION OF MRD

T. Szczepański[1], A.W. Langerak[1], I.L.M. Wolvers-Tettero[1], G.J. Ossenkoppele[2], G. Verhoef[3], M. Stul[4], E.J. Petersen[5], M.A.C. De Bruijn[1], M.B. Van 't Veer[6], J.J.M. Van Dongen[1]. *[1]Dept. of Immunology; [6]Dept. of Hematology, Erasmus University Hospital Rotterdam; [5]Dept. of Hematology, University Hospital Utrecht; [2]Dept. of Hematology, Free University Hospital, Amsterdam, The Netherlands; [3]Dept. of Hematology; [4]Center for Human Genetics, University of Leuven, Belgium*

In order to get insight into immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements in adult ALL, we studied 48 patients (26 with precursor-B-ALL and 22 with T-ALL) enrolled in the HOVON-18 study and compared the results to previously analyzed large series of pediatric ALL patients. Southern blotting (SB) with multiple DNA probes for the *IGH, IGK, TCRB, TCRG, TCRD,* and *TAL1* loci revealed rearrangement patterns largely comparable to pediatric ALL, but several differences were found for precursor-B-ALL patients. Firstly, adult patients showed a lower level of oligoclonality in the *IGH* gene locus (5 out of 26 patients; 19% vs. ~40% in pediatric precursor-B-ALL) despite a comparable incidence of *IGH* gene rearrangements (24 out of 26 patients; 92%). Secondly, the *IGK* gene deletions were found in only ~35% of adult patients vs. ~50% of pediatric cases. All detected *IGK* gene deletions (n ≈ 12) concerned rearrangements of the kappa deleting element (Kde) to Vκ gene segments, which represent two thirds of the Kde rearrangements in pediatric precursor-B-ALL. Thirdly, a striking predominance of immature Dδ2-Dδ3 cross-lineage recombinations was observed (7 out of 16 *TCRD* rearrangements; 44%), whereas more mature Vδ2-Dδ3 gene rearrangements occurred less frequently (6 out of 16 *TCRD* rearrangements; 38% vs. >70% in pediatric precursor-B-ALL). Together these data suggest that the Ig/TCR genotype of precursor-B-ALL is more immature and more stable in adults than in children.

We also evaluated whether heteroduplex analysis of PCR products of rearranged Ig and TCR genes can be used for identification of molecular targets for minimal residual disease (MRD) detection. Using five of the major gene targets (*IGH, IGK, TCRG, TCRD* and *TAL*1 deletion), we compared the SB data and heteroduplex PCR results. High concordance between the two methods ranging from 96 to 100% was found for *IGK, TCRG,* and *TAL*1 genes. The concordance was lower for *IGH* (70%) and *TCRD* genes (90%). Based on the heteroduplex PCR data we conclude, that MRD monitoring is possible in almost 90% of adult precursor-B-ALL and >95% of adult T-ALL patients.

SS20. Molecular changes in leukemia

O-1107 QUANTITATION OF MRD IN CHILDREN WITH OLIGOCLONAL ALL SHOWS THAT THE CLONES THAT GROW OUT DURING RELAPSE SHOW THE SLOWEST REDUCTION ALREADY DURING INDUCTION THERAPY

V. De Haas[1,2], O.J.H.M. Verhagen[2], H. Van den Berg[1], A.E.G.Kr. Von dem Borne[3], C.E. Van der Schoot[2]. *[1]Emma Kinderziekenhuis/Kinder AMC; [2]Central Lab. of the Netherlands Red Cross and the Lab. of Exp. and Clin. Immunol., University of Amsterdam; [3]Dept. of Haematology, Academic Medical Center, Amsterdam, The Netherlands*

Polymerase Chain Reaction (PCR) for immunoglobulin heavy chain and T-cell receptor rearrangements can be applied for the detection of Minimal Residual Disease (MRD) in B-precursor Acute Lymphoblastic leukemia (ALL). The presence of PCR-detectable malignant cells at the end of induction therapy is strongly correlated with the occurrence of a relapse. Precise quantitation of residual disease will increase the predictive value of the PCR results. Oligoclonality with respect to antigen receptor gene configuration is the major drawback of MRD detection by means of IgH/TCR rearrangements, because the outgrowing clone at relapse may have changed its rearrangement. However, it is not known whether oligoclonality is hampering MRD detection already during induction therapy. Therefore we selected 4 children with oligoclonal ALL and quantified the different subclones in bone marrow samples taken at diagnosis, at the end of induction therapy, as well as in a sample taken at relapse in the 3 relapsed patients. Nested PCRs, specific for the each subclone, were tested on 2-fold diluted DNA samples (20 replicates). Because this PCR can detect a single cell, the number of positive PCR reactions at a certain dilution end-point measures the number of residual leukemic cells, calculated by Poisson statistics. In all patients, we were able to follow at least 4 clonal rearrangements. In two patients we performed single-cell analysis on FACS-stored leukemic cells to show which of the rearrangements were obtained from the different alleles present in the same cell. We found that within each patient the subclones as characterized by the rearrangements were behaving differently in response to therapy. In all 3 patients, the clones that grew out during relapse showed relatively the slowest reduction. We conclude that precise quantitation of MRD during induction therapy can be investigated by IgH/TCRθ-PCR in a limiting dilution assay, but it is necessary to use multiple markers for the various clones detected at diagnosis.

O-1108 EXPRESSION OF BCR-ABL IN M1 CELLS INDUCES DIFFERENTIATION WITHOUT ARRESTING PROLIFERATION

N. Cambier, Y. Zhang, G. Vairo, K. Kosmopoulos, D. Metcalf, N.A. Nicola, A.G. Elefanty. *The Walter and Eliza Hall Institute of Medical Research Melbourne, Australia*

The undelying mechanism leading to the preponderance of maturing myeloid cells which characterises chronic myeloid leukaemia (CML) remains obscure. Because of its ability to mimic the proliferative and cell survival functions of haematopoietic growth factors, we hypothesized that the oncogene activated in CML, BCR-ABL, may also influence differentiation. To test this hypothesis, we examined the effects of BCR-ABL on the myeloid differentiation of murine M1 cells, which cease dividing and differentiate into macrophages in the presence of LIF or IL-6. We found that BCR-ABL induced morphological features of macrophage differentiation in M1 cells, accompanied by increased expression of macrophage cell surface markers and the acquisition of phagocytic ability. Interestingly, clones of M1 cells which expressed BCR-ABL remained in cell cycle and were refractory to the growth inhibition and apoptosis induced by IL-6 or LIF. Low levels of constitutively phosphorylated stat 3 in M1.210 cells provided a possible molecular mechanism for the differentiative response to BCR-ABL. M1.210 cells constitutively expressed CIS-1 and SOCS-1 mRNAs, which may have contributed to their impaired responses to LIF and IL-6. These cells also expressed inappropriately high levels of c-MYC mRNA for their degree of differentiation, which may have been important in maintaining cellular proliferation. These data suggest that the stimulation of both differentiation and proliferation by BCR-ABL may be involved in the genesis of the phenotype observed in CML.

O-1109 $p16^{INK4a}$ ABROGATION BY A MUTANT CYCLIN DEPENDENT KINASE 4 (CDK4) CHANGES CML PROGENITOR CELL KINETICS

W. Chinswangwatanakul, J.L. Lewis, S.B. Marley, N.C.P. Cross, J.M. Goldman, M.Y. Gordon. *Department of Haematology, Imperial College School of Medicine at Hammersmith, London, UK*

$p16^{INK4a}$, a putative tumour suppressor gene, is one of the cell cycle control genes. $p16^{INK4a}$ usually forms a binary complex with CDK4/6 and acts as a negative regulator by displacing them from cyclin D. $p16^{INK4a}$ deletions have been detected in 50% of lymphoid blast crisis CML patients and at least two CML blast crisis cell lines, K562 and BV173, but $p16^{INK4a}$ alterations have not been reported in chronic phase CML. We have shown previously that restoring the $p16^{INK4a}$ gene in K562 and BV173 cells could partially reverse their malignant phenotypes by inhibiting growth and prolonging cycling time. To confirm the $p16^{INK4a}$ suppressive function and its role in CML evolution, we designed studies to block $p16^{INK4a}$ expression in CD34+ cells separated from CML patients in chronic phase using retrovirus-mediated gene transfer of a mutant CDK4. CDK4-K35M, a catalytically inactive CDK4 mutant, can sequester $p16^{INK4a}$ and therefore mimic $p16^{INK4a}$ deletion. As CDK4-K35M can also sequester cyclin D, wild type CDK4 (wtCDK4) was used as a control. Both CDK4 cDNAs were cloned into a retroviral vector, pBabeNeo (pBN) and transfected into GP+*env*AM12, an amphotropic packaging cell line. We used a transwell system to achieve a high percentage of transduced cells with low producer cell contamination. After co-incubation with pBN, pBN-CDK4-K35M, pBN-wtCDK4, and unpackaged producer cells, transduced CD34+ cells were plated for CFU-GM and BFU-E in the presence or absence of G418. Video recording of individual CFU-GM colonies was undertaken daily for 14 days to follow the kinetics of colony formation. We achieved approximately 50% transduction efficiency confirmed by PCR for Neo®. The CDK4-K35M transduced CFU-GM showed a faster growth rate and shorter doubling time compared to controls ($p < 0.0001$). In addition, the mutant CDK4 transduced CFU-GM colonies had an earlier onset of colony formation ($p < 0.0025$). Analysis of BFU-E colony formation showed that $p16^{INK4a}$ abolition by CDK4-K35M reduced the numbers of subcolonies in erythroid brusts which is indicative of an increased probability of differentiation (reduced probability

of self-replication). We conclude that abrogation of the normal function of $p16^{INK4a}$ in CML alters progenitor cell kinetics and thereby mimics blastic transformation. The data provide support for the notion that deletion of p16 may play a causal role in progression of CML.

O-1110 MLL-TANDEM DUPLICATIONS CAN BE FOUND IN THE HEMATOPOIESIS OF ALMOST ALL HEALTHY INDIVIDUALS

S. Schnittger, B. Wörmann, W. Hiddemann, F. Griesinger. *Hematology/ Oncology, Göttingen, Germany*

Rearrangements of the MLL-gene (for mixed lineage leukemia) by reciprocal translocations involving chromosome band 11q23 are well described in infants and adults with acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL). In addition, partial tandem duplications of the NH_2 region of the MLL-gene have been associated with trisomy of chromosome 11 in AML and recently, have also been reported for karyotypically normal AML. We addressed the question whether MLL-duplications are leukemia specific alterations or whether they occur outside the context of malignancy in normal hematopoietic cells. Analysis of 65 peripheral blood samples from healthy individuals by two step (nested) RT-PCR with 35 cycles, each, revealed MLL-duplication transcripts in almost all samples. Duplication transcripts were also identified in mRNA isolated from bone marrow of two healthy individuals, indicating that at least some of the duplication transcripts are potentially functional. The results were confirmed in a second series of experiments with completely new reagents and RNA in a distant lab building. The length of the transcripts varied from sample to sample, with up to 5 different duplication transcripts per individual. Sequencing of RT-PCR products revealed fusions of exons 9, 10, and 11 with exon 3 identical to those found in a subset of AML patients that are positive for the MLL duplication as indicated by single step PCR and Southern blot. In addition, several fusion products were identified which have not been described in AML. Four of these fusions have been characterized by sequencing and revealed exon9/exon4, exon9/exon2, as well as exon13/exon3 fusions. One fusion transcript included a pseudoexon from intron 1 fused to exon3. All transcripts with the exception of the exon9/exon4 fusion, are in frame and potentially translatable. Dilution series indicated that the frequency of duplication transcripts is 4 logs less frequent in normal individuals than in AML patients positive for MLL duplication in primary PCR. Genomic seminested PCR using intronic primers revealed MLL-duplications also at the DNA level. The results of sequencing indicated that the duplications are products of Alu mediated recombinations. Our data strongly suggest that MLL duplications occur in a subset of cell in normal hematopoisis at the genomic level. The biological function of this phenomenon is still unclear.

O-1111 RETROVIRAL INSERTION IN *CB2 (EVI11)* IN CASBR-M MULV-INDUCED LEUKEMIAS FREQUENTLY COINCIDES WITH A NOVEL COMMON INTEGRATION SITE *EVI12*

P.J.M. Valk, Y. Vankan, M. Joosten, B. Löwenberg, R. Delwel. *Institute of Hematology, Erasmus University Rotterdam, Rotterdam, The Netherlands*

Evi11 has been shown to be a frequent common retroviral integration site (cVIS) in CasBr-M MuLV-induced leukemias. In two myeloid cell lines, i.e. NFS107 and NFS78 and in 14 CasBr-M MuLV-induced primary tumors, retroviral insertion was found in the *Evi11* locus. The target gene in *Evi11* is *Cb2*, a gene which encodes the peripheral cannabinoid receptor. This receptor is tissue specific and expressed in all blood cell lineages. Furthermore, it has been shown that *Cb2* is important in growth regulation of hematopoietic cells. How retroviral insertion in *Evi11* may affect *Cb2* protein expression and leukemogenesis is subject of current investigations. Leukemogenesis is a multistep process involving various genetic defects. In an attempt to identify novel cVISs and proto-oncogenes which may cooperate with *Cb2* in tumor development, retroviral flanking cDNA fragments were isolated from the "*Cb2* positive" cell line NFS107. By Southern blot analysis it was demonstrated that one of those fragments identified a new cVIS, which we designated *Evi12*. *Evi12* rearrangements were present in 17% of the CasBr-M MuLV-induced primary tumors. More interestingly, in 8/14 "*Evi11/Cb2* positive" primary leukemias, virus insertions were found in *Evi12* as well. These data strongly suggest cooperation between the proto-oncogenes in *Evi12* and *Evi11*. The retroviral integrations in *Evi12* were all within a 1.6 kb genomic fragment, located on mouse chromosome 10, 5′ of a candidate target gene *Grp94*. *Grp94* is a molecular chaperone which is involved in folding and maturation of proteins. Although this protein is ubiquitously expressed, recent studies by J.W. Brewer et al. (EMBO 16:7207, 1997) demonstrated that in myeloid cells (*Evi12* negative) the expression of *Grp94* is tightly regulated by growth factors, e.g. Epo or IL3. Preliminary results indicate that in the "*Evi12* positive" IL3 dependent cell line NFS107, *Grp94* mRNA expression is elevated. Currently, studies are being caried out to investigate whether as a result of retroviral insertion in *Evi12*, the regulation of *Grp94* expression by IL3 or other growth factors is disturbed. Our data suggest that in "*Evi11/Evi12* positive" leukemias aberrant expression of *Cb2* and *Grp94* may be key defects in the multistep proces of tumor progression.

SS21. Chronic lymphoproliferative disorders

O-1112 COLLABORATIVE META-ANALYSIS OF RANDOMISED TRIALS IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

J.L. Binet, G. Dighiero, D. Catovsky, M. Hansen, B. Jaksic, E. Kimby, E. Montserrat, S. Richards, P. Wiernik. *On behalf of the CLL Trialists' Collaborative Group, CTSU, Harkness Building, Radcliffe Informary, Oxford OX2 6HE, England; French Cooperative Group on CLL; MRC; CTSU, UK; Danish CLL Study Group; IGCI, Croatia; Lymphoma Group of Central Sweden; PETHEMA, Spain; ECOG, USA*

It has been difficult to assess the relative effects of immediate versus deferred treatment for early stage CLL, and of combination chemotherapy versus chlorambucil for later stage disease. A systematic review, involving a collaborative meta-analysis, was performed to obtain reliable estimates of these effects. Individual patient data were sought for all relevant randomised trials, and were centrally checked and analysed.

Six trials, involving 2000 patients with early CLL, randomised deferred treatment versus immediate treatment with chlorambucil or chlorambucil plus a steroid. 10-year survival was 44% with immediate versus 47% with deferred treatment (difference = 3% with standard deviation (SD) 3%; non-significant (NS)).

Fewer than 400 patients in any stage have been randomised in trials of chlorambucil versus chlorambucil plus a steroid. Survival was similar with both treatments. Four trials, involving 760 patients, randomised COP (cyclophosphamide, vincristine plus prednisone) versus chlorambucil (with or without a steroid), and five trials, involving 840 patients, randomised CHOP (COP plus doxorubicin) versus chlorambucil (with or without a steroid). There was no evidence of any difference between individual trials in their estimate of the relative effect of these treatments and, overall, 5-year survival was 48% SD 2% both with combination chemotherapy and with chlorambucil.

These results suggest that treatment of early stage disease should be deferred until necessitated by symptoms, and that when treatment is required, there may well be little or no advantage to using combination chemotherapy instead of chlorambucil.

O-1113 EARLY USE OF CHLORAMBUCIL IS UNABLE TO INFLUENCE SURVIVAL IN STAGE A CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS. LONG TERM RESULTS FROM TWO RANDOMIZED TRIALS OF THE FRENCH COOPERATIVE GROUP IN CLL

G. Dighiero, K. Maloum, B. Desablens, B. Cazin, M. Navarro, R. Leblay, M. Leporrier, J. Jaubert, G. Lepeu, B. Dreyfus, F.-L. Turpin, G. Tertian, A. Bichoffe, J.-L. Binet, P. Travade. *For The French Cooperative Group in CLL - Institut Pasteur, Paris, France*

Background: To determine whether immediate treatment with chlorambucil is of benefit for early stage CLL patients, the French Cooperative Group ran two randomized trials in untreated stage A CLL patients, involving 1535 patients. Because median survival can be expected to be as much as ten years, long follow-up was required.

Patients and Methods: In CLL-80, 609 patients were randomized to daily continuous chlorambucil (CB) or no treatment; in CLL-85, 926 patients were randomized to either intermittent CB plus prednisone (CP) or abstention. Median follow-up is now >11 and >6 years, respectively. Endpoints were overall survival, treatment response and disease progression.

Results: No benefit of early treatment was observed in either trial (relative risk of death = 1.14; p = 0.23 {confidence interval (CI: 0.92–1.41} for CLL-80 and relative risk of death = 0.96; p = 0.74 {CI: 0.75–1.23} for CLL-85). In the CLL-80 and 85 trials, 76% and 70% of patients respectively responded to therapy ({Complete hematological remission (CHR) + partial remission (PR)} and these patients displayed better survival than patients failing to respond (FAI). Although a benefit of CB in slowing disease progression was observed, no effect on overall survival was found. In the abstention group from the CLL-80 trial, 49% of patients did not evolve and did not need any therapy after a follow-up >11 years. However, 37% of stage A patients have died of causes related to disease and 41% progressed to stages B and C.

	No of patients	No of deaths	No of deaths related to CLL	Overall 7-year survival	7-year without progression to B and C	7-year survival according to treatment response		
						CHR	PR	FAI
CLL-80 (AB)	308	169	115	62%	65%			
CLL-80 (CB)	301	175	128	66%	77%	78%	69%	50%
CLL-85 (AB)	466	126	101	69%	78%			
CLL-85 (CP)	460	121	82	69%	83%	84%	77%	58%

Conclusion: Neither of the two CB schedules prolonged survival in these patients. Since deferring therapy until it is required because of disease progression to stages B or C does not compromise survival, initial therapy could have been appropriately deferred.

O-1114 PRELIMINARY RESULTS OF THE UK MEDICAL RESEARCH COUNCIL TRIAL IN CHRONIC LYMPHOCYTIC LEUKAEMIA - CLL3

D. Catovsky, T. Hamblin, S. Richards. *On behalf of the MRC Working Party in Adult Leukaemia. Royal Marsden Hospital, London; Royal Bournemouth Hospital; CTSU, Oxford, UK*

The Medical Research Council (MRC) CLL3 trial was a multi-centre randomised trial of standard chlorambucil versus chlorambucil plus epirubicin for patients with B-cell chronic lymphocytic leukaemia (CLL). Patients with Binet stage A progressive disease, stage B or stage C were eligible provided they required treatment and were either untreated, or had been treated without anthracyclines and were not considered resistant to chlorambucil. Schedules were chlorambucil 10 mg/m^2 days 1–6 or epirubicin 50 mg/m^2 i.v. on day 1 plus chlorambucil 10 mg/m^2 days 2–7, repeated every 28 days. Treatment was given for at least 6 months unless there was evidence of progression. Patients who did not respond by 6 months were eligible for treatment with fludarabine in the MRC non-responder study.

418 patients were randomised between May 1990 and August 1997 and have been followed up to October 1997. At entry, 77 patients were Binet stage A, 159 stage B and 182 stage C. Most patients were previously untreated (395/418). There was no clear difference in response rates at 6 or 12 months:

	6 months		12 months	
	C	C + E	C	C + E
No response	55 (30%)	50 (27%)	18 (13%)	15 (10%)
Partial response	113 (62%)	110 (59%)	95 (68%)	94 (65%)
Complete response	15 (8%)	27 (14%)	27 (19%)	35 (24%)
Total currently evaluable	183	187	140	144

There is no significant difference in survival between the randomised groups: 95 deaths/208 patients in the chlorambucil group and 89/210 in the chlorambucil plus epirubicin group (reduction in the odds of death = 10%, standard deviation = 15%, 2 p = 0.5) Survival at 5 years is 45%, 49% for chlorambucil, chlorambucil plus epirubicin respectively.

O-1115 DYSREGULATION OF FAS/FAS LIGAND-APOPTOTIC PATHWAY IN CD3+ LARGE GRANULAR LYMPHOCYTE (LGL) LEUKEMIA

T. Lamy, J.H. Liu, T.H. Landowski, W.S. Dalton, T.P. Loughran, Jr.. *H. Lee Moffitt Cancer and Reseach Institute, Tampa, Florida, USA*

Fas/(CD95)-induced apoptosis plays a critical role in the elimination of activated lymphocytes and induction of peripheral tolerance. Defects in Fas/Fas-Ligand (Fas-L)-apoptotic pathway have been recognized in autoimmunc lympho-proliferative diseases (ALPS) and *lpr* or *gld* mice, and attributed to Fas and Fas-L gene mutations, respectively. Large granular lymphocyte (LGL) leukemia is a chronic disease characterized by a proliferation of antigen-activated Cytotoxic T lymphocytes. Autoimmune features such as, hyperγglobulinemia, rheumatoid factor, and circulating immune complexes are common features in LGL leukemia and ALPS. Therefore we hypothesize that expansion of leukemic LGL may be secondary to a defective Fas apoptotic pathway. In this study, we investigated expression of Fas and Fas-L in 11 CD3+ LGL leukemia and explored the apoptotic response to anti-Fas MAb. We found that leukemic LGL from each patient expressed constitutively high levels of Fas/Fas-L, similar to those seen in normal activated T cells. However, cells from 9 of these 11 patients were totally resistant to anti-Fas-induced apoptosis. Similar results were seen after anti-CD3-MAb-triggered cell death. Lack of anti-Fas-induced apoptosis was not due to mutation in the Fas antigen. Leukemic LGL were not intrinsically resistant to Fas-dependent death, as LGL from all but one patient underwent apoptosis after phytohaemaglutinin (PHA)/interleukin 2 (IL2) activation. The patient whose leukemic LGL were intrinsically resistant to Fas had an aggressive form of LGL leukemia which was resistant to combination chemotherapy. These findings that leukemic LGL are resistant to Fas-dependent apoptosis despite expressing high levels of Fas are similar to observation made in Fas-L transgenic mice. These data suggest that LGL leukemia may be a useful model of dysregulated apoptosis causing human malignancy and autoimmune disease.

O-1116 PCR ANALYSIS OF Vλ GENE USAGE IN B CELL DISORDERS

A. Abusedra, A. Bybee, M. Laffan, J.F. Apperley. *Department of Haematology, Imperial College School of Medicine, London, UK*

Although approximately 40% of the antibodies found in human serum contain Ig λ light chains, the human λ locus was until recently poorly characterised at the molecular level. Despite recent progress in characterising the structure of this locus. studies on rearrangement remain scarce, particularly with regard to use of the Vλ repertoire. We have therefore attempted to amplify rearranged Vλ genes from 160 cases of lymphoproliferative disorders (117 CLL, 14 multiple myeloma (MM), 18 AL amyloidosis, 8 hairy cell leukaemia (HCL) and 3 lymphoma) in whom immunophenotyping showed lambda restriction. Using a Vλ consensus primer and a J λ consensus primers products were generated in 108 of these patients (67.5%). These were confirmed by sequence analysis to be rearranged Ig λ sequences. Clonal λ gene rearrangements were detected in 90 patients with CLL (77%) but in only 5 of the MM patients (35%) and 6 of those with AL amyloidosis (33%). The high success rate in CLL is probably due

the high proportion of clonal cells in the samples, lower somatic mutation rates in this disease, and good quality DNA. Of 8 patients with HCL, 6 (75%) were PCR positive, and sequence analysis in these cases also showed no or little mutation from germ-line. The lower success rates in MM and AL amyloidosis are probably due to a combination of a high rate of somatic mutation and a lower percentage of clonal cells in the samples. 13 of the 30 known Vλ genes were utilised in these cases. Of the 90 λ CLLs in whom a rearrangement was sequenced, 38 used the gene segment known as IGVL3S2 (3h) and 20 used DPL11 (2a2). GVLX4.4 (10a) and DPL5 (1b) segments were each used in 6 patients. Segments DPL3 (1g). DPL23 (3r) and DPL24 (4c) were each used in four patients. Segment DPL8 (1e) was used three times, segment DPL16 (31) twice and segments DPL2 (1c), DPL4 (1d) and DPL1 (1a) were each used once. Comparison of these results with the normal B cell repertoire suggests that there may be a preferential usage of IGVL3S2 and DPL11 in CLL.

O-1117 IMMUNOPHENOTYPE AND DNA PLOIDY ANALYSIS: IMPLICATIONS FOR MINIMAL RESIDUAL DISEASE INVESTIGATION IN MULTIPLE MYELOMA

J.F. San Miguel[1], J. Almeida[2], G. Mateo[1], M. Ocqueteau[1], M.D. Caballero[1], R. García-Sanz[1], M. González[1], J. Bladé[3], M.J. Moro[4], I. Hernández[5], A. Orfao[6]. *[1]Servicio Hematología, Hospital Universitario, Salamanca; [2]Departamento de Medicina, Universidad de Salamanca; [3]Hospital Clínico de Barcelona; [4]Hospital Virgen Blanca León; [5]Hospital General de Segovia; [6]Servicio General de Citometría, Universidad de Salamanca, Salamanca, Spain*

Intensive treatment strategies, including bone marrow transplantation, result in a high complete remission (CR) rate in multiple myeloma (MM) patients (up to 60%) and a three year post-diagnosis probability of event-free survival of 40–60%. Relapses are the major cause of treatment failure, due to the persistence of minimal residual disease (MRD), which is undetectable by morphology. Accordingly, more sensitive techniques are needed to evaluate the effectiveness of these new therapies in MM patients. The aim of the present study was to explore the applicability of immunophenotype and DNA ploidy analysis for monitoring MRD in MM patients. Sixty-one bone marrow (BM) samples from untreated MM patients and 10 healthy BM samples were stained by direct immunofluorescence using a large panel of McAb in triple combinations: CD38, CD56, CD19, CD40, CD28, CD80, CD138, CD10, CD13, CD33, CD20, CD45, CD22, CD23, CD117, CD34, HLA-DR, sIg, FMC7 and CD9. In order to increase the sensitivity of the technique, a double-step acquisition was made (FACSort cytometer, Becton/Dickinson): first, a total of 10^4 events/test were acquired and in a second step, acquisition through a "live gate" drawn on SSC/CD38++ (where PC are located) was performed. Overall, in 87% of MM patients the PC displayed an aberrant phenotype with respect to normal PC, that would allow the investigation of MRD: over-expression of CD56 (62%), CD28 (16%) and CD33 (6.5%) and asynchronous expression of CD117 (28%) sIg (21%) and CD20 (10%). Serial dilutional experiments performed in 14 patients – in order to stablish the sensitivity level of the technique – allowed the identification of one aberrant PC among 10^4–10^5 normal BM cells. A simultaneous staining for PC (CD38 and CD138) and DNA was performed to analize the DNA content. In 63% of MM patients PC displayed DNA aneuploidy. Thus, this marker would allow the distinction between normal and myelomatous PC. Dilutional experiments performed in 6 cases showed that it was possible to detect up to one aneuploid cell among 10^4 diploid cells. The simultaneous use of both techniques allowed the investigation of MRD in 93% of MM patients. According to these results, phenotype and DNA ploidy analysis would allow the monitoring of MRD in most MM cases and their sensitivity levels compare favourably with the morphological approach and almost reaches the levels obtained by PCR technique.

SS22. Hemoglobinopathia and thalassemia

O-1118 ANTIBODY BLOCKING OF SICKLED RED BLOOD CELL ATTACHMENT TO CULTURED ENDOTHELIUM

K.A. Brown, R. Mahmalji, T.C. Pearson[1], F.A. Spring[2], S.F. Parsons[2], D.J. Anstee[2]. *Departments of Immunology and [1]Haematology, United Medical and Dental Schools of Guy's and St Thomas' Hospital, London; [2]The Bristol Institute for Transfusion Sciences, Bristol, UK*

Binding of irreversibly sickled red blood cells (RBCs) to endothelium activated with TNFα may lead to microvascular occlusions and the painful crises of sickle cell disease. This study investigated whether the abnormal binding of sickled RBCs to endothelial cells could be inhibited by antibodies against RBC glycoproteins that may act as adhesion molecules. Adherence was measured by a radiometric assay in which RBCs were overlaid onto monolayers of human endothelial cells. Stimulation of the monolayers with TNFα increased their adhesiveness for sickled RBCs by approximately 200% ($p < 0.005$). Irreversible sickled RBCs isolated from 28 patients with sickle cell disease were treated with antibodies against CD44, CD47, glycophorin A, Lutheran, LW or Kell glycoproteins prior to the assay. Irrelevant isotype-matched antibodies acted as controls. Sickled cell binding was inhibited only by antibodies against glycophorin A, the R18 epitope but not Wr^b, (mean 17±9%; $p < 0.05$), Lutheran (24±15%; $p = 0.05$), LW glycoprotein (27±17%; $p = 0.05$) and CD47 (35±19%; $p < 0.02$). The findings demonstrate that CD47, glycophorin A, Lutheran and LW glycoproteins are contributing to the attachment of sickled RBCs to endothelium and that antagonising their expression could interfere with the formation of microvascular occlusions in sickle cell disease.

O-1119 HYDROXYUREA INDUCED ERYTHROPOIETIN SECRETION IN SICKLE CELL SYNDROMES MAY UNDERLIE THE OBSERVED HbF INCREASE

I. Papassotiriou, A. Stamoulakatou, E. Voskaridou[1], E. Stamou, D. Loukopoulos[2]. *Hematology Laboratory, "Aghia Sophia" Children's Hospital; [1]Thalassemia Unit, "Laikon" Hospital; [2]First Dept of Medicine, University of Athens, Athens, Greece*

The mechanism of the significant increase of HbF which appears in patients with SCD or thalassemia after 4–8 weeks of treatment with hydroxyurea (HU) is not yet completely understood. Most accepted is the view that the drug causes an acute depletion of the normally maturing HbS synthesizing erythroid precursors followed by massive "recruitment" of a population of earlier precursors which maintain the program for HbF synthesis but remain dormant in the normal marrow. Over the last years we have treated with HU several patients with various SCD. Results have been presented on various occasions and they all confirm both the increase of HbF and the dramatic clinical improvement of the patients. The present paper summarizes our findings with regards to the levels of serum erythropoietin determined at various intervals after initiation of therapy. The hormone assays were carried out by chemiluminescence; all measurements were done in duplicate. HbF was determined by ce-HPLC. The pattern of response was similar in almost all cases. Baseline serum EPO values (at least two measurements prior to starting therapy) varied from 33–570 IU/L. 2–4 weeks after initiation of treatment with HU (usually 1.5 g daily over 7 days/week) EPO levels increased gradually to reach values 3–31 times the basal ones after 8–16 weeks of continuous administration of the drug. The increase of HbF was almost parallel. Following this peak, the EPO values started decreasing again and stabilized at an intermediate (but definitely higher that the initial one) level throughout the rest of therapy. EPO levels have been observed to increase in many instances following cancer chemotherapy with pattern similar to the one described above. The underlying mechanisms have not been elucidated. On the other hand, administration of high doses of exogenous r-huEPO in animals has been followed by dramatic increases of HbF. We suggest that the increase of HbF in SCD following HU administration may reflect this mechanism.

O-1120 METABOLIC DEFECTS IN β-THALASSEMIA INTERMEDIA ERYTHROCYTES RELATE TO MEMBRANE OXIDATIVE DAMAGE

D. Tavazzi, M.D. Cappellini, L. Duca, G. Graziadei, F. Turrini[1], P. Arese[1], G. Fiorelli. *Centro Anemie Congenite, Osp. Magg. Policlinico IRCCS, Dip. Medicina Interna, Univ. di Milano; [1]Dip. Genetica, Biologia, Genetica Medica, Università di Torino, Italy*

Beta-thalassemia (β-thal) is characterized by ineffective erythropoiesis, iron overload and reduced survival of erythrocytes (RBC). Globin precipitation and oxidative membrane damage were already well investigated and impaired RBC redox metabolism was previously reported, however a direct relation with membrane abnormalities has not yet been established. Here we report the relation between RBC membrane alterations and metabolic defects in 15 untransfused β-thal intermedia patients. Membrane-bound hemichromes (HCR) were separated by gel-filtration from ghosts and quantitated spectrophotometrically. Band 3 and bound IgG were also determined in protein aggregates. Erythrophagocytosis was measured in cultured monocyte-derived macrophages. Glutathione (GSH) and NADPH were quantitated by HPLC. Basal and stimulated hexose-monophosphate shunt (HMPS) and glucose consumption through the HMPS were evaluated as CO_2 production. Results are listed as medians (min-max) in the table:

	Thal int (n = 15)	**Controls** (n = 10)
HCR (nmol/mL RBC)	6.05 (1.42–16.80)	0.06 (0–1.12)
band 3 (% of total)	3.08 (0.21–12.81)	0 (0–2.86)
GSH (mol/RBC × 10^{-17})	2.68 (1.14–3.98)	6.39 (5.43–8.41)
NADPH (mol/RBC × 10^{-17})	0.27 (0.11–0.35)	0.179 (0.15–0.26)
bHMPS (mol/RBC × 10^{-21})	14.15 (3.98–31.10)	6.54 (5.16–7.56)
sHMPS (mol/RBC × 10^{-21})	96.52 (27.8–200.90)	115.30 (97.1–148.7)
s/b HMPS ratio	7.17 (2.46–24.73)	17.72 (15.53–28.12)
b glucose (% of total)	8.05 (3.80–18.91)	4.95 (4.30–7.00)
s glucose (% of total)	43.90 (16.00–65.52)	58.15 (44.4–78.0)
s/b glucose ratio	4.80 (2.32–12.80)	11.54 (7.50–16.59)

Band 3 aggregation strongly correlated to HCR deposition ($p < 0.001$) and IgG were always present on β-thal RBC membranes. Erythrophagocytosis increased (1.87 vs 0.39 RBC/macrophage, $p < 0.010$) and correlated both with HCR ($p < 0.010$) and aggregated band 3 ($p < 0.013$) but not with IgG. GSH deficiency and a trend in NADPH increase were observed, but these reductants are not related to membrane damage. HMPS was overstimulated and lowered with increasing membrane damage. Moreover, HCR negatively correlated both with HMPS stimulation (s/b HMPS ratio, $P < 0.026$) and increment in HMPS glucose consumption (s/b glucose, $p < 0.019$). Globin precipitation was more severe when HMPS is less reacting. Furtherly, a role of HCR-induced band 3 aggregation in opsonization and clearance of RBC was demonstrated.

O-1121 CGP 72 670: A NEW, POTENT, ORALLY ACTIVE IRON CHELATOR

H.P. Schnebli. *Pharmaceuticals Research, Novartis Pharma, Basel, Switzerland*

CGP 72 670, is a representative of a new class of tridentate and iron-selective chelators, the bis-hydroxyphenyl-triazoles. Forty-four compounds of the triazole series with different molecular properties were evaluated both for their pharmacological effects and tolerability, together with some 700 other compounds from five additional chemical classes. CGP 72 670 emerged from this selection process as the compound which best combines high oral potency and tolerability.

It has a higher affinity for iron III (pM is 3 log units higher than that of L1) and its iron complex is kinetically more stable than that of L1, both *in vitro* and *in vivo*.

In the bile duct cannulated rat CGP 72 670 has a rapid onset of action and, at equivalent (molar) doses, is ten-fold more potent than L1 in iron elimination. As with most other iron chelators the duration of its effect increases with increasing dose. In a chronic (12 week) study in severely iron-loaded rats, CGP 72 670 was twice as effective as s.c. Desferal and far more effective than L1.

In the iron overloaded marmoset, the most relevant model for the characterization of iron chelators, CGP 72 670 induces iron excretion within a few hours of administration. Very considerable amounts of iron are still excreted in the 24 to 48 hour period, particularly at higher dosages. Iron excretion induced by CGP 72 670 is predominantly by the faecal route and only a few % are excreted in the urine. CGP 72 670 is highly selective for iron, and does not induce the excretion of zinc and copper. In the marmoset the "effective oral dose" for CGP 72 670 is about 22 mg/kg. At equivalent (molar) doses the total iron excretion induced by oral CGP 72 670 is more than 10-fold higher than that induced by L1.

CGP 72 670 is better tolerated than L1. In marmosets which received the compound daily for 4 weeks, no adverse effects were seen at 3 (males) and 6 fold (females) the "effective dose". Nephrotoxicity, seen at very high dosages, both in rats and marmosets, appears to be a consequence of severe iron deprivation, as it was a much reduced feature in iron overloaded animals. In the context of its safety evaluation, it was furthermore shown that CGP 72 670 does not promote the absorption of dietary iron.

O-1122 PHASE I, SINGLE DOSE TRIAL ON DEPOT DESFERRIOXAMINE (CGH 749B) IN TRANSFUSION-DEPENDENT β-THALASSEMIC PATIENTS

J.B. Porter[1], D. Alberti, I. Hassan, A. Racine, B. Davis[1], V. Voi[2], A. Piga[2]. *[1]University College London, UK; [2]University of Turin, Italy; Novartis Pharma Ltd., Basle, Switzerland*

We have evaluated a new depot formulation of desferrioxamine (CGH 749B) aimed at providing continuous chelation without the need of a pump. The tolerability profile, pharmacokinetics and urine iron excretion (UIE) were assessed over 72 h (19 patients) and 144 h (10 patients) after the administration of single subcutaneous bolus injections of CGH 749B (five escalating doses of 2.5, 5.0, 7.5, 10.0 and 12.5 mg/kg). The same assessments over 48 h were also performed on Desferal® (DFO) treatment (40 mg/kg, single subcutaneous infusion lasting 8 h).

28 pts on CGH 749B and 4 pts on DFO complained of mild or moderate adverse events at the injection site. Prolonged release of desferrioxamine from CGH 749B in comparison with Desferal® infusion and a lower peak concentration were observed. Mean AUC values versus CGH 749B dose showed excellent linearity ($r^2 = 0.927$). The linear regression is given by: AUC ratio = −0.03 + 0.20 (CGH 749 B dose ($P < 0.0001$). A similarly good linear relationship was also observed over the 144-hour period (AUC ratio = −0.75±0.35 (CGH 749 B dose - $P = 0.03$). A CGH 749 B dose of 5.2 mg/kg led to an FO AUC (0–72 and 0–144 h) that was approximately similar to that of Desferal® 40 mg/kg.

The linear relationship between the ratio of the urinary iron excretion over a period of 72 hour after the s.c. injection of CGH 749B to that of Desferal® 40 mg/kg over a period of 48 hours, and the administered dose of CGH 749B was given by: ratio = 0.26±0.11 × (CGH 749B dose); $P = 0.006$. A CGH 749B dose of 6.7 mg/Kg was therefore equivalent to a Desferal® dose of 40 mg/kg in UIE. The CGH 749B-induced iron excretion was about 1 mg daily between 72 hours and 144 hours after administration at the dose level of 7.5 mg/Kg. Our results show a promising efficacy and tolerability profile of CGH 749B after single s.c. bolus injections, which supports the rationale to start repeated administration studies.

O-1123 SYNTHETIC ALLOSTERIC MODIFIERS OF HEMOGLOBIN THAT COULD BE USEFUL IN THE TREATMENT OF SOME HEMOGLOBINOPATHY DISEASES

J. Kister[1], I. Papassotiriou[2], T. Boyiri[3], N. Griffon[1], D.J. Abraham[3], D. Loukopoulos[4], M.C. Marden[1], C. Poyart[1]. *[1]INSERM U473 Le Kremlin-Bicêtre, France; [2]Hematology Laboratory "Aghia Sophia" Childrens Hospital, Athens; [4]First Dep. of Medicine, Athens University, Athens, Greece; [3]Dep. of Medicinal Chemistry, Virginia Commonwealth University, Richmond, Virginia, USA*

The regulation of the allosteric equilibrium of hemoglobin (Hb) is of interest in medicine. Agents that produce a high affinity Hb ("left-shifter" compounds) have been clinically evaluated as antisickling agents while those that produce a low affinity Hb ("right-shifter" compounds) may be useful for the treatment of thalassemia and ischemic problems arising from stroke and cardiovascular diseases. The allosteric modifier approach consisting of lowering or increasing the oxygen affinity might be of utility for the treatment of these hemoglobinopathies and ischemic diseases. In this study, we investigated the effect of a bezafibrate derivatives RSR-4, [2-[4-[[(3,5-dimethylalanilino)carbonyl]methyl]-phenoxy]-2-methylpropionic acid], on the

oxygen affinity of fresh red blood cells (RBC) suspensions from patients with high F-thalassaemia intermedia (β^0CD39/$\delta\beta$) or with Hb H disease (showing a Hb H level of 33.5%) compared to that of normal controls. This allosteric modifier crosses the red cell membrane and binds to the α-chains of deoxy-Hb, decreasing Hb's oxygen affinity. RSR-4 is the most potent allosteric modifier discovered to date that shift the oxygen equilibrium curve to the right ("right-shifter" compounds) in whole blood and *in vivo*. We have also tested some new "left-shifters" compounds (TB88 and TB120) that are able to induce a large increase in the oxygen affinity of fresh red blood cells suspensions from normal (AA), untreated heterozygous (AS), and homozygous (SS) sickle patients. For each sample, we have performed the deoxygenation curve followed by the reoxygenation curve. In the deoxygenation procedure, these two modifiers induce a large increase of the oxygen affinity in the different types of RBC's suspensions (by a factor of 2 and 10 for TB120 and TB88 respectively). In the reoxygenation process, the effects of these drugs were less than for the deoxygenation (by a factor of 2 to 3 for TB88 and by a factor of 1.3 to 2 for TB120) indicating a large hysteresis. These preliminary results indicate that these new allosteric modifiers are able to induce a significant increase of the oxygen affinity of the RBC from different origins. Thus, we hope that these new "left-shifters" compounds may be useful as antisickling agents.

SS23. Myelodysplastic syndrome

O-1124 IN VITRO MODULATION OF CASPASE ACTIVITY IN MYELODYSPLASTIC SYNDROMES

L. Campos, D. Boudard, O. Sabido, A. Viallet, S. Piselli, C. Vasselon, D. Guyotat. *Faculté de Médecine Jacques Lisfranc, St Etienne, France*

Myelodysplastic syndromes (MDS) are characterized by abnormal growth of committed progenitors in clonogenic assay, with reduced number of colonies and decreased colony/cluster ratio. It has been recently suggested that an excess of apoptosis was the cause of marrow failure in MDS. We studied the expression and role of caspases interleukin-1β-converting enzyme (ICE) and CPP32/apopain in a series of 168 MDS cases classified as refractory anemia with excess of blasts (RAEB) (N = 63), RAEB in transformation (RAEB-T) (N = 26), chronic myelo-monocytic leukemia (CMML) (N = 19), refractory anemia (RA) (N = 35) and RA with sideroblasts (N = 25). Growth pattern of committed progenitors (CFU-GM day 7 and 14, CFU-E and BFU-E) in methylcellulose was studied in 83 cases. The percentage of CPP32 positive cells was significantly higher in RA (65±17) and RAS (90±8) than in CMML (59±20), RAEB (40±16) and RAEB-T (30±14) ($p < 10^{-5}$). Although less dramatic a significant difference was also observed for the percentage of ICE-positive cells (42±21, 51±22, 35±26, 38±17, 33±20, $p < 10^{-3}$). Spontaneous growth of day 7 CFU-GM was associated with higher percentage of blasts, with RAEB, RAEB-T or CMML subtypes and with lower expression of CPP32 and ICE). The yield of CFU-E, BFU-E, and day 7 and 14 CFU-GM (in the presence of appropriate growth factors) was overall decreased by comparison to normal marrow, but large individual differences were observed in all cytological categories. However CFU-E number was particularly low in cases with high expression of CPP32, and in RA and RAS as compared to RAEB, RAEB-T and CMML. Inhibition of ICE and CPP32 by specific inhibitors (Ac-YVAD-CHO and DEVD-CHO respectively) resulted in a significant increase of the yield of all types of colonies (up to 50-fold of control). This was particularly dramatic with CPP32 inhibitor, which normalized the number of BFU-E and CFU-E in most cases of RA and RAS. ICE and CPP32 inhibition also resulted in an increase of the colony/cluster ratio. By contrast, caspases inhibitors had no significant effect on normal marrow progenitors. It is concluded that ICE and CPP32 may have a role in the increased apoptosis observed in MDS.

O-1125 EXPOSURE TO MYELOTOXIC AGENTS AND MYELODYSPLASIA: CASE-CONTROL STUDY AND CORRELATION WITH CLINICOBIOLOGIC FINDINGS

G.M. Rigolin, A. Cuneo, M.G. Roberti, A. Bardi, R. Bigoni, N. Piva, C. Minotto, P. Agostini, C. De Angeli, L. Del Senno[1], G.L. Castoldi. *Department of Biomedical Sciences, Hematology Section; [1]Department of Biochemistry and Molecular Biology, University of Ferrara, Italy*

To better define the role of exposure to myelotoxic agents in the genesis of myelodysplastic syndrome (MDS), this analysis was performed consisting of: a) a case control study for the determination of the relative risk (RR) of developing MDS, including 178 consecutive patients and 178 controls matched for sex and age; b) a study of clinicobiologic features in MDS arising after occupational exposure to myelotoxic agents and in MDS in "non-exposed" patients. The definition of the "exposure" status was based on interviews using a predetermined questionnaire, with calculation of an "exposure" index (hours/day X days/year X years). Cumulative exposure to pesticides or to organic solvents, for more than 2,400 hours, was recorded in 48 and 25 MDS patients, respectively, compared to 27 and 4 controls ($p < 0.00001$; RR 3.74; 95% confidence interval: 2.02-5.37). Older age and an excess of refractory anemia with ringed sideroblasts and refractory anemia with excess of blasts was noted among "exposed" MDS-patients (group 1), compared to non-exposed MDS-patients (group 2). 68.3% patients in group 1 had clonal chromosome changes, compared with 43.2% patients in group 2. Complex karyotypes, -7/7q-, -5/5q-, +8, 7p and 17p aberrations were seen more frequently in group 1, whereas a normal karyotype, isolated 5q- or 20q-occurred more frequently in group 2. The association of exposure to myelotoxic agents with older age at presentation and with unfavourable chromosome changes accounted for the shorter survival that was observed in "exposed" patients. These data show that occupational exposure to pesticides and organic solvents in our region resulted into an increased RR of developing MDS and that a distinct cytogenetic profile was associated with MDS in "exposed" patients. These findings provide strong indirect evidence that these agents may play a role in the pathogenesis of MDS, preferentially targeting some of the chromosome regions which are frequently involved in therapy-related myeloid neoplasias.

O-1126 DYSPLASTIC VS. PROLIFERATIVE CMML — RETROSPECTIVE ANALYSIS OF 92 PATIENTS FROM A SINGLE INSTITUTION

T. Noesslinger[1], M. Pfeilstoecker[1], H. Tuechler[2], H. Muehlbacher[2], H. Gruener[2], E. Pittermann[1,2], R. Heinz[1,2]. *[1]3rd Med. Dept for Hematology and Oncology, Hanusch Hospital, Vienna; [2]L. Boltzmann Inst. for Hematology and Leukemia Research, Hanusch Hosp., Vienna, Austria*

CMML a heterogeneous hematological disorder with myelodysplastic and myeloproliferative features, is classified within the myelodysplastic syndromes. Two years ago the FAB group suggested to segregate two subtypes: myelodysplastic (MDS-CMML) and myeloproliferative (MPD-CMML), dependent on the white blood count (<13.000 vs. >= 13.000/mm^3). Based on a retrospective analysis of 92 initially untreated pts. with CMML diagnosed between 1982 and 1997 at our institution, we compared the presenting clinical and hematological features of both diseases and examined whether the refined classification provides information for prognosis and therapy. 60 pts. with MDS-CMML and 32 pts. with MPD-CMML were diagnosed. Median age at diagnosis (72 vs. 75 years) and sex ratio (M/F: 1.5 vs. 1.1) were not significantly different between both subgroups. Hepatomegaly (19% vs. 5%, p = 0.04) was more common in MPD-CMML, frequence of splenomegaly (38% vs. 37%) was similar. Concerning blood parameters hemoglobine level (106 vs. 108 g/l) and platelet count (93.000 vs. 92.000/mm^3) showed no difference, but pts. with MPD-CMML had significantly higher LDH values (median 339 vs. 255 U/l, p = 0.005). The percentage of bone marrow blasts (15%) was equal in both disorders. Karyotype anomalies (37 and 22 cases evaluated respectively in both subgroups) were found in 23% of pts. With MDS-CMML and in 25% of pts. with MPD-CMML (p = 0.48). Follow up data were available for 89 out of 92 pts.. The median survival was significantly longer for pts. with MDS-CMML than for pts. with MPD-CMML (31 vs. 16 months, p = 0.028). The progression rate to AML (50%) was similar in both subgroups, with all pts. receiving chemotherapeutic regimens. Remarkably the pts. with MPD-CMML and progression of disease had longer median preleukemic duration than the subgroup of pts. with MDS-CMML (22 vs. 16 months, 10 = 0.62); even after transformation in AML the median survival of the pts. with MPD-CMML was longer (5 vs. 2 months, p = 0.36). Although in course of disease anemia was more common in MPD-CMML (p = 0.016) and thrombocytopenia was more common in MDS-CMML (p = 0.07), the transfusion rate showed no difference between the two subgroups. These data suggest on the one hand that the subgroup of pts. with MDS-CMML has longer median survival, on the other hand that cytotoxic treatment after progression to AML seems to be more beneficial in myeloproliferative CMML than in the myclodysplastic variant.

O-1127 HIGH LEVELS OF APOPTOSIS CHARACTERIZE RAEB AND ARE LOST DURING LEUKEMIC TRANSFORMATION

M.R. Ricciardi, M.T. Petrucci, C. Ariola, C. Gregorj, R. Latagliata, M.C. Petti, A. Tafuri. *Dipartimento di Biotecnologie Cellulari ed Ematologia, Università "La Sapienza" Roma, Italy*

A pathogenetic role of apoptosis (APO) has been proposed in Myelodysplastic syndromes (MDS). Aim of our study was to evaluate the correlation between APO and different stage of leukemic transformation in MDS subtypes. In 23 MDS patients, 12 RAEB, 11 RAEB-t, we evaluated the spontaneous rate of APO in the overall mononuclear cells and in the CD34+ fraction. In addition APO changes induced by short term cell culture in medium enriched by FCS were also evaluated. APO was detected by two-color flow cytometric assay, costaining blasts with the CD34 antigen and Annexin V. When a flow cytometric aneuploid clone was found, double staining DNA/CD34+ was used to distinguish MDS hemopoiesis. Cell cycle distribution was also measured by the Acridine-Orange flow cytometric technique. Detection of APO levels in the overall mononuclear cell population showed a significant difference ($p = 0.006$) between RAEB (m = 14.6%) and RAEB-t (m = 3.95%). These data were confirmed by the spontaneous rate of apoptosis restricted to the CD34+ compartment. A significant ($p = 0.004$) higher spontaneous rate of APO was found in RAEB (m = 1.78%) compared to RAEB-t (m = 0.46%). Short term cell culture in the presence of FCS enriched medium protected from apoptosis only RAEB samples: the levels of APO were reduced in both the overall mononuclear population and in CD34+ cells from 14.6% and 1.78% to 8.05% and 0.99%, respectively. In contrast the levels of APO in RAEB-t increased from 6.55% and 0.52% to 12.5% and to 0.79%, respectively. In conclusion, increased levels of apoptosis are not a general feature in MDS, but characterize only the RAEB subtype. These findings suggest a functionally active APO only in dysplasias with a lower leukemic burden, as shown by the presence of an unbalanced cell death/survival ratio, still responsive to a regulatory a bidirectional (survival-apoptosis) control, while MDS in leukemic transformation lose this growth-control mechanism.

O-1128 QUININE IMPROVES RESULTS OF INTENSIVE CHEMOTHERAPY IN MYELODYSPLASTIC SYNDROMES (MDS) EXPRESSING P GLYCOPROTEIN (PGP): UPDATED RESULTS OF A RANDOMIZED STUDY

E. Wattel, E. Solary, B. Hecquet, D. Caillot, N. Ifrah, A. Brion, N. Milpied, M. Janvier, A. Guerci, H. Rochant, C. Cordonnier, F. Dreyfus, A. Veil, L. Hoang-Ngoc, A.M. Stoppa, N. Gratecos, A. Sadoun, H. Tilly, P. Brice, B. Lioure, B. Desablens, B. Pignon, J.P. Abgrall, M. Leporrier, B. Dupriez, D. Guyotat, P. Lepelley, P. Fenaux. *Groupe Français des Myélodysplasies (GFM) and Groupe GOELAMS, CHU-Lille, France*

Intensive chemotherapy (IC), in MDS, gives lower complete remission (CR) rate than in de novo AML, possibly due in part to higher incidence of Pglycoprotein (PGP) expression in MDS blast cells. We therefore designed a randomized trial of IC with or without quinine, an agent capable of reverting the multidrug resistance (mdr) phenotype, in patients aged $\leq$65 years with high risk MDS. Patients were randomized to receive Mitoxantrone 12 mg/m^2/d d_{2-5} + AraC 1 g/m^2/12 h d_{1-5}, with (Q^+) or without (Q^-) quinine (30 mg/kg/day). 131 patients were included between October 1992 and May 1996. A third interim analysis, whose results are presented here, was performed at the reference date of 30 May 1997. PGP expression analysis was successfully made in 91 patients and 42 patients (46%) had positive PGP expression. Overall, 57 patients (44%) achieved CR, including 29/62 (47%) Q+ and 28/69 of the 69 Q- (41%) patients (difference not significant). In PGP positive cases, 13 of the 25 (52%) patients who received quinine achieved CR, as compared to 3 of the 17 (18%) patients treated with chemotherapy alone ($p = 0.02$). In PGP negative cases, the CR rate was 35% and 49%, respectively in patients who received quinine or chemotherapy alone (difference not significant). Median Kaplan-Meier estimate of overall survival was 12 months. In the 42 PGP positive patients, median Kaplan-Meier survival was 13 months in patients allocated to the quinine group, and 8 months in patients treated with chemotherapy alone ($p = 0.01$). In PGP negative patients, median Kaplan-Meier survival was 14 months in patients allocated to the quinine group, and 14 months in patients treated with chemotherapy alone. Side effects of quinine mainly included vertigo and tinnitus that generally disappeared with 20% dose reduction. Mucositis was significantly more frequently observed in the quinine group. No life threatening cardiac toxicity was observed. In conclusion, updated results of this randomized study show that quinine increases the CR rate and survival in PGP positive MDS cases treated with IC. The fact that quinine had no effect on the response rate and survival of PGP negative MDS suggests a specific effect on PGP mediated drug resistance rather than, for instance, a simple effect on the metabolism of Mitoxantrone and/or AraC.

O-1129 AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR PATIENTS WITH POOR RISK MDS AND SECUNDARY AML (sAML)

T. De Witte, S. Suciu, G. Verhoef, B. Labar, E. Archimbaud†, C. Aul, D. Selleslag, A. Ferrant, P. Wijermans, F. Mandelli, S. Amadori, U. Jehn, P. Muus, H. DeMuynck, M. Dardenne, R. Zittoun, R. Willemze, A. Gratwohl, J. Apperley. *Dept. of Hematology, University Hospital Nijmegen, The Netherlands*

Treatment of patients with MDS or sAML with chemotherapy results in few longterm survivors. Therefore, we initiated a trial to assess the efficacy of intensive chemotherapy, followed by ASCT or alloBMT in a prospective study. All patients with a donor were candidates for alloBMT. The remaining patients were eligible for ASCT. These patients received an ABMT in the initial phase of the study and later blood stem cells mobilized with filgrastim during the recovery phase of the consolidation course. Entry into the study finished in March 1997 with 197 patients registered. Sufficient data is available from 173 patients. The median age was 47 years (range: 16–60). A total of 94 patients (54%) achieved CR after 1 or 2 courses of remission-induction therapy. An HLA-identical sibling was identified for 35 patients and alloBMT in first CR was performed in 23 patients. 30 of the 51 patients without a donor in first CR after the consolidation course received an autograft in CR-1. Sixteen patients received an ABMT and 13 patients received mobilized stem cells (PSCT). The median number of infused CFU-GM was 5×10^4/kg in the marrow harvests and 22×10^4/kg b.w. in the apheresis products ($p < 0.001$). The median granulocyte recovery time to $.5 \times 10^9$/l was 70 days after ABMT and 24 days after PSCT ($p = 0.04$). The median platelet recovery time to 20×10^9/l without platelet support was 116 days after ABMT and 71 days after PSCT ($p = 0.05$). The transplant-related mortality was similar in both groups. The 2-year survival was 31% and the 2-year DFS was 32%. This analysis shows that patients with poor-risk MDS may benefit from a consolidation of CR by ASCT. The hematopoietic recovery after transfusion of mobilized blood stem cells was significantly faster, but only a prospective randomized trial may show whether this will translate in a longer DFS than after ABMT.

SS24. Chronic myeloproliferative disorders

O-1130 UPDATE OF THE 810 CHRONIC MYELOGENOUS LEUKEMIA PATIENTS TREATED WITH INTERFERON α2b OR IFN α2b AND CYTARABINE

F. Guilhot, F. Maloisel, A. Guerci, M. Michallet, J.L. Harousseau, J. Brière, H. Rochant, A.J. Ciurana, J.F. Abgrall, D. Guyotat, B. Christian, H. Orfeuvre. *Department of Hematology and Clinical Oncology CHU, Poitiers, France*

The CML 91 trial was initiated in order to explore the usefullness of the combination of Interferon α2b (IFN) and Cytarabine (Ara-c) for the treatment of patients (pts) with the chronic phase chronic myelogenous leukemia (CML). All pts with a Ph chromosome aged less than 70 y, received IFN α2b starting at 5.10^6IU/m^2 subcutaneously and Hydroxyurea 50 mg/kg/day. Hydroxyurea was discontinued when complete hematologic remission was achieved. Pts randomized in the IFN + Ara-c arm received in addition courses of Ara-c at a dose of 20 mg/m^2/d. 10 d per month; the monthly courses being started within 2 weeks after randomization. The endpoints of the trial were overall survival, hematologic response at 6 months and cytogenetic response at 12 months. In case of resistance, an allogenic bone marrow transplantation was proposed or an autologous unmanipulated stem cell reinfusion after high dose chemotherapy in the absence of a suitable donor. We enrolled 810 pts over a 6 year period and 721 were studied: 360 randomly assigned to the IFN-Ara-C group and 361 to the IFN group. An update of this trial shows that the probability of having a major cytogenetic response at 24 months was significantly higher in the IFN-Ara-C group ($p = 0.006$). Also the pts in the IFN-Ara-C group survived significantly longer than those in the IFN group ($p = 0.02$). At 3 years the estimated survival rates were 85.7% and 79.1% for the IFN Ara-C group and the IFN group respectively. In this trial a relationship was also noted between cytogenetic response and survival. After adjustment for disease-related variables and the Sokal score, overall survival remained significantly higher in the IFN-Ara-C group ($p = 0.03$). The cumulative incidence of complete cytogenetic response was higher in the IFN + Ara-C group ($p < 0.01$). Pts who achieved a complete hematologic response at 6 months had a higher probability of major cytogenetic response at 12

months. The 2 year survival rates after allogenic bone marrow transplantation or autologous stem cell transplant were comparable in each group.

O-1131 MOLECULAR HETEROGENEITY IN COMPLETE CYTOGENETIC RESPONDERS ON INTERFERON-α THERAPY OF CML: LEVELS OF MINIMAL RESIDUAL DISEASE PREDICT RISK OF RELAPSE

A. Hochhaus, A. Reichert, S. Saußele, A. Reiter, M. Emig, R. Hehlmann, J.M. Goldman, N.C.P. Cross. *III. Medizinische Klinik, Klinikum Mannheim der Universität Heidelberg, Mannheim, Germany; Imperial College School of Medicine, Hammersmith Hospital, London, UK*

A substantial minority of CML patients on interferon-α (IFN-α) therapy achieve a complete response (CR), defined as the disappearance of Ph-positive metaphases and/or the disappearance of the rearranged BCR band in Southern blot analysis. We have quantified levels of residual BCR-ABL transcripts in sequential blood and bone marrow samples from 52 (34 m., 18 f.) such patients by competitive nested RT-PCR. The median age at diagnosis was 48 (range 22–70) years and complete remission was achieved between 0.4 to 6.4 (median 1.4) years after the start of IFN-α therapy. BCR-ABL transcript levels were standardized for quality and quantity of cDNA by quantification of ABL transcripts as an internal control. 49 patients had evidence of residual disease in all follow-up samples analyzed; in three patients RT-PCR was intermittently negative. The lowest level of residual disease (maximum response) detected for each patient ranged between 0 and 4.5% (median 0.11%) BCR-ABL/ABL. During a median follow up period of 1.4 years, 11 patients (21%) relapsed; in eight cases Ph-positive metaphases or M-bcr rearrangement reappeared whilst the patients were still hematologically in chronic phase and three patients developed blast crises (2 lymphoid and one myeloid). When studied previously in CR, these 11 patients had a median BCR-ABL/ABL ratio of 0.5% compared with a median ratio of 0.04% for the 41 patients who remained in CR ($p = 0.011$). Ten of the 11 patients who relapsed never had ratios lower than 0.11%. Our findings show that the amount of residual disease in complete responders spans a range over four orders of magnitude and that the degree of response is significantly related to the probability of relapse.

O-1132 METHYLATION OF THE ABL PROMOTER IN CHRONIC MYELOGENOUS LEUKEMIA (CML) CORRELATES WITH DISEASE PROGRESSION

A. Willer, A. Krämer, S. Saußele, A. Hochhaus, W. Seifarth, R. Hehlmann. *III. Medizinische Klinik Mannheim, Universität Heidelberg, Wiesbadener Straße 7-11, D-68305 Mannheim, Germany*

Methylation of the DNA plays an important role in gene expression and possibly tumorigenesis. In the case of CML, it has been shown that progressive de novo methylation of the ABL promoter is linked to disease progression (Zion M. et al., PNAS 91, 10722–26, 1994). Therefore we wanted to investigate the significance of monitoring the ABL promoter methylation in CML in comparison with other features of disease progression and quality of molecular response. 61 samples from a total of 15 patients with CML were analyzed up to 48 months from diagnosis. The patients were treated with interferon alpha and/or hydroxyurea. DNA extracted from peripheral blood cells was digested with methylation sensitive and methylation insensitive enzymes. A PCR was performed using primers derived from the ABL promotor sequence. In dependence on the methylation status, an amplification product of 170 bp was obtained after digestion with methylation sensitive enzymes in PCR. In contrast to Zion et al. who further analyzed the PCR amplificates with Southern blot, we performed the PCR with ^{35}S-ATP. Therefore, the PCR product could be visualized by a polyacrylamid sequence gel. Samples from 4 patients who were good responders to interferon alpha revealed no ABL promoter methylation over 18 months and remained negative for BCR-ABL in Southern blot. In samples from 7 other patients who showed disease progression within the observed time interval, de novo methylation of the ABL promoter could be observed. Disease progression was also observed in an increase of BCR-ABL sequences in Southern blot and quantitative PCR analysis. In samples from 4 further patients who had 100% Ph positive cells at diagnosis and who rapidly progressed to blast crisis, all samples showed methylation of the ABL promoter. We conclude that i) methylation of the ABL promoter is linked to disease progression, ii) there is a good correlation of the altered methylation status of the ABL promoter with other methods used for monitoring CML, iii) using the method described here, it is possible to analyze a large number of samples. The evaluation of the predictive value of this method with regard to disease progression, response to treatment and survival requires further analyses.

O-1133 LEUKOCYTE ALKALINE PHOSPHATASE IDENTIFIES THE TERMINALLY DIFFERENTIATED NORMAL NEUTROPHIL AND ITS LACK IN CML IS NOT DEPENDENT ON THE P210 TYROSINE KINASE ACTIVITY

G.P. Dotti, E. Garattini, G.M. Borleri, O. Spinelli, T. Barbui, A. Rambaldi. *Divisione di Ematologia Ospedali Riuniti Bergamo and Isituto di Ricerche Farmacologiche Mario Negri, Milan, Italy*

The neutrophil maturation in normal bone marrow can be studied by flow cytometry combining side and forward scatter and several differentiation markers including CD15, CD11b and CD16 antigens. We characterised a new MoAb (1B12.1) specific for the leucocyte alkaline phosphatase (LAP) (Rambaldi et al Br. J Haematol 96: 815–822; 1997) which can be used to detect, by flow cytometry, LAP expression on the cell surface of mature neutrophils. Aim of this study was to investigate the cell surface expression of LAP along the granulocytic differentiation pathway in normal bone marrow and the kinetic of LAP protein expression in neutrophils obtained from normal donors and CML patients. Indeed, in CML neutrophils LAP is decreased or absent for a defective mRNA expression which can be effectively restored by G-CSF. Moreover, we investigated the effects of *in vitro* inhibition of p210 tyrosine kinase activity with genistein (30 μM to 60 μM) or CGP57148B (0.3 μM to 1.0 μM) on LAP mRNA expression, as well as protein synthesis and transport to plasma membrane in CML granulocytes. We provide evidence that in normal marrow a significant proportion of myeloid cells are $CD11b^{dim}$ and $CD16^{dim}$ positive but still lack expression of LAP which, on the contrary, is tightly restricted to $CD11b^{brigth}$ and $CD16^{brigth}$ positive cells. We show that the incubation for 24 hours with a pharmacological dose of G-CSF (50 ng/mL) is able to increase the cells surface expression of LAP protein in normal (49% vs. 76%) and CML (10% vs. 43%) PMNs. Moreover, in CML granulocytes the inhibition of p210 tyrosine kinase activity fails to restore LAP mRNA expression, as well as protein synthesis and transport to plasma membrane. In summary, our results suggest that (i) the acquisition of LAP protein on cell surface of mature myeloid cells follows CD16 antigen expression and can be considered as the last marker of the neutrophil maturation (ii) G-CSF is able to increase the expression of LAP on the cell surface of normal and CML neutrophils (iii) the impairment of LAP mRNA and protein synthesis in CML is not dependent on the abnormal function of p210 tyrosine kinase activity and could be related to a precocious and uncontrolled release of white blood cells from the bone marrow into the blood stream.

O-1134 MYELOFIBROSIS WITH MYELOID METAPLASIA (MMM) IN YOUNG INDIVIDUALS: DISEASE CHARACTERISTICS, PROGNOSTIC FACTORS AND IDENTIFICATION OF RISK GROUPS

F. Cervantes, G. Barosi[1], J.-L. Demory[2], J. Reilly[3], R. Guarnone[1], B. Dupriez[2], A. Pereira, E. Montserrat. *Hematology Department, Hospital Clinic Clinic, Barcelona, Spain; [1]Laboratory of Medical Informatics, IRCCS Policlinico S. Matteo, Pavia, Italy; [2]Service des Maladies du Sang, CHRU, Lille, France; [3]Royal Hallamshire Hospital, Sheffield, England*

MMM is an uncommon disorder in young individuals, for whom hemopoietic stem cell transplantation offers the only possibility of cure. However, while the latter procedure is associated with significant morbidity and mortality, the clinical course of MMM is variable, with many patients showing an indolent course. Selection of young MMM patients for transplantation is difficult since no prognostic data exists for this subgroup. In the present study a number of initial clinical and laboratory parameters were evaluated for prognosis in 121 MMM patients aged 55 years or less. Median survival of the series was 128 months (95% CI: 90–172). In the Cox proportional hazard regression model three initial variables were independently associated with shorter survival: Hb < 10 g/dL ($p < 0.0001$), the presence of constitutional symptoms (fever, sweats, weight loss) ($p = 0.001$), and circulating blasts ≥1% ($p = 0.003$). Based on the above three criteria, among the 116 patients with the complete data two groups were identified: a "low-risk" group, characterized by 88 patients with 0–1 adverse prognostic factor, in whom MMM had an indolent course (median survival 176 months, 95% CI: 130–188), and a "high-risk" group, including 28 patients with 2–3 factors, who had a more aggressive disease (median survival 33 months, 95% CI: 20–42). The above prognostic scoring system can be of help in making treatment decisions in young patients with MMM.

O-1135 HLA-DR4 AND HLA-DR15 LOWER THE RISK OF THE ACQUISITION OF CHRONIC MYELOID LEUKEMIA (CML)

E.F.M. Posthuma[1], J.H.F. Falkenburg, J.F. Apperley, A. Gratwohl[1], R.F. Schipper[1], G.M.T. Schreuder[1], J. D'Amaro[1], J.H.V. Biezen[1], J. Hermans[1], R. Willemze, D. Niederwieser. *The EBMT Chronic Leukemia Working Party; [1]Depts. Hematology and Immunohematology, Leiden University Medical Center, The Netherlands*

CML is characterized by a chromosomal translocation t(9;22) resulting in the chimeric bcr-abl oncogene that encodes for the P210 fusion protein. The fusion part of this protein contains a novel aminoacid sequence. If peptides derived from this leukemia-specific part of P210 are expressed in MHC molecules on leukemic cells this may elicit immunological responses. Recent studies using synthetic peptides identical to the bcr-abl fusion region revealed that breakpoint specific peptides are capable of binding to the class I molecules HLA-A3, -A11 and -B8 and the class II molecules HLA-DR1, -DR3, -DR4, -DR11 and -DR15. Cytotoxic T cell responses have been induced against bcr-abl derived synthetic peptides in these HLA molecules. We have previously shown that individuals expressing HLA-B8 have a diminished risk of the development of CML. The possible mechanism is that antigen processing of P210 may lead to presentation of immunogenic tumorspecific peptides by these MHC molecules in vivo resulting in an autologous cytotoxic response against leukemic cells. To assess a possibly protective effect of these class II molecules we performed a large multicenter case-control study. This study (1462 patients European Bone Marrow Transplant registry and 500,596 controls) yielded significantly lower odds ratios (ORs) of 0.86 (95% CI 0.75–0.98; $p < 0.05$) for HLA-DR3, of 0.79 (95% CI 0.71–0.89; $p < 0.05$) for HLA-DR4 and of 0.84 (95% CI 0.72–0.97; $p < 0.05$) for HLA-DR15. The diminished OR for HLA-DR3 is probably due to the linkage disequilibrium of HLA-DR3 with the HLA-B8 molecule already proven to be protective against the development of CML. The OR for HLA-DR1 of 0.91 (95% CI 0.80–1.04) and for HLA-DR11 of 0.87 (95% CI 0.74–1.01) were not significant. These results indicate that HLA-DR4 and HLA-DR15 expression may result in a protective effect on the acquisition of CML probably by presentation of bcr-abl breakpoint peptides in these MHC molecules. HLA-DR1 and-DR11 appeared not to protect against CML suggesting that breakpoint derived peptides are not presented by these MHC molecule in vivo or do not lead to a significant immune response.

SS25. Thrombocytopenia and thrombopathy

O-1136 TAHINI ASSOCIATED THROMBOCYTOPENIA

J. Arnold[1], W. Ouwehand[2], G.A. Smith[3], H. Cohen[4]. *[1]Department of Haematology, St Mary's Hospital; [4]Imperial College School of Medicine @ St Mary's, London and Division of Transfusion Medicine; [2]University of Cambridge; [3]East Anglian Blood Centre, UK*

A 28 year old Caucasian female presented with a new onset petechial rash on the background of over a year's history of easy bruising and recurrent epistaxes. A blood count revealed thrombocytopenia confirmed on blood film (30×10^9/L). Her platelet count recovered spontaneously to normal within 6 days. Over a 3 month observation period her platelet count fluctuated between 30 and 180 $\times 10^9$/L before falling to 18 $\times 10^9$/L. Investigations including bone marrow aspirate were consistent with autoimmune thrombocytopenic purpura (AITP). Prednisolone was commenced at a dose of 1 mg/kg. Despite an initial improvement in her platelet count she relapsed whilst on steroid therapy with a nadir platelet count on of 4 $\times 10^9$/L. A temporal relationship was noted between dietary consumption of tahini, a pulped sesame seed product, and episodes of thrombocytopenia. This was confirmed with an oral challenge of 200 mg tahini on two occasions. The platelet count fell to a nadir of 34 $\times 10^9$/L at 24 hours, rising to 97 $\times 10^9$/L at 72 hours and 189 $\times 10^9$/L by day 8. The direct platelet immunoflourescence test (PIFT) for platelet associated immunoglobulin (PAIg) became positive for IgG within 6 hours with antibody specificity towards glycoprotein Ib/IX (CD42) demonstrated using the monoclonal antibody specific immobilisation of platelet antigens technique (MAIPA). Weak IgM and IgA positivity was also demonstrated. Although no hapten was identified the results were most consistent with a hapten mediated immune thrombocytopenia. Subsequent exclusion of sesame seeds from her diet has resulted in the maintenance of a normal platelet count for the last twelve months. Investigation of adult AITP includes assessment for underlying lymphoproliferative or autoimmune disorders and a drug history. The importance of diet as an aetiological factor in adult AITP is unknown, but a dietary history may be useful.

O-1137 CHARACTERISATION OF AUTOANTIBODIES ISOLATED FROM A PATIENT WITH POST TRANSFUSION PURPURA

N.A. Watkins[1], P. Smethurst[1], G. Smith[2], W.H. Ouwehand[1,2,3]. *[1]Division of Tranfusion Medicine, University of Cambridge; [2]National Blood Service East Anglia; [3]National Institute for Biological Standards and Control, UK*

Post-transfusion purpura (PTP) is a transfusion related syndrome characterised by the destruction of both transfused and autologous platelets. Transfused platelets are destroyed by HPA alloantibodies, however, the exact mechanism for the destruction of autologous platelets is unknown. Short lived autoantibodies have been hypothesised as a possible mechanism and we aimed to test this by using V gene phage display technology. An IgG derived V-gene phage library was constructed from the B cell mRNA of a patient in the acute phase of PTP. The patient's serum reacted strongly with HPA-1b but, when tested by ELISA, the serum cross-reacted with purified GPIIb/IIIa of the HPA-1a form. Affinity selection of phage by a modified MAIPA strategy and on purified GPIIb/IIIa identified four platelet specific single chain antibody fragments (scFv's).

All four are specific for GPIIb/IIIa, two lack binding and one has reduced binding to Glanzmanns' Thrombasthenia platelets with 50%+ GPIIIa expression. Nucleotide sequencing showed that the antibodies use a range of VH genes of different families (DP73, DP32, DP66 and V3–49) which have minimal somatic mutation and a short CDR3. These features are characteristic for the fetal repertoire and point to a T cell independent immune response. The isolation of these scFv's from a PTP patient lends credence to the autoantibody theory.

O-1138 ABNORMAL CYTOKINE GENE EXPRESSION IN THROMBOCYTOPENIC PURPURA IS PARTIALLY RESTORED AFTER IMMUNOGLOBULIN ADMINISTRATION

A. Mouzaki, M. Theodoropoulou, E. Mavroides, V. Orphanos, M. Karakantza, M.-C. Kyrtsonis, D. Koliperas, N. Beratis, A.K. Maniatis. *Laboratory Hematology and Transfusion Medicine & Dpt. of Pediatrics, Patras University School of Medicine, Patras, Greece*

The pathogenesis of autoimmune disorders such as Idiopathic Thrombocytopenic Purpura (ITP) is still unclear but the cytokine network has been implicated. Intravenous immunoglobulins (IVIG) are effective in the treatment of ITP and interference with the cytokine network has been hypothesized as one of their actions. In order to probe further this hypothesis we studied cytokine gene expression in 13 normal controls and in 10 children with acute ITP before and 5 days after IVIG administration. RNA was isolated from blood mononuclear cells directly or after in vitro cell culture with or without mitogens and gene expression studied by semi-quantitative RT-PCR. Our results are summarised in Table 1:

Genes	WBC from normal controls			WBC from ITP patients before treatment			WBC from ITP patients after IVIG treatment		
	ex-vivo	CM alone	CM + PHA + PMA	ex-vivo	CM alone	CM + PHA + PMA	ex-vivo	CM alone	CM + PHA + PMA
β_2-micro	+	+	+	+	+	+	+	+	+
IL-1α	-	±	+	±	+	++	-	+	++
IL-1β	-	±	+	+	+	++	+	+	++
IL-2	-	-	++	±	±	+	-	-	+
IL-3	-	-	+	±	±	+	-	-	+
IL-4	-	-	+	±	±	+	-	-	+
IL-5	-	-	+	-	-	±	-	-	+
IL-6	-	-	+	+	+	+	±	+	++
IL-8	+	+	+	-	-	-	+	+	++
IL-10	-	-	++	±	±	+	-	±	++
IL-13	-	-	+	+	+	+	-	±	++
IFN-γ	-	-	+	-	-	±	-	-	±
TGF-β	-	-	+	+	+	±	+	+	
TNF-α	-	-	++	-	-	±	-	-	±

In conclusion the data indicate that IVIG treatment partially restores cytokine gene expression in ITP patients, especially in their ex-vivo, uncultured cells.

O-1139 GLYCOPROTEIN IIIA TRUNCATION RATHER THAN ABSENCE OF CYSTEINE$_{406}$-CYSTEINE$_{665}$ BOND CAUSES THROMBASTHENIA IN MOST IRAQI-JEWISH PATIENTS

R. Yatuv, N. Rosenberg, R. Dardik, U. Seligsohn. *Institute of Thrombosis and Hemostasis, Department of Hematology, Sheba medical center, Tel-hashomer, Israel*

We have previously described an 11bp deletion within exon 12 of the GPIIIa gene causing Glanzmann Thrombasthenia (GT) in most Iraqi-Jewish patients. This mutation predicts a truncated protein lacking the trans-membrane and cytoplasmic domains, and abolishes Cys_{655} thus eliminating an apparently important Cys_{406}-Cys_{655} disulfide bond. To evaluate the effects of GPIIIa truncation and Cys_{655} absence, we designed a T2011 → A mutation which changes Cys_{655} → Ser (Ser_{655}), and an A2019 → T mutation which produces a stop codon at amino acid 657 ($stop_{657}$). CHO cells were transfected with cDNAs for normal GPIIB and for normal GPIIIa (WT), GPIIIa with the 11bp deletion or any of the designed mutations. Fluorescence activated cell sorting (FACS) analysis demonstrated binding of monoclonal antibodies against GPIIa (AP3) and against GPIIb/IIIa (10E5) to cells transfected with WT GPIIIa and with Ser_{655} but not to cells transfected with the 11bp deletion or $stop_{657}$. GPIIb/IIIa complex expressed on WT and Ser_{655} transfected cells supported adhesion and spreading of the cells unto immobilized fibrinogen whereas defective adhesion and no spreading were demonstrated in the $stop_{657}$ and the 11bp deletion transfected cells. Lysates of the transfected cells were subjected to immunoprecipitation by polyclonal antibodies against GPIIb and GPIIIa and to immunoblotting using monoclonal antibodies against GPIIb and GPIIIa. The results demonstrated the presence of GPIIb and GPIIIa in the WT and Ser_{655} transfected cells but not in the $stop_{657}$ and the 11bp deletion transfected cells. The results suggest that Cys_{406}-Cys_{655} bond is not essential for GPIIb/IIIa binding to fibrinogen and that GT phenotype in the patients carrying the 11bp deletion mutation is caused by the truncation of the GPIIIa.

O-1140 THROMBOCYTOPATHY CHARACTERIZED BY REDUCED THROMBIN-INDUCED PLATELET MEPACRINE RELEASE: A DENSE GRANULE SECRETION DEFECT

A. Van de Stolpe, N. Jacobs, H. Verbruggen, I.R.O. Novakova. *Department of Hematology/Central Hematological Laboratory, University Hospital Nijmegen St Radboud, Nijmegen, The Netherlands*

δ-Storage pool *deficiency* (SPD) is characterized by reduction/absence of platelet dense granules, and is associated with hemorrhagic diathesis. A defect in *secretion* of dense granules results in a similar thrombocytopathy but cannot be diagnosed by conventional diagnostic methods.

Mepacrine is selectively taken up by dense granules in platelets and released upon stimulation with thrombin, and can be tracked by its autofluorescence using flow cytometry. To investigate whether mepacrine flow cytometry can by used to identify patients with SPD and platelet secretion defects, we studied mepacrine uptake and thrombin-stimulated mepacrine release in platelets from 31 consecutive patients with symptoms suggestive of a congenital primary hemostatic disorder and with a prolonged bleeding time (Simplate), and in three patients with established SPD. Platelets in whole blood were incubated with mepacrine, subsequently fixed in paraformaldehyde or stimulated with thrombin prior to fixation, and flow cytometry was performed to measure platelet mepacrine content. In every instance a normal control was included. Platelet aggregations with collagen, ADP and arachidonic acid were performed and ADP and ATP/ADP ratio in platelets was determined.

Results: Control values for mepacrine uptake (90±9% of platelets positive fluorescence) and release (1.2±1.7% of platelets remain fluorescent) were established. Aside from the patients with known SPD, one family (two persons) and one other patient showed reduced mepacrine *uptake* and could be diagnosed as SPD. Five patients (one brother and sister) showed clearly reduced mepacrine *release*, associated with reduced collagen-induced platelet aggregation, but with normal mepacrin uptake, ADP content and ATP/ADP ratio. These five patients are likely to have an isolated dense granule secretion defect explaining their bleeding tendency, which could not be diagnosed by conventional laboratory analysis. We conclude that flowcytometric analysis of platelet mepacrine uptake and release may allow rapid diagnosis of SPD, and detect patients with (congenital) dense granule secretion defects that otherwise go undiagnosed.

O-1141 EVALUATION OF PLATELET FUNCTION WITH PFA-100® IN PATIENTS WITH CONGENITAL PLATELET SECRETION DEFECTS

A. Lecchi, M. Cattaneo, B. Agati, R. Lombardi, A.B. Federici, G. Mostarda, P.M. Mannucci. *Angelo Bianchi Bonomi Hemophilia and Thrombosis Ctr. Dept. of Internal Medicine, IRCCS Ospedale Maggiore, University of Milano, Italy*

Objective: Techniques measuring platelet aggregation under the high shear rate conditions that can be found in the microcirculation could reflect the status of primary hemostasis better than turbidimetric techniques. We have evaluated platelet function at high shear with the PFA-100® system in patients with δ-storage pool deficiency (δ-SPD) or primary secretion defects (PSD: platelet secretion defects not due to abnormalities in platelet granules or in the platelet arachidonate pathway), before and after the i.v. infusion of desmopressin.

Design and Methods: Closure times with the PFA-100® system were determined for both the collagen/ADP and the collagen/epinephrine cartridges in 7 patients with δ-SPD, 10 patients with PSD and 30 controls. Measurements were repeated 1 and 4 hours after the i.v. infusions of desmopressin (0.3 μg/Kg) in 6 patients with δ-SPD and 7 with PSD. At all time points, vWF plasma levels, the bleeding time (Symplate II) and platelet aggregation induced by combinations of collagen, ADP and epinephrine in Born's aggregometer were also measured.

Results: Baseline closure times were longer in δ-SPD and PSD patients than in controls with the collagen/epinephrine cartridge. In contrast, closure times with the collagen/ADP cartridge were normal in both δ-SPD and PSD patients. Treatment with desmopressin increased the plasma vWF levels, shortened the prolonged bleeding times, shortened the closure times with the collagen/ADP cartridge and normalized the closure times with the collagen/epinephrine cartridge. Platelet aggregation in Born's aggregometer was not normalized by desmopressin.

Conclusions: The PFA-100® test could be useful in the diagnosis and therapeutic monitoring of patients with defects of platelet secretion. The collagen/epinephrine cartridge is more sensitive than the collagen/ADP cartridge to defects of platelet secretion.

SS26. Immunorecognition and immunotherapy

O-1142 ABSENCE OF COSTIMULATION AND SECRETION OF TH1 CYTOKINE INHIBITORS CONTRIBUTE TO EVASION OF ANTI-LEUKAEMIC IMMUNITY

A. Buggins, W. Hirst, D. Darling, J. Gäken, A. Pagliuca, F. Farzaneh, G.J. Mufti. *Departments of Haematological Medicine & Molecular Medicine, King's College School of Medicine and Dentistry, London, UK*

Effective immunotherapy requires a clear understanding of the mechanisms by which leukaemic cells avoid rejection by cell-mediated immunity (CMI). We have studied two potential mechanisms for this: i) absence of costimulatory molecules (i.e. B7.1) and ii) immunosuppressive soluble factors secreted by leukaemic cells. U937 cells (AMMoL) express many molecules required for antigen presentation but are B7.1 -ve. We have generated high B7.1 expressing cells (U937-B7.1) with levels of B7.1 expression higher than normal resting dendritic cells (DC) used as a +ve control, but with similar levels of MHC I & II. Using normal allogeneic PBMNC or T-cells as responders, irradiated B7.1 positive leukaemic cells stimulated up to 15 fold greater proliferation compared to the mock infected control cells but less than DCs. HTLp frequency with B7.1+ leukaemic cells as stimulators showed up to a 5 fold increase compared to control cells. Analysis of cytokines produced in the reactions showed that DCs stimulated the expected CMI (Th1) profile (γ-IFN+, IL-2+, IL-4−) whereas leukaemic cells induced either no measurable cytokines or a humoral (Th2) profile (γ-IFN-, IL-2−, IL-4+), which was greater with B7.1 expression. Of note B7.1+ leukaemic cells were able to induce low levels of IL-10. Intracellular cytokine analysis of T-cells stimulated in the presence of leukaemic cells in a transwell demonstrated that leukaemia-derived soluble factors can suppress Th1 cytokine production, notably γ-IFN. U937 cells secrete IL-10 (a Th1 suppressor) which may explain this phenomenon. Thus, B7.1 expression by leukaemic cells enhances T-cell proliferation by activation of increased numbers of precursor T-cells and greater stimulation of activated T-cells, but does not provide the necessary microenvironment to induce CMI. This may be explained by the inability of leukaemic cells to provide the necessary cytokines normally secreted by professional APCs (e.g. IL-12). However, release of inhibitory factors (e.g. IL-10) by leukaemic cells can inhibit or switch production of cytokines by T-cells, redirecting the response from protective (Th1) to tolerant (Th2). Therefore, strategies employing leukaemic cells for clinical immunotherapy will, in addition to costimulatory signals, need to provide the appropriate microenvironment (i.e. supplying positive, e.g. IL-2 and blocking negative signals, e.g. IL-10) to control the direction of the immune response towards protective CMI.

O-1143 MECHANISMS OF TARGET CELL RESISTANCE AGAINST THE INDUCTION OF CYTOLYTIC CELL DEATH BY NATIVE AND IL12/IL2-ACTIVATED NATURAL KILLER CELLS

C. Lehmann, L. Uharek, M. Zeis, B. Glass, M. Krönke[1], N. Schmitz. *University of Kiel, Dept. of Int. Medicine II and [1]Dept. of Immunology, Kiel, Germany*

Many tumor cell targets demonstrate resistance against NK-cell-mediated cytotoxicity. In some but not all resistant neoplasms, effective target cell lysis can be achieved by activation of NK cells with IL2 and IL12. Using NK-sensitive (K562) and NK-resistant (ML-2) leukemia cell lines, we started to investigate the underlying mechanisms. CD56+ effector cells were isolated by immunomagnetic selection from peripheral blood mononuclear cells of healthy donors and treated for 24 hours with IL2 (500 U/ml) and IL12 (100 U/ml). NK-mediated cytotoxicity and induction of apoptosis were analysed by flowcytometry. Cytotoxicity was assayd by labeling of target cells with DiO (a green fluorescent membrane dye) and - after lysis by NK cells - counter-staining with propidium-iodide (a red DNA dye which only penetrates dead cells). Induction of apoptosis was assessed by staining with Annexin-V. In contrast to K562 cells, ML-2 cells were not killed by untreated CD56+ cells (lysis <5%, e:t ratio 10:1). NK cells activated with Il-2/Il-12, however, induced tumor cell death in 63% of ML-2 cells. Since activation leads to increased expression of CD95 on NK cells, it has been speculated IL2/IL12-activated NK cells may become more susceptible to tumor cell induced apoptosis. With K562 cells as target, we could indeed demonstrate a significant increase of NK cells undergoing apoptosis but failed to detect a similar relationship using NK-resistant ML-2 cells (indicating that this mechanism was not relevant for resistance). Significant differences were detected in the time course of target cell death. While the lysis of K562 cells was complete after less than 1 h, maximum lysis of ML-2 targets by activated NK cells took more than 4 hours. suggesting the recruitment of different pathways. Whereas blocking of granzyme and perforin secretion by EDTA completely inhibited the lysis of K562, approximately 50% of susceptible ML-2 cells still underwent cytolytic cell death. Using isolated cytotoxic granules from NK cells, we observed the same results as with NK cells itself, indicating that the resistance of ML-2 cells was not based on deficient granule exocytosis. Thus, our data provide evidence that resistance against NK-cell mediated killing can be based on specific target cell properties not related to the lack of activatory cellular interactions. Other pathmays (like Fas) which are activated by IL-2 and IL-12 may become important if the tumor cell shows resistance against granzyme/perforin-mediated apoptosis.

O-1144 IDENTIFICATION OF LEUKEMIA-ASSOCIATED MINOR HISTOCOMPATIBILITY ANTIGENS

H. Dolstra[1], H. Fredrix[1], F. Maas[1], T. De Witte[1], C. Figdor[2], E. Van de Wiel-van Kemenade[1]. *Department of [1]Hematology; [2]Tumorimmunology, University Hospital Nijmegen, The Netherlands*

Cytotoxic T lymphocyte (CTL) reactivity against minor histocompatibility antigens (mHag) plays an important role in prevention and cure of leukemic relapses after HLA-identical bone marrow transplantation (BMT). Despite their great significance in this graft-versus-leukemia reactivity, mHag epitopes and their encoding genes are not well characterized. The objective of our project is to identify genes that encode mHag that exhibit restricted or preferential expression in leukemia cells and to exploit the identified antigens for the induction of leukemia-specific CTL activity in vivo. Therefore, we have investigated whether leukemia cell restricted mHag-specific CTL are present within the T cell repertoire of leukemia patients treated by HLA-matched BMT. Until now we have been able to isolate three CTL lines recognizing mHag with a limited tissue distribution. Using one of these CTL we have identified the encoding polymorphic gene of a novel mHag, named HB-1, by cDNA library screening. The HB-1 gene is expressed in high levels by B-ALL cells and EBV-transformed B cells, which results in CTL recognition. In contrast, normal B and T cells are not lysed by HB-1 specific CTL due to low expression of the HB-1 gene. Therefore, the HB-1 antigen may be a useful rejection antigen for specific immunotherapy to treat patients with B cell malignancies.

O-1145 MALIGNANT PLASMA CELLS EXPRESS GENES OF THE MAGE FAMILY

N. Van Baren, F. Brasseur, G. Hames, D. Godelaine, C. Landry, A. Ferrant, K. Thielemans, T. Boon, P. Coulie. *Ludwig Institute for Cancer Research and Université Catholique de Louvain, Brussels, Belgium*

The MAGE genes encode antigens made of peptides presented by HLA class I molecules, and recognized by cytolytic T lymphocytes (CTL). These genes are expressed in a variety of solid tumors but are silent in normal tissues, except in male germinal cells, which do not carry HLA molecules. The corresponding antigens are therefore truely tumor-specific, and can be used in clinical trials to induce tumor rejections. MAGE gene expression was initially reported to be absent from malignant blood diseases, except the uncommon adult T-cell leukemia. We used reverse transcription and PCR to analyze the expression of genes MAGE-1, 2, 3, 4, 6 and 12 in bone marrow samples from myeloma patients. More than half of the stage III myeloma samples appeared to express at least one of these genes. In addition, activation of MAGE was detected in several myeloma cell lines. The EJM cell line, which carries HLA-A1 molecules, was found to express gene MAGE-3 at a high level. The EJM cells were recognized and lysed by a CTL clone specific for the MAGE-3.A1. These results are a first step towards the development of immunotherapy trials aimed at inducing cellular immune responses against MAGE antigens in advanced myeloma patients.

O-1146 INDUCTION OF ANTITUMOR IMMUNITY BY IDIOTYPE-PULSED DENDRITIC CELLS AGAINST ESTABLISHED MULTIPLE MYELOMA

M. Zeis, L. Uharek, B. Glass, M. Krönke[1], J. Steinmann[1], N. Schmitz. *University of Kiel, Dept. of Int. Medicine II; [1]Dept. of Immunology, Kiel, Germany*

Despite high-dose therapy regimen and autologous stem cell transplantation multiple myeloma (MM) remains a malignant hematological disorder with poor outcome. Recent evidence suggests new therapeutic approaches by dendritic cell (DC)-based enhancement of immune responses. We loaded bone marrow-derived DCs with myeloma-immunoglobulin ($IgG2a^{HOPC}$) to induce protective antitumor immunity in a mouse myeloma model: Balb/c mice were

inoculated i.p. with graded numbers (5–10 × 10^5) of HOPC myeloma cells (Balb/c origin). Animals were immunized with three injections of 5 × 10^5 DCs pulsed with the $IgG2a^{HOPC}$. To achieve a setting resembling minimal residual disease (MRD), a subgroup of myeloma-bearing animals received total body irradiation (7.5 Gy) and a subsequent transplantation of 2 × 10^7 syngeneic peripheral blood progenitor cells (PBPC). Immunization with $IgG2a^{HOPC}$ - pulsed DCs did not prolong survival of mice bearing a high tumor load (10 × 10^5) but led to survival rates of 40% for mice with low tumor burden (5 × 10^5). The combination of PBPC transplantation and subsequent DC-therapy resulted in 85% in long term survival. In addition, treatment with idiotype-pulsed DC induced specific T cell responses against HOPC myeloma in vitro. These data provide a rationale for the use of DC-based vaccines in clinical trials with multiple myeloma patients at the stage of MRD.

O-1147 INTERLEUKIN-2 MAY INDUCE COMPLETE REMISSION IN PATIENTS WHO RESPOND ONLY PARTIALLY TO DONOR LYMPHOCYTE INFUSION (DLI) FOR RELAPSE FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION

F. Dazzi, J. Kaeda, E. Kanfer, J.F. Apperley, J.M. Goldman. *Department of Haematology, ICSM at Hammersmith Hospital, London, UK*

The results of DLI for the treatment of leukaemia in relapse after allogeneic BMT has suggested a potential area for the use of interleukin-2 (IL-2) as it may enhance the effects of this cellular immunotherapy. We have shown previously that some patients given DLI in escalating doses may respond only partially. Eight such patients [6 with chronic myeloid leukaemia (CML), 1 with acute myeloid leukaemia (AML), and 1 with multiple myeloma (MM)] received IL-2 treatment after DLI. Among the CML patients, 1 was in molecular relapse, 3 had cytogenetic relapse, and 2 others were in accelerated and blastic phase, respectively. The patient with AML had extramedullary relapse post DLI, and the patient with MM had shown progressive rise in paraprotein levels despite apparent marrow remission following DLI. All patients received subcutaneous IL-2 injections at a dose of 9 to 18 MU daily for 3 days every two weeks. Three pts (one with AML, and 2 with AP or BP CML) received IL-2 in conjunction with a second course of DLI. The other five patients received IL-2 alone (median time from DLI to subsequent IL-2 therapy was 120 days). Six patients demonstrated a response. Of the 6 CML pts, 2 achieved a molecular remission, and 1 a cytogenetic remission; the patient in BP showed a short-lived substantial response (BCR-ABL/ABL transcript ratio dropped to 6%) but subsequently died of progressive disease. The other 2 patients with CML did not respond. In the AML patient, the combined treatment (DLI/IL-2) cleared the extramedullary disease. In the remaining patient with MM a 41% decline in paraprotein levels was observed following 3 courses of IL2. The median time to achieve the response was 61 (range 10–167) days. We conclude that IL2 can enhance the efficacy of donor lymphocyte therapy.

SS27. Experimental stem cell transplantation

O-1148 VISUALISATION OF CMV-SPECIFIC AND H-Y-SPECIFIC CYTOTOXIC T CELLS WITH FLUORESCENT HLA-PEPTIDE COMPLEXES

G. Gillespie, T. Mutis, J. Bell, E. Goulmy, P.A.H. Moss. *Institute of Molecular Medicine, University of Oxford; Department of Haematology, Birmingham, UK; Department of Immunohaematology, Leiden, Holland*

CMV-specific cytotoxic T cells (CTL) are thought to be important in protection from CMV disease following stem cell transplantation. Quantitation of such CTL following transplantation may be of value in predicting the risk of CMV disease in individual patients. At present methods for the detection of virus-specific CTL are relatively slow and indirect so we have used fluorescent HLA-peptide complexes to detect T cells specific for a peptide from CMV pp65 bound to HLA-A2. Using this reagent we find that around 1 in 2000 CD8+ T cells are specific for the peptide in healthy seropositive individuals and that such cells have a heterogeneous phenotype. We are currently staining blood samples from patients following transplantation to study the kinetics of the reconstitution of anti-CMV immunity.

CTL directed against minor histocompatibility antigens such as H-Y or HA-1 may play a role in GvHD or the GvL effect. We have produced a complex of HLA-A2 with a peptide from the H-Y protein, smcy. This reagent stains T cell clones and will be used to study the development of H-Y-specific CTL following transplantation.

Such reagents offer a new approach to studying immune reconstitution post-transplantation and could be of clinical value in predicting the need for therapeutic intervention or directly allowing manipulation of antigen-specific CD8+ T cell populations.

O-1149 T CELL RECOVERY FOLLOWING STEM CELL TRANSPLANTATION IN THYMECTOMIZED AND THYMUS BEARING MICE

Th. Guillaume, D.B. Rubinstein, P. Staquet, P.M. Symann. *Catholic University of Louvain, Brussels, Belgium; Boston University, Boston, USA*

Immune reconstitution, particularly that of the T cell compartment may be delayed for months following high-dose chemotherapy and autologous hematopoietic stem cell transplantation. This delay is in part due to the recipient's decreased thymic capacity to regenerate new, fully functional T cells. T cells contained in the graft can be expanded in the recipient with defective thymic function. Autologous peripheral blood stem cells (PBSC) has replaced bone marrow (BM) as the source hematopoietic stem cell support following high-dose chemotherapy and results in prompt recovery of circulating granulocytes and platelets. It is unclear however whether recovery in cell numbers correlates with prompter T cell functional integrity. To approach this question, a murine model was used to compare sources of stem cell grafts containing varying numbers of T cells and correlate that with the promptness of immune reconstitution following transplantation. Both thymectomized mice and thymus-bearing mice were transplanted following lethal irradiation with either mobilized PBSC, BM or T cell depleted BM. In the spleen, a faster recovery of $CD3^+$ cells in PBSC recipients was seen in comparison with BM recipients. No difference in the time course of $CD8^+$ cell recovery was observed regardless of recipient type. Concanavalin-A-induced IL-2 production and proliferation in response to mitogen by $CD4^+$ cells at one month and three month following transplantation were similar in the different groups of recipients. These findings indicate that at least in the murine model, PBSC can accelerate T cell numerical recovery in the early period following transplantation while having little effect on T cell function. Although the model of thymectomized mice may contribute to our understanding of immune recovery following transplantation, it does not represent a direct counterpart of what is observed in the clinic.

O-1150 EX VIVO EXPANSION OF NOD/SCID-REPOPULATING CELLS AND CAFC-wk.6 FROM UMBILICAL CORD BLOOD

N. Kusadasi, P.L. Van Soest, R.E. Ploemacher. *Institute of Hematology, Erasmus University Rotterdam, The Netherlands*

In view of the limited potential of umbilical cord blood cells (UCB) for transplantation in adults we have attempted to expand the graft content of primitive stem cells by culturing $CD34^+$-selected UCB cells for periods upto 11 weeks with various cytokine combinations including FL + TPO (w/wo stromal feeders); FL + TPO plus SCF and/or IL-6; SCF + IL-6; and combinations including IL-3. At weeks 0, 2, 5, 10/11 cultures we analyzed by phenotyping, CFC and CAFC assays and 5-6 wk multi-lineage repopulation of NOD/SCID mice (Scid-Repopulating Cell, SRC). The CAFC frequencies in individual UCB's varied between 1–50/10^5 nc, while also SRC activity differed greatly. In addition, the number of CFC generated in long-term flask cultures per CAFC at wk. 6 showed a maximal 5000-fold difference between UCB grafts. For these reasons, we have only used pooled UCB grafts (5–16 grafts) for ex vivo expansion. The addition of IL-3 led only to maintenance or loss of CAFC wk. 6 in 7 day cultures, although CAFC wk. 2, CFC and $CD34^+$ could be significantly expanded.

FL + TPO, but not SCF + IL-6, were required and sufficient to give upto 20-fold increase of multilineage SRC after 2 wks of culture. In general, at wk. 5, SRC were around or less then input values while they were undetectable at wk. 10. In 2-wk cultures the CAFC wk. 6 content was 1–10-fold input values in cytokine combinations without IL-3. The highest expansion of CAFC wk. 6 in 11 wk cultures (86-fold) was found with FL + TPO + stroma, while CAFC wk. 6 were below 40% of input when stimulated with SCF + IL-6, or FL + TPO + SCF. CFC generation increased in all groups upto week 4 of culture when a 100–900-fold expansion was observed whereafter the weekly CFC output stabilized. After 10 weeks of culture, FL + TPO + stroma supported a 2000-fold CFC expansion, while only a 100-fold increase over input was observed with FL + TPO alone. In all cytokine combinations without IL-3 there was a continuous increase in the absolute numbers of $CD34^+$ cells weekly generated with 10^3–10^4-fold the input values at wk. 10, while cells with the primitive phenotype $CD34^+38^-$ were 10^4–5.10^5-fold expanded at that time.

Our results show that SRC can be significantly expanded ex vivo within 2 weeks. If the SRC assay is a measure of in vivo repopulating stem cells in men these findings would suggest a major improvement in the preparation of UCB grafts to meet the requirements for rapid repopulation in the clinical setting. The data also indicate that the different surrogate methods to quantitate hemopoietic stem cells (SRC, CAFC, CFC generation and $CD34^+CD38^-$ phenotype) show great incongruency. Thus, especially flowcytometry and CFC generation seem to greatly overestimate expansion of primitive stem cells in 10/11-wk cultures.

O-1151 CLINICAL SCALE EXPANSION OF MEGAKARYOCYTE PROGENITOR CELLS AND MATURE MEGAKARYOCYTES FROM UMBILICAL CORD BLOOD

R. Briddell[1], P. Kerzic[1], G. Stoney[1], B. Kern[1], I. McNiece[1,2]. [1]*Amgen Incorporated, Thousand Oaks, California;* [2]*University of Colorado, Denver, Colorado, USA*

Patients transplanted with umbilical cord blood (CB) cells following high-dose chemotherapy have prolonged delays in platelet recovery often requiring platelet transfusions for up to 100 days post transplant. In vitro expansion of megakaryocyte progenitor cells (megakaryocyte-colony forming cells; MK-CFC) and/or mature megakaryocytes (MK) may provide a transplant product that results in more rapid platelet recovery. We have developed clinical scale expansion culture conditions with GMP grade reagents for myeloid progenitor cell expansion. We have evaluated these culture conditions for expansion of MK-CFC and mature MK. CD34+ cells isolated from frozen CB were cultured for 10 days in 5% CO_2 in 100% humidified air with 100 ng/mL each of stem cell factor, granulocyte-colony stimulating factor and megakaryocyte growth and development factor in expansion media (Amgen Incorporated) in teflon bags. No mature MKs were present in the CD34+ cells prior to expansion as determined by immunohistochemistry staining for CD41/CD42b/CD61. The CD34+ cells were also assayed for MK-CFC using a serum depleted fibrin clot assay which resulted in a median of 6,885 MK-CFC (range = 1,380–31,500; n = 10). After 10 days of culture in the expansion conditions described above, the cell product contained a median of 11.4% mature MK (range = 5.3%–15.2%; n = 10) and a median of 701,000 MK-CFC (range = 14,300–4,110,000; n = 10). This represents a 76 median fold increase (range = 4–257; n = 10) in MK-CFC from expansion cultures. For transplantation in a 20 kg individual, the expansion culture would provide a median of 35,050 MK-CFC/kg (range = 715–205,000; n = 10). Previously we have reported that 20,000 MK-CFC/kg results in rapid platelet recovery in breast cancer patients transplanted with mobilized peripheral blood progenitor cells. This data suggests that the clinical scale expansion of CB should contain sufficient MK-CFC and mature MK to provide rapid platelet recovery in CB transplant recipients, resulting in decreased platelet transfusion requirements.

O-1152 SELECTIVE DEPLETION OF ALLOANTIGEN ACTIVATED T CELLS

A.M.C. Van Dijk, F.L. Kessler, L.F. Verdonck, G.C. De Gast, H.G. Otten. *Jordan Laboratry, University hospital Utrecht, Utrecht, The Netherlands*

One of the major problems following bone marrow (BM) transplantation is the response of donor T cells against the recipient, leading to graft-versus-host-disease (GVHD). Therefore, it would be a major improvement if these host reactive T cells could be specifically depleted while other specificities are maintained. Currently, an often used treatment to prevent GVHD is an aspecific depletion of about 99% of the T cells in the BM. What we aim at is a specific depletion of about 0.1%; the alloantigen specific T cells. This will result in a faster reconstitution of the BM and T cell responses against for instance pathogens remains intact. To optimize depletion we first coated PHA activated T cells with a monoclonal antibody for CD25, an activation marker, and subsequently depleted for antibody positive cells using either Dynabeads or separation columns and the magnetic cell sorter (MACS). In a series of experiments, the MACS proved to be more efficient than Dynabeads in depleting T cells expressing low levels of CD25, whereas the efficiency of depleting T cells with high level CD25 was comparable. The mean efficiency for MACS was 95.3% versus 87.2% when Dynabeads were used. The specificity of alloantigen activated T cell depletion was investigated in several functional assays. T cells were prestimulated by culturing them with B7-1 transfected keratinocytes (KC^{B7-1}), subsequently T cells were coated with either anti-CD25 or control IgG, and depleted for antibody positive cells. Proliferation of T cells against KC^{B7-1} after CD25 depletion was reduced by 46% whereas control IgG depletion had no effect. The frequency of T cells reactive with 3rd party stimulator cells remained unchanged. In contrast, the frequency of KC^{B7-1} reactive T cells on average showed a 5 fold reduction after CD25 mediated depletion of activated T cells. This method is therefore specific in depleting alloantigen activated T cells and has potential to be used for preventing GVHD following allogeneic bone marrow transplantation.

SS28. Stem cell biology

O-1153 SEEDING-EFFICIENCY OF HUMAN STEM CELLS IN NOD/SCID MICE: CONSEQUENCES FOR FREQUENCY ASSESSMENT

P.B. Van Hennik, A.E. De Koning, R.E. Ploemacher. *Institute of Hematology, Erasmus University Rotterdam, The Netherlands*

The NOD/SCID (Non Obese Diabetic/Severe Combined Immuno Deficiency) mouse is a frequently used animal model in experimental hematology. Recently, the NOD/SCID mouse model has been used to determine the frequency of a stem cell subset which is believed to be more immature than the human Cobblestone Area Forming Cell (CAFC) wk.6, namely the SCID-Repopulating-Cell (SRC). The SRC frequency in Umbilical Cord Blood (UCB) was set at 1:930,000 nucleated cells (nc); 1:3,000,000 nc in Bone Marrow (BM) and 1:6,000,000 nc in Peripheral Blood Stem Cells (PBSC) (J.C.Y. Wang et al., Blood 1997). However, as it may be assumed that many infused stem cells are sequestrated upon infusion and will not reach the proper niches in the recipient, actual frequencies could be considerably higher. For this reason we have assessed the seeding-fraction (f) of NOD/SCID BM nc and human UCB, BM and PBSC nc in the bone marrow and spleen of the NOD/SCID mouse. NOD/SCID mice were irradiated with 9 Gy in order to destroy recipient hematopoiesis. The unsorted nc were injected 2–5 hours post-irradiation. Transplants varied between 33×10^6 and 111×10^6 nc. The recipient mice were killed the next day and the femora and spleens were harvested. The number of stem and progenitor cells seeding to these organs within 24 hours was assayed by CAFC and CFC. While we have previously reported that in other congenic mouse recipients 8–10% and 18–20% of transplanted murine CAFC lodge to the spleen and total bone marrow, respectively, NOD/SCID CAFC had lower f in spleen (median, 2.9% [range, 0.7–4.0]) and bone marrow (8.7% [2.0–9.1]). For human UCB, BM and PBSC, the f for CAFC wk.6 in the bone marrow of NOD/SCID mice was 4.4 [3.5–6.3], 0.8 [0.3–1.7] and 5.3 [1.4–13.6] percent, respectively. If these f's are applied to the SRC frequencies

mentioned above, then the stem cell frequency in UCB is 1: 39,600; in BM 1: 24,000 and in PBSC 1: 318,000. In comparison, CAFC wk.6 frequency in UCB is 1:8,333, in BM 1: 4,348 and in PBSC 1: 10,000. Our data show that the recently postulated low frequencies of stem cells may partly result from their low seeding-efficiency in the bone marrow of NOD/SCID mice. In addition, as outgrowth of human cells in NOD/SCID mice will also be determined by the fraction of stem cells proliferating in the marrow and their growth rate, the human stem cell frequencies could be even be higher than assessed by the SRC assay.

O-1154 GENE TRANSFER IN HEMATOPOIETIC STEM CELLS WITH *IN VIVO* LONG-TERM REPOPULATING ABILITY CAN BE ASSESSED *IN VITRO* USING THE COBBLESTONE AREA FORMING CELL (CAFC) ASSAY AND GREEN FLUORESCENT PROTEIN

J.M.J.M. Zijlmans, M.F.A. Bierhuizen, E. Jumelet, P.B. Van Hennik, A.W. Wognum, G. Wagemaker, B. Löwenberg, R.E. Ploemacher. *Institute of Hematology, Erasmus University Rotterdam, The Netherlands*

Somatic gene therapy of the most primitive subset of hematopoietic stem cells with *in vivo* long-term repopulating ability is not very efficient. The development of more efficient transduction procedures for these primitive stem cells is hampered by the long-term followup (6 months) of the *in vivo* assays. Here, we have developed and validated an *in vitro* assay with a short followup (5 weeks) to assess the transduction efficiency of hematopoietic stem cells with short-term and long-term repopulating ability. Donor mice (C57/Ly5.1$^+$) were treated with 5-Fluorouracil (150 mg/kg) on day −6 and bone marrow cells were harvested on day 0. Intermediate density cells (1.069–1.078 g/ml) were incubated in serum-free medium in the presence of mSCF, hFlt3-ligand, mIL-12, mIL-3, hTpo and blocking anti-TGFβ moab's. On day +2, cells were transferred to fibronectin coated dishes and cultured for an additional two days in the presence of virus supernatant derived from an ecotropic retroviral producer cell line (GP + E86) transfected with the Enhanced Green Fluorescent Protein (EGFP) as a reporter gene. Following the transduction procedure the mean percentage of EGFP$^+$ cells assessed with flow cytometry was 74% (n = 5, range 62–87%). The transduction efficiency of subsets of hematopoietic stem cells was determined *in vitro* in the stroma-based CAFC assay and *in vivo* following transplantation in Ly5.2$^+$ sublethally irradiated (7.5 Gy) recipients. Recipients were analyzed at 4–35 weeks after transplantation with multiparameter flow cytometry to assess Ly5.1 and EGFP expression in B220$^+$, CD4$^+$, CD8$^+$, Gr1$^+$ and Mac1$^+$ subsets of blood cells. In the CAFC assay, the transduction efficiency of early and late cobblestones was very easily assessed by direct inspection of the cultures using a reverse fluorescence microscope. Transduction efficiency of early cobblestones (week 1–3) was 80–95%, and of late cobblestones (week 4–5) 50–65%. *In vivo* in the recipient mice, within the Ly5.1$^+$ donor-derived subpopulation of B−, T-cells, granulocytes and monocytes, the percentage of EGFP$^+$ cells was 79% (65–87) at 4 weeks and 56% (45–68) at 35 weeks after transplantation. In conclusion, the transduction efficiency of hematopoietic stem cells with *in vivo* short-term or long-term repopulating ability can be accurately assessed *in vitro* in the CAFC assay and EGFP as a reporter gene. Therefore, this assay system may allow for optimization of gene transduction protocols.

O-1155 THE SCL GENE SPECIFIES HAEMANGIOBLAST DEVELOPMENT FROM EARLY MESODERM

M. Gering, A. Rodaway[1], B. Göttgens, R.K. Patient[1], A.R. Green. *Department of Haematology, University of Cambridge; [1]Randall Institute, King's College, London, England*

The SCL (TAL-1) gene encodes a bHLH transcription factor that is essential for normal primitive and definitive haematopoiesis. SCL is also expressed in endothelial cells, but its function there is not essential since the vasculature forms normally in homozygous null mutant mice and the role of SCL in endothelial development is therefore obscure. We have isolated the zebrafish SCL homologue and show that it is co-expressed in early lateral mesoderm with markers of primitive haematopoietic (GATA-2), endothelial (Flk-1) and pronephric (Pax-2) cells. Ectopic expression of SCL mRNA in zebrafish embryos resulted in overproduction of haematopoietic and endothelial precursors with a concomitant loss of pronephric and somitic tissue. Notochord and neural tube formation were unaffected. These results provide the first evidence that SCL specifies formation of haemangioblasts, the proposed common precursor of blood and endothelial lineages. Our data also underline the striking similarities between the role of SCL in haematopoiesis/vasculogenesis and the function of other bHLH proteins in muscle and neural development.

O-1156 MUTANT *N-RAS* BLOCKS GRANULOCYTIC DIFFERENTIATION OF HUMAN CD34$^+$ CELLS

R.L. Darley, T.G. Hoy, L. Robinson, A.K. Burnett. *LRF Differentiation Unit, Department of Haematology, University of Wales College of Medicine, Cardiff, Wales, UK*

RAS oncogenes are commonly associated with myeloid leukaemia, yet we have a poor understanding of how mutant *RAS* contributes to the process of leukaemogenesis. To unravel the effect of this oncogene on normal haematopoietic development we have retrovirally transduced CD34$^+$ cells with mutant *N-RAS* together with the selectable marker gene, *lac-Z*. Expression of the *lac-Z* gene product, β-galactosidase, allows immediate identification and study of *N-RAS*-expressing cells following incubation with a fluorogenic substrate for β-galactosidase which gives rise to a fluorescent signal in the infected cells. By using multiparameter flow cytometry *N-RAS*-expressing cells can therefore be studied throughout development. Mock- or control-infected cells (expressing *lac Z* alone) exhibited a normal pattern of granulocytic differentiation in response to G-CSF and GM-CSF over a 2 week culture period, characterised by reduction or loss of expression of markers associated with primitive cells (CD34$^+$, HLA-DR, CD71) together with up-regulation of cell-surface antigens associated with granulocytic maturation (CD15, CD11b, CD32). Mutant *N-RAS*-expressing cells did not undergo terminal differentiation under these conditions in that they failed to normally up-regulate maturation-associated antigens, while expression of transferrin receptor (CD71) was maintained at high levels throughout the culture period. In addition, expression of other cell-surface antigens (CD13, CD11a) was also aberrant. Morphological analysis of *N-RAS*-expressing cells after a 2 week culture period revealed that most of these cells had retained blast/promyelocyte morphology. Despite the maintenance of this immature phenotype we found no evidence that mutant *N-RAS* promoted the proliferative capacity of these cells. These data recapitulate many of the dysplastic features of leukaemic and preleukaemic cells. Taken together with our previously reported observation on the effect of mutant *N-RAS* on erythropoiesis (*J. Exp. Med.* (1997) **185**, 1337) these results suggest that this oncogene may promote leukaemogenesis by preventing completion of the normal haematopoietic development programme.

O-1157 CLONING OF A NOVEL HEMATOPOIETIC GROWTH AND DIFFERENTIATION FACTOR

P. Dörmer, E. Kremmer[1], B. Ehring, R. Mailhammer, A. Brechtel, E. Glas. *GSF-Institut für Experimentelle Hämatologie; [1]GSF-Institut für Immunologie, 81377 München, Germany*

In the supernatant of WEHI-3 cells grown in serum-poor medium an activity was detected inducing hemoglobin synthesis in murine Friend erythroleukemia and human K562 cells. A comparable activity was present in supernatants from previously irradiated human bone marrow stromal cell lines L87/4 and L 88/5. Gel chromatographic fractionation indicated a molecular size of 40–60 kDa for the major peak of activity besides several smaller active fractions. By expression cloning of WEHI-3 cDNA and screening of 25,000 clones an active supernatant from COS-1 cells was obtained. The sequence of the respective clone does not contain known motifs and spans an open reading frame of 209 amino acids. The activity was preliminarily designated EDR for Erythroid Differentiation Regulator.

Northern blot analysis revealed the major sites of *edr* mRNA expression in thymus, fetal liver, spleen and bone-marrow. At the poly (A)+ level all tissues so far studied are positive. A whole array of mRNA species with different sizes is present and preferentially located in the nucleus, all exhibiting an identical 3′ end containing the open reading frame. In the case of need the readable mRNA apparently is spliced out of the various mRNA species. Western blots show that the protein is dimerized.

Transfection of the murine *edr* cDNA sequence into CX2 cells increased their seeding efficiency. Therefore, cell lines were screened for growth factor response to EDR. The Burkitt's lymphoma cell line BL-70 growing strictly stroma-dependent showed a strong stroma cell replacing effect of EDR when administered together with low doses of α-thioglycerol.

O-1158 MOLECULAR CLONING OF NEW GENES DIFFERENTIALLY EXPRESSED DURING HUMAN EMBRYONIC HEMATOPOIESIS

M. Teyssier, S. Prost, M. Kirszenbaum. *CEA-DRM-SRHI-Groupe de Recherche sur la Régulation et l'Expression des Gènes, Hôpital St-Louis, Paris, France*

During human embryogenesis, at 3.5–4 weeks of gestation, two distinct sites of hematopoiesis generate first HSCs: extraembryonic yolk sac (YS) and intraembryonic aorta-gonads-mesonephros (AGM) region than the HSCs migrate to embryonic liver where all hematopoietic activities start. In order to understand the molecular mechanisms that control human early hematopoiesis, we have studied the identification of the genes expressed differentially in hematopoietic tissues during embryogenesis. We have compared the expression of mRNAs from the tissues presenting different levels of hematopoietic differentiation as YS (week 4), AGM region (weeks 3.5–4.5), liver (weeks 4–7), and umbilical cord blood (UCB) using RNA Differential Display (RDD) technique. The differentially expressed cDNAs were cloned, sequenced and compared to the GenBank database. We have cloned two genes differentially expressed in early hematopoiesis. The first (36-10) is highly expressed in AGM region and early liver then its expression decreases and increases in UCB; in YS the expression is very low. The sequence of 36-10 clone is tightly homologous to DNA break repair genes family. Second clone (36-16) is highly expressed in YS and early liver then rapidly decreases, its expression in AGM as in UCB is low. The sequence of 36-16 clone did not present any homology with GenBank database. The full-length cDNA was cloned and the predicted protein presents a new membrane molecule with five-transmembrane domain. The simultaneous expression of 36-10 clone in AGM and early liver may indicate the migration of HSCs from AGM nevertheless the other HSCs sub-population from YS expressing 36-16 clone may as well colonize the liver. Moreover the differential expression of the genes involved in DNA break repair may be relevant in the early human hematopoiesis.

ISH presidential symposium

PS-1159 MOLECULAR PATHOLOGY OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

T. Kinoshita, Y. Murakami, J. Takeda. *Research Institute for Microbial Diseases and Medical School, Osaka University, Osaka, Japan*

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disorder of the hematopoietic stem cell characterized by clonal hematopoietic cells with defective biosynthesis of glycosylphosphatidyl-inositol (GPI). The GPI deficiency causes defective surface expressions of at least twenty proteins that are normally anchored to the cell membrane via GPI. Somatic mutation in the X-linked gene PIG-A that is essential for the first step in GPI biosynthesis accounts for GPI deficiency in all patients with PNH characterized to date. Current research focuses on a mechanism of dominant hematopoiesis by affected clone(s). Analyses of chimeric mice generated with Piga-knock out, GPI-deficient ES cells demonstrated that disruption of Piga alone does not cause domination of the GPI-deficient cells. These analyses, however, were compromised by the presence of GPI-deficient non-hematopoietic cells that caused a high frequency of lethality and might also have affected a potential of GPI-deficient hematopoietic stem cells to expand. To avoid this problem, we knocked out Piga early in embryogenesis by means of Cre-loxP system and transplanted GPI-deficient fetal lever cells into lethally irradiated mice. Many chimeric mice that have GPI-deficient cells only in hematopoietic lineages were generated. The GPI-deficient hematopoietic cells contributed to hematopoiesis for longer than eight months without showing expansion, confirming that other factor(s) must be involved in expansion of affected cells.

PS-1160 THE CYTOKINE-INDUCIBLE SH2 PROTEIN (CIS) FAMILY: NEGATIVE REGULATORS FOR JAK SIGNALING PATHWAYS

A. Yoshimura, H. Sakamoto, M. Masuhara, R. Sizuki, A. Matsumoto. *Institute of Life Science, Kurume University, Aikawa-machi 2432-3 Kurume 839-0861, Japan*

We have reported two JAK-signaling modulators, CIS (cytokine-inducible SH2 protein) and JAB (JAK2 binding protein), which are structurally related. Here we cloned three additional CIS family genes (CIS2, CIS3, and CIS4) on the basis of an expression sequence tag (EST) database search. We also found at least two additional candidates of this gene family in the database. These genes were induced by erythropoietin and granulocyte-macrophage colony stimulating factor in certain hematopoietic cell lines. The SH2 domain as well as a C-terminal 40 amino acids region, designated as CIS homology domain (CH domain), are highly conserved in this family, while the N-terminal regions of these proteins share little similarity. Yeast two hybrid assay as well as *in vitro* and *in vivo* binding assays revealed that in addition to JAB, CIS3 bound to the JAK2 tyrosine kinase domain (JH1), although the interaction of CIS3 with the JAK2-JH1 domain was much weaker than that of JAB. JAB and CIS3 inhibited kinase activity of JAK2 *in vitro*, indicating that these are novel class of tyrosine kinase inhibitor. Transient expression of JAB and CIS3, but not other CISs, strongly inhibited LIF-induced STAT3-reporter gene activation in 293 cells. Furthermore, constitutive overexpression of JAB and CIS3 in M1 leukemia cells prevented leukemia inhibitory factor (LIF)-induced differentiation and growth arrest. Although the physiological function remains to be investigated, CIS family genes could play a role in negative regulation of cytokine signaling by interacting with specific targets.

PS-1161 REGULATION OF MEGAKARYOPOIESIS

K. Kaushansky, P. Rojnuckarin, C.E. Carow, J.G. Drachman. *University of Washington, Seattle, WA 98195, USA*

Much has been learned of the biological effects of thrombopoietin (TPO) since its cloning four years ago. This talk will focus on the mechanisms responsible for megakaryocyte (MK) development. Both *in vitro* and *in vivo* studies have revealed TPO to affect all aspects of MK development, from its support for survival and enhanced proliferation of hematopoietic stem cells, to fostering the shedding of platelets from mature MKs. All of these actions appear to be secondary to binding and inducing dimerization of the Mpl receptor. One of the first events induced by TPO binding is recruitment and transphosphorylation of members of the JAK kinase family. Two such kinases are so activated in Mpl-bearing cell lines and normal MKs, JAK2 and TYK2. Using cell lines deficient in one or the other kinase, we have found JAK2 but not TYK2 essential for subsequent signaling events. Many of the substrates of JAK2 have been identified, and include the latent transcription factors STAT3 and STAT5, the adapter proteins Grb2 and Shc, the guanine nucleotide exchange factors SOS and Vav, and phosphatases such as SHIP and SHP2. More recently, much attention has focused on the role played by MAP kinases in MK development. Using normal murine MKs we have begun to dissect the molecular pathways leading to activation of members of the MAP kinase family, and will present evidence that TPO-induced activation of the MAP kinase kinase, MEK, is a critical event in the generation of highly polyploid MKs. Finally, much work has also focused on endomitosis, the process by which MKs become polyploid. We and others have found endomitosis to be an aborted mitosis, not the absence of this cell cycle step, and to employ an active cdc2/cyclinB kinase complex. Thus, although still an enigmatic cell, a greater understanding of MK biology is upon us.

POSTER SESSIONS III. Wednesday, 8 July

Molecular diagnosis and minimal residual disease

P-1162 REAL-TIME QUANTITATION OF PHILADELPHIA POSITIVE LEUKEMIA BY PCR USING A SINGLE TUBE ASSAY

A.T.F. Van de Locht, E.H.P. Stevens-Linders, E.J.B.M. Mensink. *Central Hematology Laboratory, University Hospital Nijmegen, The Netherlands*

We developed a single tube assay to determine residual disease in patients with Philadephia positive leukemia (CML and ALL) using the ABI PRISM 7700 Sequence Detection System for real-time quantitative PCR. The expression of the Bcr-Abl hybrid oncogene was determined and normalized in a multiplex PCR using one of two different endogenous references; the Pbgd housekeeping gene mRNA or 18S ribosomal RNA. The Bcr-Abl mRNA level was determined from standard curves using cDNA derived from a Philadephia-positive cell line K562 diluted in cDNA from a negative cell line HL-60. The endogenous reference level was determined using a dilution of K562-cDNA in water. The ratio of the calculated quantities was used to normalize the different patient samples. The sensitivity of the assay was determined and the Bcr-Abl dosage present in 250 fg of K562 RNA could still be detected in a background of 50 ng of total RNA. The dynamic range of the method spanned six orders of magnitude. The analysis showed that both endogenous references can be used to quantitate hybrid mRNA expression levels in Philadephia positive

leukemia. Reproducibility was tested by analysis of ten identical assays on K562 RNA and resulted in a variation of 15% in starting quantity. Comparison of the single tube assays with the same reactions in separate tubes showed a complete concordance of results. We conclude that both Pbgd and ribosomal RNA are suitable quality control references. This semi-automated method is fast, sensitive, accurate and allows high throughput of samples.

P-1163 DETECTION OF BCR-ABL TRANSCRIPTS IN CHRONIC MYELOID LEUKEMIA (CML) USING A NOVEL "REAL TIME" QUANTITATIVE RT-PCR ASSAY (Taq MAN CHEMISTRY)

C. Preudhomme, L. Hornez, A. Merlat, D. Pruvot, A.L. Laine, A. Cosson, J.P. Peyrat, P. Fenaux. *CHU, Centre Oscar Lambret and U124, Lille, France*

RT-PCR can be used to detect BCR-ABL fusion transcripts in CML. Since BCR-ABL transcripts can still be found by RT-PCR in pts with long term cytogenetic complete remission (CR) after Allogeneic Bone Marrow Transplantation (allo BMT) or after interferon, quantitative competitive RT-PCR techniques have been developed. However, these techniques did not give linear results for more than two logs, induced high probability of contamination, and finally were quantitative only when used with a competitor carrying only minor modifications. In this work, we have developed BCR-ABL quantification by real time RT-PCR using the ABI PRISM 7700 (Perkin Elmer), a new technique which allows simple and rapid quantification of a Target Sequence during the extensive phase of PCR amplifications. A probe labeled with a fluorescent dye at the 5′ end hybridizes to the target sequence on the third exon of the ABL gene. The nucleolytic activity of the Taq DNA polymerase cleaves the probe and releases the reporter dye, resulting in a increase in the fluorescence signal. The absolute copy number of a target sequence (BCR-ABL) or control gene (ABL) in an unknown sample can then be calculated using a fluorescence curve prepared from a set of BCR-ABL RNA standards, obtained after in vitro transcription of a cloned BCR-ABL fragment in a Bluscript plasmid, ranging from 0.056 to 5600 pg. Each sample is normalized to the absolute quantity of the ABL gene transcript and the results are expressed as a BCR-ABL/ABL ratio. In our hands, the sensitivity of a serial dilution of total RNA from a positive cell line (K562) in a negative cell line (HL60) is 10^{-4}. 10 CML pts in cytogenetic CR, including 2 autografted pts, 3 pts treated by IFN and 5 allografted pts were studied sequentially. The autografted pts showed high level of BCR-ABL transcripts in all samples. 2 of the 3 pts treated by IFN showed high constant BCR-ABL transcript level but the third pt showed a progressive decrease, with a negative result in the last sample, taken 60 months after the onset of IFN. For the 5 pts allografted, 3 started to show a decrease in transcript levels 5, 7 and 8 months respectively after allo BMT, and had negative results 8, 10 and 11 months after allo BMT. They remained in hematological and cytogenetical CR, after 19, 27 and 94 months respectively, with negative results. The remaining two allografted pts had progressive increase of BCR-ABL level transcript; one developed cytogenetic relapse after 10 months and the 2nd pt remained in cytogenetic CR (60 mitoses examined) after 8 months but had developed an granulocytic sarcoma. **In conclusion,** real time RT-PCR appears to offer advantages over previously proposed quantitative RT-PCR methods. Our preliminary results suggest a good correlation between quantitative real time PCR and clinical and cytogenetic data. However, the sensitivity of the current technique (10^{-4} appears to be relatively low.

P-1164 OVERPRODUCTION OF THE BCR/ABL TRANSCRIPTS IN HUMAN LEUKEMIA CELL LINES K562 AND BV173

J. Moravcová, S. Muchová. *Institute of Hematology and Blood Transfusion, Praha, Czech Republic*

The human leukemia cell line K562, bearing the BCR/ABL fusion gene - a typical marker of chronic myeloid leukemia (CML), is widely used in scientific investigations. Cells of this line are also very often used to estimate PCR sensitivity. The cell line K562 is known to produce additional or atypical Ph chromosomes and multiply the BCR/ABL gene [1]. This must result in overproduction of BCR/ABL transcripts. As in our laboratory cells of lines K562 and BV173 are used for preparation of competitors for quantification of the b2a2 and b3a2 fusion BCR/ABL transcripts, respectively, we estimated the rate of this overproduction in both cell lines. Six patients at CML diagnosis with 100% of Ph chromosomes and the BCR/ABL to BCR transcript ratio equal to 1 were taken as 100% standards. Three of them bore the b2a2 and three others the b3a2 fusion gene. By competitive RT-PCR, the overproduction of BCR/ABL transcripts was found to be 82-fold and 14-fold in K562 and BV173, respectively. In both cell lines, there is a great variability that manifests itself even during repeated cultivations of the cells. Both cell lines recently cultivated in our laboratory showed further increased overproduction of BCR/ABL transcripts. Many researchers use leukemic cell line K562 or BV173, but poorly a few of them are aware of overproduction of their BCR/ABL transcripts. If the cell line K562 is used for estimation of RT-PCR sensitivity this can be strongly overrated. On the other hand, the amount of antisense oligonucleotides or other agents blocking the BCR/ABL function can be underrated.

This work was supported by grant No. 3568-3 of IGA MZ CR.

(1) Wu et al., 1995, Leukemia, 9: 858–862.

P-1165 EVALUATION OF MINIMAL RESIDUAL DISEASE USING REAL TIME QUANTITATIVE RT-PCR (TaqMAN CHEMISTRY) IN t(8,21) AML PATIENTS

T. Smetsers, J.M. Cayuela, F. Morschhauser, S. Martini, E.J.B.M. Mensink, P. Fenaux, C. Preudhomme. *University Hospital Nijmegen, CHU Lille and Paris St Louis, The Netherlands*

Several groups have reported positive results of minimal residual disease (MRD) from RT-PCR analysis of t(8,21) AML patients who remain in haematological and cytogenetic long term remission (CR). Recently, real time quantitative PCR technology was suggested to be more useful in clinical management. We used real time PCR (ABI prism 7700, Perkin Elmer) to test blood and bone marrow samples from AML patients (pts) with t(8,21) previously studied by 2 conventional RT-PCR methods in 2 different labs (sensitivity of 10^{-6} in one lab and 10^{-5} in the other) in order to compare these 2 technologies.

Methods: the rapid AML1-ETO quantification was based on the threshold value (first time the signal becomes visible). The absolute copy number of the target sequence (AML1-ETO) or of the house keeping gene (PBGD) in an unknown sample was then calculated using a calibration curve. For AML1-ETO, the curve was obtained by serial dilution of total RNA from the t(8,21) positive Kasumi cell line in RNA from HL60 and also from a synthetic construct. For PBGD, the curve was obtained by dilution of RNA from K562 in water. Each sample was normalized to the absolute quantity of the PBGD transcripts and the results were expressed as a AML 1-ETO/PBGD ratio. 8 patients (pts) were sampled following induction, consolidation or intensification and every 3 to 6 months after completion of therapy. The median number of bone marrow sample per patient was 7 (range 5 to 10). All had more than 12 months of follow-up (median 24 months, range 12 to 36). Consolidation treatment included high dose Ara C (7), and allo BMT (1).

Results: We found that the sensitivity of real time PCR was around 5.10^{-5} which equals 10 molecules of AML1-ETO transcripts. All patients had a decrease of transcript quantity of 5 orders of magnitude within the first 6 months of treatment. These patients also converted early to PCR negativity with two conventional RT-PCR methods (sensitivity of 10^{-5} and 10^{-6}).

In Conclusion: preliminary results on patients in CR suggest a good correlation between conventional and real time quantitative PCR. Increased sensitivity could probably be obtained via concentration of the cDNA sample. Analysis in pts who progressed or relapsed are in progress in order to study the kinetic profile and try to determine a quantitative threshold predicting relapse versus CR

P-1166 QUANTITATIVE DETECTION OF RESIDUAL FOLLICULAR NHL CELLS CARRYING t(14;18) BY A DOUBLE PROBE REAL-TIME QUANTITATIVE PCR

J.P.P. Meijerink, C.M.P.W. Mandigers, E.L.R.T.M. Tönnissen, A.T.F. Van de Locht, J.M.M. Raemaekers, E.J.B.M. Mensink. *University Hospital Nijmegen, Nijmegen, The Netherlands*

Quantitative data on residual malignant cells during the course of disease may result in individualization of therapy. Although quantitative competitive PCRs have been developed, their usefullness is limited due to labour intensity and contamination problems. A new type of PCR which makes use of taqman chemistry has recently become available. In this real-time analysis, the amount of PCR product is quantified by measuring the cycle number at which reporter-group (FAM) emissions due to degradation of an internal probe exceeds the 10 times standard deviation of baseline emissions, i.e. the threshold cycle (Ct). We use this type of PCR to measure the amount of residual t(14;18) carrying malignant cells during the course of disease of follicular NHL patients. Since the Ct occurs in the exponential phase of the reaction, positive patient samples are compared to a single reference sample. As reference, we used a t(14;18) construct which is diluted in normal DNA and can mimic both the MBR and MCR breakpoints such as found in patients. To correct for minor differences in the amount of DNA (about 1 µg) and potential PCR inhibitory factors, we normalized the t(14;18) signal to the albumine PCR signal. The amplification of the albumine locus, as measure for amount of DNA, is performed within the same amplification reaction using an JOE-labeled albumine internal probe. The albumine PCR is performed under conditions at which it is able to discriminate for minor differences in DNA content and at which it does not hinder the t(14;18) specific amplification.

P-1167 REAL-TIME PCR FOR QUANTITATIVE DETECTION OF MINIMAL RESIDUAL DISEASE (MRD) IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

M.J. Pongers-Willemse[1], O.J.H.M. Verhagen[2], G.J.M. Tibbe[1], J.M. Wijkhuijs[1], V. De Haas[2], E.R. Van Wering[3], C.E. Van der Schoot[2], J.J.M. Van Dongen[1]. [1]*Dept. of Immunology, Erasmus University Rotterdam;* [2]*Dept. of Immunohematology, Central Laboratory of the Netherlands Blood transfusion Service, Amsterdam;* [3]*Dutch Childhood Leukemia Study Group, The Hague, The Netherlands*

PCR analysis of immunoglobulin (Ig) and T cell receptor (TCR) genes has shown that risk groups can be identified with a different outcome based on MRD information at two successive time points early in treatment. Assessment of the amount of residual cells appeared to be important for risk group assignment. However, current PCR techniques do not allow accurate quantification. Therefore we have tested 'real-time' quantitative PCR (RQ-PCR) with the TaqMan technology for MRD detection.

The junctional regions of rearranged of Ig/TCR genes define the specificity and sensitivity of the PCR-based MRD detection in ALL. Usually the sequence information of the junctional region is used to design a patient specific probe, which detects DNA from the leukemia in follow-up samples, after PCR amplification of the Ig/TCR gene rearrangement. For the application of the TaqMan technology we have chosen for the same approach. The fluorochrome labeled TaqMan probe is designed at the position of the junctional region. Real time information is obtained from each PCR cycle by degradation of the TaqMan probe by the exonuclease activity of the Taq-polymerase resulting in a fluorescent signal. This approach of MRD detection was compared with classical techniques, i.e. dot-blot hybridization or liquid hybridization after PCR amplification of the Ig/TCR target.

For six MRD-targets in three different patients, TaqMan probes were designed for RQ-PCR analysis; this concerned three *IGH*, two *TCRD*, and one *IGK* gene rearrangement. Sensitivities of the PCR-targets were defined by $\log_{10}$ dilutions of DNA from diagnosis into DNA from normal cells and varied between 10^{-3} and 10^{-5}. These results were comparable with the dot-blot technique, while the liquid hybridization usually obtained one $\log_{10}$ higher sensitivity. Bone marrow follow-up samples of one patient were analyzed by two different targets together with the diagnosis dilution series. Some PCR positive samples were quantified in parallel by limiting dilution and showed comparable tumor loads as found with the TaqMan approach.

The RQ-PCR represents an improvement on current MRD techniques because it is quick and allows easy quantitative MRD detection, which is essential for clinical application of MRD information. Nevertheless, the method should be further optimized to obtain sensitivities of at least 10^{-4}.

P-1168 DETECTION OF T-CELL RECEPTOR γ GENE REARRANGEMENT USING POLYMERASE CHAIN REACTION (PCR)

A. Sahar. *Rambam Medical Center, Haifa, Israel*

Detection of clonality by gene rearrangement in lymphoproliferative diseases provides information of practical diagnostic value in distinguishing neoplastic from reactive disease.

The detection of T-cell receptor (TCR) gene rearrangement has been performed by Southern blot analysis for many years. Recently the use of polymerase chain reaction (PCR), becomes more and more the initial diagnostic technique of choice to detect monoclonality for routine needs. In this procedure, the PCR products are analyzed on denaturing gradient gel electrophoresis (DGGE) or ontemperature gradient gel electrophoresis (TGGE). We have introduced the use of polyacrylamide gel electrophoresis (PAGE) for the detection of the PCR products and found it much simpler and rapid and yet allows high sensitivity. Clonality was detected in 13/15 (87%) of T-ALL cases, in 6/9 (67%) of T-lymphoma cases and also in 5/7 (71%) of B-ALL cases. (TCR γ rearrangement is not lineage-specific). We also performed analysis of skin biopsies of patients with Mycosis Fungoides (MF) (cutaneous T-cell lymphoma), and as a negative control - patients with inflammatory dermatoses. In this group - clonality was demonstrated in 12/17 (70%) of MF cases and in none (0/18) of the dematoses cases. We conclude that PAGE analysis of PCR products is a better way for the routine detection of TCR-γ clonality of lymphoid tumors.

P-1169 FREEZING OF MASTER MIXTURE FOR REAL-TIME QUANTITATIVE PCR RETAINS FULL AMPLIFICATION ACTIVITY AND FACILITATES PCR STANDARDISATION OF MOLECULAR DIAGNOSTICS

T.F.C.M. Smetsers, E.H.P. Stevens, L.T.F. Van de Locht, E.J.B.M. Mensink. *Department of Hematology and Central Hematology Laboratory, University Hospital Nijmegen, The Netherlands*

The detection and quantitation of fusion transcripts by reverse transcriptase PCR is often troubled by the lack of a standardised procedure. In order to improve the reproducibility of the we tested the effect of freezing of PCR reagents on the efficiency of the PCR using the highly reproducible real-time quantitative PCR method (ABI/PRISM™ 7700 system). The reactions tested were reverse transcriptase PCR reactions for the detection of AML-1/ETO transcripts in acute myeloid leukemia and two transcripts of the porphobilinogen deaminase (PBGD) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping genes. The PCR master mix contains all PCR components including Taq polymerase, primers, Taqman probe and template. In triplicate experiments we quantitated the number of transcripts for the different genes at several time points. We conclude that even after 60 days of freezing there is no loss of amplification efficiency. Repeated freezing and thawing showed a gradual reduction of the amplification activity. Therefore freezing and storage of PCR master mixture for a long time is possible without subsequent loss of activity. We also sent the PCR mixes with template to different PCR laboratories and investigated the reproducibility and difference in sensitivity. The sensitivity in the different institutes was comparable although there were some differences most likely due to the differences in PCR machine. Some PCR reactions seem to be more sensitive to different reaction conditions than others. In conclusion; the use of frozen PCR mixtures will speed up PCR handling, enhance reaction reproducibility, facilitate standardisation and reduce the risk of PCR contamination in PCR routine diagnostics.

P-1170 PROGNOSTIC VALUE OF IGH REARRANGEMENT DETECTION IN PERIPHERAL BLOOD STEM CELL (PBSC) HARVESTS OF PATIENTS WITH MULTIPLE MYELOMA UNDERGOING PBSC TRANSPLANTATION

L.G. Robinson, S.J. Austin, C.E. Owen-Jones, A. Al-Sabah, R. Bailey-Wood, K.I. Mills, A.K. Burnett. *University of Wales College of Medicine, Cardiff, CF4 4XN, UK*

Multiple Myeloma (MM) remains elusive to definitive treatment despite the introduction of intensive chemotherapy with PBSC rescue. The optimal mobilisation, conditioning regimes and benefits of selection techniques are yet to be fully established. Rearrangements of the IgH gene are present in many patients and may be used to monitor minimal residual disease in PBSC harvests and following transplantation. 16 patients with MM mean age 54

yrs, underwent sequential high dose therapy with Cyclophosphamide 4 g/m^2, Etoposide 2.4 mg/m^2 and Melphalan 130 mg/m^2 followed by Busulphan-Cyclophosphamide PBSCT. PBSC's were collected following the initial cycle of cyclophosphamide with G-CSF to optimise collection. All patients had harvests of >2 $\times$ 10^8 MNC's/Kg. PBSC's were retrospectively analysed for the presence of IgH gene rearrangements by RT-PCR using the ABI genetic analyser fluorescent detection system. 11/16 patients had clonal rearrangements detectable and of these, 4 have subsequently relapsed and died of progressive disease, 3 remain in complete remission (CR) and 4 in partial remission (PR), 2–4 yrs post PBSCT. Of those patients with no detectable rearrangement 2 remain in CR and 3 in PR post PBSCT with no episodes of disease progression. These results suggest that tumour contamination of PBSC collections does not preclude a favourable outcome to PBSCT but the risk of disease progression is increased and may reflect tumour burden at the time of PBSC collection.

P-1171 HIGHLY REPRODUCIBLE PCR QUANTITATION OF MYELOMA TUMOR CELLS USING THE ABI PRISM™ 7700

P.M.W. Willems, L.J. Groothuis, E.J.B.M. Mensink, C.E. Van der Schoot[1], O.J.H.M. Verhagen[1], R.A.P. Raymakers. *Department of Hematology and Central Hematology Laboratory, Academic Hospital Nijmegen; [1]Department of Immunohematology, Central Laboratory of BloodTransfusion, Amsterdam, The Netherlands*

The unique immunoglobulin heavy chain rearrangement (IgH) of myeloma plasma cells can be used as a marker for the myeloma tumor clone. Currently allele specific oligonucleotide (ASO)-IgH PCR is the most sensitive method to detect myeloma tumour cells. We constructed ASO-PCRs for four patients using sense (CDR1) and antisense (CDR3) ASOs and family specific probes. For quantitative analysis, we tested a recently developed integrated system for thermal cycling, real time fluorescent detection of PCR product and subsequent calculation of starting quantities (Perkin Elmer-ABI PRISM™ 7700 Sequence Detector System). Real time ASO-PCR consistently detected 1 DNA target or 1 tumor cell in a background of 80.000 normal cells (sensitivity $\geq$1.25 10^{-5}). The ABI PRISM™ 7700 system proved highly reproducible. Using a 5 log range of template concentrations within a single PCR we found an average 1.7 fold variation in starting quantity per sample. We found an average 2.6 fold day to day variation in this assay. For quantitation of ASO-PCR product with conventional densitometrical analysis we calculated an average 8.8 fold variation in starting quantity per sample. For a quantitative limiting dilution assay where we subjected *10 dilutions to PCR in quintuplicate we calculated an average 8.4 fold variation in starting quantity per sample. This variation reduced to 3.0 fold provided that all reactions were performed in ten fold. We present a standardized method that allows sensitive quantitation of myeloma cells with higher reproducibility than other available quantitation methods. In addition this PCR method allows high throughput of samples with a minimal contamination risk since there is no post PCR sample handling. We conclude that quantitative real time IgH PCR is an accurate and sensitive tool to monitor treatment response of most B cell malignancies.

P-1172 THE FEASIBILITY OF A ROUTINE MOLECULAR DIAGNOSTIC LABORATORY: A THIRD WORLD EXPERIENCE

W.S. Stevens, G. Stevens, B. Mendelow. *Department of Haematology, School of Pathology, University of the Witwatersrand and S.A.I.M.R., South Africa*

Introduction: Molecular technology using techniques like PCR have proved to be important in the setting of basic and applied research as well as invaluable in the diagnosis, early intervention and monitoring of a patient's response to numerous diseases. We initiated a routine molecular diagnostic facility at the Chris Hani Baragwanath hospital in Soweto. Much of our initial focus has been on lymphoma diagnostics using various techniques to categorise non-Hodgkin's lymphoma into distinct biologic entities. Our emphasis has however changed to address the needs of our population in particular the AIDS epidemic which is substantial and worsening. We are in the process of developing further tests that are rapid, cost effective and relevant. Assays of both a quantitative and qualitative nature are currently performed. The awareness that the use of non standardised assays at numerous sites results in variable sensitivity, specificity and reproducibility of these assays has led us to initiate an external quality assurance programme.

Conclusion: Despite limited resources we feel that molecular techniques like PCR are feasible, cost effective and important for the rationalisation of therapy in the third world. Standardised procedures and the use of common algorithms are however, essential for ensuring reliable results. The ever increasing number of samples received bears witness to the usefulness of this service.

P-1173 A SIMPLE MICRODISSECTOR-ASPIRATOR FOR THE RAPID PROCUREMENT OF SINGLE CELLS FROM CYTOLOGY SPECIMENS

C. Beltinger, K.-M. Debatin. *Universitäts-Kinderklinik Ulm, Germany*

Molecular analysis of suspicious cells in cytology specimens would help to establish a diagnosis in ambiguous cases. To avoid contamination with normal background cells confounding molecular analysis mechanical and laser assisted methods have been described for the selective procurement of cells from histology slides. They have the drawback of either being technically demanding or expensive. Furthermore, it is unclear whether they can be applied to cytology specimens. Finally, few of these techniques are able to procure single cells.

We therefore developed a simplified combined microdissection-aspiration device for the rapid procurement of single cells from clinical cytology specimens. The principle of this device, called the cytopicker, is the combination of the microdissection tool, a steel cannula, with the aspiration tool, a glas capillary connected to vacuum into one device. Steel cannulas are optimal for microdissection of cells from the hard matrix of cytology specimens but aspirate poorly. Glas cannulas, on the other hand, are suboptimal for dissecting but aspirate very well. Combining both tools into one by inserting the capillary into the cannula allows optimal dissection using the cannula (with the glass capillary withdrawn and thus protected), followed by optimal aspiration using the capillary (after being advanced through the cannula). These axial as well as all other movements of the device are controlled by just one micromanipulator, thus making the cytopicker inexpensive to operate. We show that the cytopicker can rapidly and simply procure single cells such as lymphoblasts from cytology specimens such as peripheral blood smears. Thus, the cytopicker might facilitate molecular analysis in the routine cytology laboratory.

P-1174 RAPID AND SIMPLE METHODS TO GENERATE POSITIVE CONTROLS FOR SSCP

C. Beltinger, Klaus-Michael Debatin. *Universitäts-Kinderklinik Ulm, Germany*

The single strand conformation polymorphism assay (SSCP) is the most common applied method when screening clinical samples for the presence of mutations since it is simple and rapid. However, to achieve a sufficient sensitivity SSCP conditions have to be optimized for each different stretch of DNA analyzed. Positive controls for each DNA fragment are therefore crucial for setting up a SSCP assay, especially when the expected frequency of mutations is low, a common situation when screening a gene implicated in human disease. Unfortunately, the availability of mutant patient DNA is often limited. Therefore the capability to generate positive controls in vitro would greatly facilitate SSCP analysis. We hence investigated the feasibility to generate positive controls for SSCP and other methods of mutation detection using PCR-based mutagenesis.

The CD95(APO-1/Fas) gene was amplified with primer pairs covering and flanking the coding region using radioactive PCR. Mutant PCR products were first made by substituting a single base within one primer of each primer pair, creating either an inversion or a transversion. The PCR products were subjected to SSCP analysis. PCR amplification was successful in every PCR reaction with a mutant primer, regardless whether the mutated primer had had a transition (n = 5) or a transversion. Since both transitions and transversions were equally successfully amplified no constraints have to be applied concerning the chemistry of the mutant base within the primer. Overall, 15 of 18 (83%) of the mutant PCR products created by single-base mismatched primers were distinguished from their corresponding wild type PCR product by SSCP.

To circumvent the need for mutant primer and to create mutations that are spread across the whole amplicon we performed PCR with normal primers but with 5-bromodeoxyuridine (BrdU) in addition to unmodified bases. BrdU is known to create A:T = > G:C transitions in cells. We reasoned that this mechanism should also be operational in vitro during PCR amplification. SSCP and sequence data using this approach are presented.

P-1175 DETECTION OF CLONAL EVOLUTION IN A CASE OF T-CELL LYMPHOMA BY T-CELL RECEPTOR Vβ CDR3 "SPECTRATYPE"

S. Bonfigli, P. Pistidda, L. Guiso, F. Dore[1], M. Longinotti. *Istituto CNR per lo Studio della Patologia del Sangue, Sassari; [1]Istituto di Ematologia, Università di Sassari, Italy*

TCR gene rearrangement pattern is usually used as a marker for clonality in T-lymphoproliferative disorders. Moreover PCR-based methods for TCR junctional diversity analysis can prove whether a recurring disease represents a true relapse or a secondary malignancy.

We used a RT-PCR assay to analyze the size heterogeneity of clonotypic Complementarity Determining Regions (CDR3) in the 24 TCR Vβ gene family transcripts (i.e. CDR3 spectratyping), in a case of anaplastic T-cell lymphoma which showed a change in immunophenotype from diagnosis to relapse. At presentation the lymphoma cells had an early thymic precursor phenotype (TdT^+, $CyCD3^+$, $CD7^+$, $CD2^+$ and CD4-CD8 double negative) and the Vβs CDR3 spectratype showed a unique clonal rearrangement in the Vβ2 subfamily. After complete remission, achieved by MACOP-B regimen, the patient relapsed six months later and an intralineage shift occurred in the tumor cells which resulted to have a cortical thymocyte immunophenotype (TdT^+, $CD3^+$, $CD7^+$, $CD2^+$, $CD5^+$ and CD4-CD8 double positive). At this time an additional minor clone in the Vβ6 sub-family was found. Cloning and sequencing of the Vβ2 and Vβ6 PCR products could confirm the diversity of the junctional region sequences. These findings, pointing out a continuing TCR gene rearrangements among the malignant T-cells, suggest that the best way to monitorize T-lymphoproliferative disorders should be by clonotyping based assays.

P-1176 UTILITY OF GALLIUM-67 SCINTIGRAGHY (67 GA) IN THE FOLLOW-UP OF LIMPHOMA PATIENTS

R.B.S. Afonso, C. Pinto, A. Teixeira, M. Vivan, F. João, E. Sousa, A. Doménech, F. Plácido. *Department of Hematology and Nuclear Medicine, University Hospital, Coimbra, Portugal*

Aim of the Study: To evaluate the correlation between data provided by 67 Ga and other diagnostic tests, namely CT scan, in conjunction with clinical examination.

Material and Methods: 46 patients were enrolled in this study (34 men and 12 women), from which 35 had Hodgkins's Disease and the remainder had Non Hodgkin Malignant Limphoma.

Results: Cintigraphy was in concordance with both CT scan and clinical data in 27 cases (58%); from these, 9 presented evidence of active disease and 18 did not show signs of active desease.

In 18 patientes there was no concordance between 67 Ga and clinical and imagiological data. The rate of false positive results was superior to the rate of false negative results.

Sensibility and specificity of 67 Ga was, respectively, of 83% and 91%.

Conclusions: These results confirm that 67 Ga is a sensitive and specific technique to detect relapse and residual tumor as well as to assess efficacy of therapy; nevertheless, it must be interpreted with caution, since 67 Ga frequently overestimates the presence of residual viable tumor.

Animal models

P-1177 HEMATOPOIESIS DEFICIENT IN GPI-LINKED PROTEINS IN MICE CHIMERIC FOR A NON-FUNCTIONAL *pig-a* GENE

C. Dominguez, G. Tremml, V. Rosti, M. Bessler. *Div. Hematology Washington University School of Medicine, St. Louis, MO; Dept. Human Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

Blood cells from patients with paroxysmal nocturnal hemoglobinuria (PNH) are deficient in all proteins linked to the cell membrane by a glycosyl phosphatidylinositol molecule (GPI-anchor) due to a mutation in the X-linked *PIG-A* gene. The mutations explain the phenotype and some of the clinical features. What remains unclear is the mechanism that enables a hematopoietic stem cell deficient in so many proteins to expand. To investigate the cell fate of hematopoietic cells that lack all GPI-linked molecules we produced mice chimeric for a non functional *pig-a* gene using spatio-temporally controlled site-specific mutagenesis. Cre mediated *pig-a* recombination was aimed to occur only in the early preimplantational embryo by expressing the recombinase under the control of the adenoviral EIIa promoter. Pig-a^- hematopoiesis was assessed by flow cytometry using monoclonal antibodies towards GPI-linked surface molecules. Using this strategy we previously reported that in offspring with inactivation of the maternally derived *pig-a* gene between 1 and 5% of red cells and about 1–5% of lymphocytes lack GPI-linked surface molecules at the age of 4 weeks [1]. Using the opposite breeding scheme through which inactivation occurs in the paternally derived *pig-a* gene we now find in newborns up to 55% of red cells that lack CD24. At the age of 4 weeks the percentage of *pig-a*$^-$ red cells and lymphocytes still exceeds 20% in some animals and seems to be stable during an observation period of 3 months. The increased rate of recombination is due to the specific expression pattern of the EIIa promoter and to the fact that also in mice *pig-a* maps to the X-chromosome. The price for the increased contribution of pig-a^- cells to hematopoieis is an increased prenatal lethality (50%) due to increased contribution of pig-a^- cells to other organ systems. However, using this strategy we are able to obtain sufficient numbers of animals with a substantial proportion of pig-a^- blood cells that will allow us to perform functional studies comparing normal hematopoiesis with hematopoiesis of cells that lack GPI-linked proteins. These experiments will increase our understanding in the pathophysiology and pathogenesis of PNH.

(1) Tremml et al. 1997, Blood, 90. 407a. (abstr. 1810).

P-1178 THE INFLUENCE OF PROTEINASES ON *IN VIVO* BLASTIC TRANSFORMATION IN RAT SPECIES SD/Ipcv WITH SPONTANEOUS LYMPHOBLASTIC LEUKEMIA

M. Wald[1], T. Olejár[2], P. Poučková[2], M. Zadinová[2]. *[1]Department of Surgery, 2nd Medical Faculty, Charles University, Prague; [2]Department of Biophysics, 1st Medical Faculty, Charles University, Prague, Czech Republic*

The influence of proteolytic enzyme mixture was proved on spontaneous lymphoblastic leukemia development in our in vivo experiment in rats species SD/Ipcv. In the group treated by a mixture of trypsin, chymotrypsin and papain per rectum a significantly lower rate of blast cells in the peripheral blood during disease development was noticed. The average survival time in the experimental group was 150 +/- 45.62 days, in the control group 43.83 +/ - 24.52 days since the appearance of the first blast cells. One half of the treated animals survived even until the end of our experiment (366 days) and was finally sacrificed. In the treated group two cases with no blast cells and in one occasion a very small number of blast cells were found at the end of experiment. The proteolytic enzyme mixture has also had a positive influence on the stable weight gain in animals in contrast to the weight loss of the control group in the terminal stage of the disease. The maximum count of all leukocytes in the time of exitus ranged between 9.9×10^9/l and 29.8×10^9/l in experimental animals and only in one animal the periphery was flooded by 37% of blast cells. In the control group the numbers ranged between 21.6×10^9/l - 58.0×10^9/l and the presence of blast cells in the periphery was around 30–72%.

Genetic disorders

P-1179 GENOTYPING OF COMMON HEREDITARY HAEMOCHROMATOSIS MUTATIONS IN MICROTITER PLATES

C. Oberkanins[1], L. Kazemi-Shirazi[2], F. Kury[1], C. Polli[2], Th. Maier-Dobersberger[2], C. Yeganehfar[2], P. Ferenci[2]. *[1]ViennaLab Labordiagnostika GmbH, Vienna; [2]Department of Internal Medicine IV, University of Vienna, Austria*

Hereditary haemochromatosis (HH) is a very common autosomal recessive disorder of iron metabolism. Among individuals of Northern European descent the carrier frequency is estimated 1 in 10, while up to 1 in 200 are affected by the disease. HH is characterized by progressive accumulation of iron in various organs (e.g. liver, heart), leading to liver cirrhosis, diabetes, arthritis or cardiomyopathies. If diagnosed early enough the disease symptoms can be prevented simply and very effectively by regular therapeutic bleeding (phlebotomy).

A candidate gene for HH with close similarity to MHC class I genes has been identified on chromosome 6p and termed HLA-H or HFE. Two point mutations (C282Y and H63D) are found in more than 85% of HH patients.

We have developed a rapid and simple test for these mutations based on PCR and DNA hybridization in ELISA plates. The procedure includes three successive steps: DNA is isolated from anticoagulated blood by a rapid and convenient method. Then, relevant HLA-H gene sequences are *in vitro* amplified and terminally labelled with a reporter molecule. Finally, amplification products are selectively hybridized to allele-specific ("wild type"

or "mutant") oligonucleotide probes in separate cavities of a microwell plate and detected by immunoreaction. The genotype of a particular sample can easily be identified from the staining pattern of corresponding microwells.

The method has been validated on samples of known genotype. We have used the new test to investigate the prevalence of the C282Y mutation in 460 asymptomatic individuals from Eastern Austria. We have identified 44 heterozygous (9.6%) but no homozygous carriers among this population.

P-1180 IRON AS RISK FACTOR FOR HEPATOCELLULAR CARCINOMA

S. Fargion, M. Mattioli, M. Sampietro, G. Rossi[1], L. Saltarin, L. Lupica, A.L. Fracanzani, E. David[2], L.R. Fassati[1], G. Fiorelli. *Dipartimento di Medicina Interna; [1]Istituto di Chirurgia Sperimentale, Ospedale Maggiore IRCCS, Università di Milano; [2]Dipartimento di Scienze Oncologia Umana, Università di Torino, Italy*

It has been suggested that iron, when in excess, may facilitate cancer occurrence. To test this hypothesis we evaluated HFE, the candidate gene for genetic hemochromatosis (GH), and iron concentration in non-neoplastic tissue of explanted livers of 47 patients (pts) who underwent liver transplantation for primary liver cancer (PLC) in 43 cirrhotic and in 4 normal livers occurring. Liver iron concentration (LIC) was determined by atomic spectrophotometry absorption in 5 samples of every explant and hepatic iron index (HII = LIC/age), diagnostic for GH when $\geq$1.9, calculated. HFE mutations (mut) were studied by PCR of the relevant exons, and restriction with RsaI and BclI. LIC > normal value (29 umol/g d.w.) was detected in 10/47 pts (21%), in the 8 cirrhotic and 2 non cirrhotic pts,. and HII > 1.9 in 4 (8%). Histology of PLC was hepatocellular carcinoma in cirrhotic and hepatoblastoma in the two non-cirrhotic pts. In 7/8 cirrhotic pts HCV infection was present. Analysis of HFE gene revealed the absence of C282Y mut in all cases, whereas H63D mut was detected in 5/8 pts. These results indicate that a high number of pts who develop PLC have increased LIC, which in 8% is compatible with a diagnosis of GH. This prevalence is more than 20-fold higher than that expected, in Italy, in a normal population. The presence of H63D mut which is less clearly associated to iron overload than C282Y mut indicates the existence of a subtle alteration of iron metabolism. These results suggest that increased iron in the liver could act as a promoter of neoplastic transformation in the presence of carcinogenic factors.

P-1181 HEREDITARY HYPERFERRITINEMIA-CATARACT SYNDROME (HHCS)

J.A. Garcia-Erce, T. Cortés, G. Pérez-Lungmus, C. Salvador-Osuna, M. Giralt. *Dept. of Haematology, Hospital Miguel Servet, Zaragoza, Spain*

Introduction: Recently have been described several families of italian origin with nuclear congenital cataracts and hyperferritinaemia without signs of iron overload or HLA relationship. HHCS is associated with mutations on the gen of the L-ferritin of the chromosome 19 (19q13.3–13.4). We present the first spanish family diagnosed of HHCS.

Case Reports: Symptom-free brother and two sister, referred to exclude haemochromatosis, were found to have increased serum ferritin levels. All of them had nuclear congenital cataracts, as had their mother. Physical examination was normal in all siblings.

Proband nº1: Female, 40 year old, with nuclear cataracts, recidivant pneumothorax and anaemia treated several times with oral iron. She was sent to us presenting mild hyperferritinaemia. Blood values: Hb 112 g/L, Hct 0.35L/L, VCM 90 fL, Plts 330 $\times$ 10^9/L, WBC 7.5 $\times$ 10^9/L, corrected reticulocytes (ret) 0.8%, iron 73 mcg/dL, transferrin 369 mg/dL, ferritin 809 ng/dL, SI 19%, free ecrytrocytic protoporphyrine (FEP) 30 mcg/dL PRC. Biochemical profile: normal. HLA: A2B7/A2B7.

Proband nº2: Female, 38 year old, with nuclear congenital cataract, and treated with oral iron at the first pregnancy. Pregnant at diagnosis (4th month): Hb 121 g/L, Hct 0.36 L/L, VCM 100 fL, ret. 0.8%, iron 87 mcg/dL, transferrin 366 mg/dL, ferritin 984 ng/dL, SI 19.7%, FEP 21 mcg/dL PRC, biochemical profile normal. HLA A2B7/A24B7. At the 8th month of her second pregnancy she presented: Hb 87 gr/L, iron 34 mcg/dL, transferrin 515 mg/dL, ferritin 1422 ng/dL, SI 5.1%, FEP 47 mcg/dL PRC. According to the diagnosis of iron deficiency anaemia, she was treated with oral iron, and one month after the delivery blood values were normal: Hb 130 gr/L, iron 46 mcg/dL, transferrin 255 mg/dL, SI 14%, ferritin 1224 ng/dL.

Proband nº3: Male, 41 year old, with nuclear congenital cataracts, otoesclerosis and cured B hepatitis, is controlated since april 1997 by hyperferritinaemia without signs of iron overload. Blood values at diagnosis: Hb 140 g/L, Hct 0.43 L/L, ALT 51 UI/L, iron 78 mcg/dL, SI 19%, ferritin 1257 ng/dL. HLA A2B7/A11B35. FEP 19 mcg/dL PRC.

Remarks: In our opinion HHCS has a world-wide distribution. Its suspicion is important in order to avoid incorrect treatment (flebotomy). The patients affected of HHCS are susceptible to suffer iron deficiency anaemia as the general population.

P-1182 ANALYSES OF FANCONI ANEMIA GENES IN JAPANESE PATIENTS

M. Futaki, T. Yamashita, M. Yabe, S. Kato, A.D. D'Andrea, S. Asano, T. Nakahata. *Inst of Medical Science, Univ of Tokyo, Tokyo; Tokai Medical School, Yokohama, Japan; Dana-Farber Cancer Inst, Boston, USA*

Fanconi anemia (FA) is an autosomal recessive disease characterized by progressive bone marrow failure, congenital anormalities, and a predisposition to malignancy. There are five known complementation groups (A–E). The genes for group A (FAA) and group C (FAC) were cloned. In Western countries, the percentages of FA (A) and FA (C) patients are estimated as 60–70% and 10–15%, respectively. However, various ethnic groups are reported to have different genetic abnormalities. Regarding Japanese FA patients, genetic basis remains to be clarified. We screened 28 Japanese FA patients (22 unrelated patients and 6 patients in three families) by SSCP and found 8 homozygotes for IVS4 + 4A toT IVS4), a splice mutation in intron 4 that has only been found in patients of Ashkenazi-Jewish ancestry. Auerbach and her group recently reported that Jewish patients with IVS4 show severe phenotype, that is, early onset of hematological abnormalities, multiple anomalies and poor survival, in comparison with FA (C) patients with exon 1 mutations and non C patients. On the contrary, our results indicate that there is no significant difference between IVS4 patients and other (non C) patients in onset of median age of hematological abnormalities (IVS4, 6.21 yrs. vs. non C, 6.0 yrs., p = 0.879) or mean number of anomaly (IVS4, 1.3 vs. non C, 0.9, p = 0.453). Ethnic difference may affect clinical phenotype in FA patients with the same mutation. We also tried to screen FAA abnormalities by immunoblot analyses, since most FA (A) cells were reported to show aberrant expression of FAA protein. Among five lymphoblst lines derived from Japanese FA patients, three lacked FAA protein expression, and one expressed a truncated form, whereas lymphoblasts from aplastic anemia patients showed normal FAA expression. These results suggest that group A FA patients are common in Japan. We are now genotyping FAA in these cells. In conclusion, the only FAC mutation detected in Japanese FA patients is IVS4, the so-called "Ashekenaji Jewish" mutation, and immunoblot analysis is a useful screening to detect FAA abnormalities. These findings may be useful for screening FA gene abnormalities not only in Japan but also in other Asian countries.

P-1183 DEMOGRAPHIC AND CLINICAL ASPECTS OF TYPE 1 GAUCHER DISEASE (GD) IN ARGENTINA

M.C. Aggio, V.V. Fernández, N. Chamoles. *Hospital Interzonal Jose Penna, Bahía Blanca, Fundación para el Estudio de las Enfermedades Neurometabólicas, Buenos Aires, Argentina*

As of December 1997, there were 103 cases of GD diagnosed in Argentina by enzymatic assay and/or genotype. Median age 17 years (range 1–57). Male/female ratio 1:1. Geographical distribution agrees with the distribution of the inhabitants along the country. Ethnicity: Italians 28.8%; Spaniards 30.7%; askhenazym Jews 11.9%; Arabians 1.9%; Saxons 1.9%; south American Indians (2 guaraníes, 1 mapuche), 6.9%; mestizos 17.9%.

Presenting symptoms were abdominal distension, either isolated or associated with pallor or fatigue (58%), hepatosplenomegaly (21%), and bruisability. Bone pain was infrequent. Haematology: pancytopenia in 39%, anaemia and trombocytopenia in 36%, isolated anaemia in 13%, and isolated thrombocytopenia in 6%. Isolated leukopenia was never observed. Normal values in 6%.

Clinical findings were: hepatosplenomegaly (50%), plus bone lesions (39%); isolated splenomegaly (6%), plus bone lesions 3%. Skeletal changes were found in the hip, either isolated (36.1%) or combined with femoral and tibial abnormalities. One patient had pulmonary hypertension. Most frequent mutations (from 31 unrelated patients) were N370S (46.8%) and RecNcil (21%).

Seventy three cases are on treatment with enzyme replacement; doses range from 2.5 U/kg three times a week up to 60 U/kg every two weeks.

Comment: Presentation reflects the heterogeneity of the disease; almost all ages and degrees of severity, as well as intrafamilial variations are present. Since the prevalence was lower than that expected for a multiracial country with 33 million inhabitants, it is probable that mild forms, mostly seen in older people, are underdiagnosed. Distribution and ethnicity closely reflect the composition of the Argentinean population, suggesting that the Gaucher mutations are proportionately distributed among the different racial groups and regions. As there are autochtonous south Americans Indians affected, uncommon and yet unknown mutations could have anthropological value.

Data were obtained through attending physicians and the National Gaucher Foundation.

P-1184 A NEW MUTATION IN AN OLD DISEASE OR HOW TO LIVE WITH X-LINKED AGAMMAGLOBULINEMIA

F. Staehelin[1], A. Meindl[2], T. Kühne[1]. [1]*University Children's Hospital, Basel, Switzerland;* [2]*Unit of pediatric genetics, University Hospital, Munich, Germany*

X-linked agammaglobulinemia was first described in 1952 by Bruton, and called "Bruton's disease". The X-linked gene defect was found in 1986 and located on chromosome Xq22, coding for Bruton's agammaglobulinemia tyrosine kinase (BTK). Till today at least 280 different mutations were found and registered in a database, the "BTKbase" at Helsinki. We present the description of the genotype with the phenotypic features of a now 6.5 year old boy with an excellent clinical course. The mutation was found in exon 9: nuc pos 810 del C. The boy is treated with monthly infusions of immunoglobulin G (IgG).

The diagnosis of agammaglobulinemia was made at the age of 7 months. The boy was admitted at hospital with fever for 10 days, cough and recurrent diarrhea. He was a full-term newborn baby, with a birth weight of 3300 g. Pregnancy and delivery were uneventful. Family history is negative. In the first months of life he presented with recurrent episodes of diarrhea and some infections of the upper airways.

Initial value of immunoglobulins were IgG 0.1 g/L, IgM 0.16 g/L and IgA 0.07 g/L. A transient neutropenia was observed with a normal bone marrow aspirate. Growth hormone was normal. After implantation of a Port-a-cath™-system, monthly infusions of 400 mg/kg IgG were initiated. In 1997 the above described mutation was found. Severe bacterial infections did not occur. Within 5 1/2 years, the boy was treated 9 times in total with oral antibiotics for otitis and infections of the upper airways. Psychomotor development and growth is regular. This newly described genotype reveals a mild course probably reflecting the significance of regularly administered infusions of IgG.

P-1185 A NEW TECHNIQUE FOR THE ISOLATION OF FETAL ERYTHROBLAST FROM MATERNAL BLOOD

G. Sitar[1], G. Mazur[2], L. Manenti[1]. [1]*Dept. of Internal Medicine and Oncology, University of Pavia, Italy;* [2]*Dept. of Haematology, Medical University, Wroclaw, Poland*

One of the goals of modern genetics is the developement of safe and reliable prenatal diagnostic test which does not constitute a risk to the fetus. Currently, the safety of available methods are limited by the necessity to obtain fetal tissue for analysis by invasive means, such as amniocentesis and chorionic villus sampling (CVS), which present a finite risk to the fetus. One potentially non-invasive approach for obtaining fetal material for diagnosis is the use of fetal cells in maternal circulation. The main obstacle to overcome is the rarity of fetal cells in maternal blood. Many investigators have explored fetal cell enrichement techniques and, so far, have concentrated on four types of fetal cells as target for enrichment. Nucleated red blood cells (NRBCs) are by far the most encouraging candidate cell types for enrichment. This approach first proposed by Bianchi et al and is based on the principle that NRBCs constitute a significant proportion of the red blood cells in fetal blood, but are very rare in peripheral adult blood. Indeed, circulating NRBCs comprise about 10% of the red blood cells in the 11 week old fetus and 0.5% in the 19 week old fetus.

We have isolated fetal erythroblasts from maternal peripheral blood using sequentially physical procedures followed immunomagnetic isolation by means of beads coated with specific antibody. Maternal blood samples of woman between 7 and 11 weeks of gestation undergoing termination of pregnancy were studied. From 25 ml of blood we obtain a cell suspension enriched in erythroblast between 30% and 90%. The total number of erytroblasts recovered was ranging between 200.000 and 1200000. To asses the fetal origin of these cells FISH was performed using chromosome-X and -Y specific centromeric probes. In all cases fetal sex were correctly determined when FISH results were compared to cytogenetic analysis of trofoblast recovered at the time of the determination of pregnancy. The total number of fetal erythroblasts recovered from 25 ml of blood were between 1000 and 2000, much higher compared to that obtained by all the other procedures till now experienced.

Red cell disorders

P-1186 A NOVEL APPROACH TO THE QUANTIFICATION OF ERYTHROCYTE AGGREGATION IN CLINICAL SITUATIONS

N. Meyerstein[1], D. Meyerstein[2,5], E. Katorza[1], D. Mankuta[3], D. Zilberman-Kravits[1], L. Hatzkelzon[4], A. Pribush[1,2]. [1]*The Dr. J. Kaufmann Hematology Laboratory, Physiology Dept., Faculty of Health Sciences;* [2]*Chemistry Dept., Faculty of Natural Sciences;* [3]*Depts. of Gynecology and Obstetrics and* [4]*Hematology, the Soroka University Medical Center, Ben-Gurion University of the Negev, Beer-Sheva;* [5] *the College of Judea and Samaria, Ariel, Israel*

Alterations in red blood cell (RBC) aggregation are one of the main reasons for hemorheological abnormalities in a broad spectrum of hematological and non-hematological diseases. A novel method based on dielectric properties of cells provides a unique opportunity to quantify this RBC property in whole blood of normal, preeclamptic pregnant women and patients suffering from diabetes mellitus (IDDM and NIDDM), multiple myeloma, β-thalassemia and iron deficiency. The blood admittances at a frequency of 0.5 MHz, measured 5 and 120 sec after the stoppage of shearing, characterize the kinetics of RBC aggregation and the steady-state size of the aggregates. Both indices indicate increased aggregation in the following order: control $\approx$ IDDM < β-thalassemia < iron deficiency < NIDDM < multiple myeloma. The differences between the mean values are statistically significant ($p \leq 0.01$). Larger alterations in the aggregation indices correspond to a bigger size of the aggregates in blood smears. The aggregation indices change gradually with the gestational age in both normal and pathological pregnancy. RBC aggregation in preeclampsia is considerably larger than that in normal pregnancy at the same gestational age. For NIDDM patients, no correlation was found between the aggregation indices and clinical severity of this disease. Investigations of the aggregation process in artificial media and measuring the levels of plasma constituents suggest that enhanced aggregation is caused by both alterations in plasma composition (pregnancy) and membrane transformations (diabetes). The sensitivity of the proposed method is higher than that of all the existing techniques. Thus, the results of this study demonstrate the ability of this sensitive method to detect abnormal RBC aggregation in different clinical situations.

P-1187 INFLUENCE OF ANTIOXIDANTS ON OXIDATIVE DAMAGE IN SENSITIVE RED BLOOD CELLS

M. Spivak, D. Mazor, N. Meyerstein. *The Dr. J. Kaufmann Hematology Laboratory, Physiology Department, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel*

Mature red blood cells (RBCs) are vulnerable to oxidative stress induced by oxidants. Antioxidants protect the cells from the deleterious effects of free radical attack. The aim was to repair oxidant-induced damage to RBCs by antioxidants and to avoid oxidant-induced damage by preincubation with antioxidants. Sensitive to oxidation RBCs from iron deficiency anemia (IDA) and from newborns, were treated with t-butyl hydroperoxide (BHP), before or after exposure to captopril or butylated hydroxytoluene (BHT). Following oxidation, IDA and newborn RBCs had high malondialdehyde (MDA) levels, 84.5±9.6 and 83.7±0.9 nmol/gHb respectively, and high Methemoglobin (Met) levels 21.4±2.8 and 19.1± 4.8%. Preincubation with BHT decreased MDA levels to 25.4±6.7 in newborn RBCs and to 12.05±1.7 in IDA. Preincubation with captopril decreased MDA levels to 48.3±1.6 and increased thiol levels from 0.4 ± 0.1 to 1.95±0.6 mM in newborn RBCs. The same preincubation decreased MDA levels to 46.9±10.0 and increased thiol levels from 0.7±0.1 to 2.4±0.2 mM in IDA cells. Captopril added after BHP, increased thiol levels. No change was observed in the levels of GSH, Met and MDA if BHT was added after the oxidation. Although IDA and newborn RBCs were more oxidation sensitive than control cells, preincubation with BHT and captopril attenuated membrane oxidative damage. Captopril prevented the decrease in thiol levels, when added before or after oxidation.

P-1188 EVALUATION OF THE BLUE FORMAZAN SPOT TEST FOR THE SCREENING OF GLUCOSE 6 PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY

A. Pujades[1], R. Zarza[1], R. Alvarez[1], E. Rull[1], S.M. Lewis[2], A.M. Salvati[3], H. Fujii[4], S. Miwa[4], J.L. Vives Corrons[1]. [1]*Haematology and Laboratory Departments from:* [2]*Hospital Clinic i Provincial, University of Barcelona, Spain;* [3]*Imperial College School of Medicine, Hammersmith Hospital, London, UK;* [4]*Instituto Superiore di Sanita, Roma, Italy; Okinaka Memorial Institute for Medical Research Tokyo Women's Medical College, Tokyo, Japan*

Glucose 6 phosphate dehydrogenate (G6PD, EC 1.1.1.49) deficiency is the most frequent enzymopathy in humans affecting more than 100 million people around the world. Inheritance is sex-linked as the enzyme is controlled by one gene locus in the X chromosome.

Screening tests for G6PD deficiency depend upon the inability of cells from deficient subjects to convert an oxidized substrate to a reduced state. Whereas enzyme deficiency in hemizygous (male) or homozygous (female) individuals may be readily detected by these tests, it is more difficult to detect heterozygous (female) carriers. Several screening tests have been so far reported and a standardized method was proposed by the International Council for Standardization in Haematology (ICSH). Which screening test is used in any particular laboratory will depend upon the number of factors such as cost, time required, temperature, humidity and availability of reagents.

In 71 cases (50 haematologically normal volunteers, 9 hemizygous G6PD deficient males and 12 heterozygous deficient females) a comparison between the blue formazan spot test (BFST) and two other screening tests for G6PD deficiency: the blue methylene reduction test (BMRT) and the fluorescent spot test (FST), has been performed. In all cases, the result obtained with the three screening tests was correlated with the enzyme activity assayed spectrophotometrically. In *hemizygous G6PD deficient males* all cases were equally detected with the three methods: BFST (4.7–6.64. Controls: 11.1–13.4), BMRT (score 3 in all 9 cases) and FST (no fluorescence in 9 cases). In *heterozygous G6PD deficient females*, two methods allowed to detect 7 out of 12 cases (BFST: 8.71–11.75. Controls: 11.1–13.4; BMRT: score 3 in 7 cases) whereas the FST missed all the 12 cases which presented a variable degree of fluorescence.

In conclusion, the three G6PD deficiency screening tests here compared appear to be equally useful for the detection of G6PD deficient hemizygotes (males). For heterozygote carriers (females), however, whereas BFST and BMRT allowed to detect 7 out of 12 cases, no case was detected with FST. Is must be noted that although the sensitivity for G6PD deficient carrier detection is the same for the BMRT and the BFST, the latter has advantage because it is semi-quantitative and not merely qualitative as the former. Unfortunately, none of the three screening tests compared here allowed the detection of the 100% heterozygote carrier state of G6PD deficiency.

Acknowledgements: Supported in part by Grants SGR. Generalitat de Catalunya and FIS-96/0764 Spanish Ministry of Health.

P-1189 HEREDITARY NONSPHEROCYTIC HEMOLYTIC ANEMIA DUE TO RED BLOOD CELL GLUTATHIONE SYNTHETASE DEFICIENCY. CLINICAL AND BIOLOGICAL FINDINGS IN FOUR UNRELATED SPANISH PATIENTS

R. Alvarez[1], R. Zarza[1], A. Pujades[1], J. Estella[2], E. Oliva[1], G. Lasheras[4], M. Callis[5], A. Ribes[6], E. Beutler[7], J.L. Vives Corrons[1]. [1]*Haematology Units and Red Cell Pathology Laboratories from:* [2]*Hospital Clinic i Provincial, Barcelona;* [3]*Hospital Sant Joan de Déu, Barcelona;* [4]*Hospital Verge del Monte Toro, Maó;* [5]*Hospital de San Juan de Dios, Martorell;* [6]*Hospital General de Catalunya, Sant Cugat del Valles;* [7]*Institut de Bioquímica Clinica, IDIBAPS, Barcelona, Spain; Department of Molecular and Experimental Medicine, The Scripps Institute, La Jolla CA, USA*

In four unrelated patients with chronic haemolysis and markedly reduced red blood cell (RBC) glutathione (49.5%, 12.6%, 11.5% and 15% respectively) a severe glutathione synthetase (GSH-S, EC 6.3.2.3) deficiency was found. One case presented a neonatal haemolytic anaemia associated with oxoprolinuria, but without neurological manifestations. The family study revealed GSH-S activity in both parents to be around half the normal level, a finding consistent with the presumed autosomal recessive mode of inheritance of this enzymopathy. Two cases exhibited a well-compensated haemolytic syndrome without anaemia or splenomegaly at steady state. One of these cases was diagnosed after an episode of acute haemolytic anaemia after fava bean ingestion. The remaining patient suffered from moderate to severe chronic nonspherocytic haemolytic anaemia (CNSHA) and splenomegaly, and required occasional blood transfusion for a haemolytic crisis associated with drug ingestion. In this patient the anaemia was corrected completely after splenectomy. In addition to GSH-S, a panel of 16 other RBC enzyme activities was also studied in all the patients. Hexokinase, aldolase, glucose-6-phosphate dehydrogenase and pyruvate kinase activities were all increased; as expected given the rise in the number of circulating reticulocytes. In two patients, the incubation of RBCs with hydrogen peroxide (H_2O_2) revealed an enhanced production of malonyldialdehyde (MDA).

Acknowledgements: Supported in part by Grants SGR. Generalitat de Catalunya and FIS-96/0764 Spanish Ministry of Health.

P-1190 ERYTHROCYTE GLUCOSE -6- PHOSPHATE DEHYDROGENASE DEFICIENCY IN ANTALYA, TURKEY: A STUDY ON THE 563 MUTATION OF THE G6PD GENE

H. Bağcı[1], D. Canatan[2], S. Samakoğlu[1], C. Çam[1], Ö. Güzeloğlu[1], S. Gümüşlü[3], G. Bağcı[1], N. Temimhan[2]. [1]*Departments of Genetics and* [3]*Biochemistry, Faculty of Medicine, Akdeniz University; Research and Treatment Center for Inherited Blood Diseases;* [2]*State Hospital of Antalya, Antalya, Turkey*

Studies on the mutation 563^T of the glucose-6-phosphate dehydrogenase (G6PD) gene in Antalya, a coast city in the Mediterranean region of Turkey, were performed in 20 families affected with G6PD deficiency classified - according to WHO - as group 2 G6PD deficiency referred by state hospital with the clinical manifestations of acute haemolytic anemia corresponded to favizm.

Mutation was determined by restriction enzyme (Mbo II) analysis of PCR amplified DNA which was isolated from the blood samples of deficient individuals.

This point mutation (563^T) was found in all families and Turkish origin. It is postulated that at least part of the Mediterranean populations has the same genetical defect of G6PD gene in our region.

P-1191 THE PREVANCE OF G6PD VARIANTS IN A POPLATION OF JORDANIAN BEDOUINS

M.M. Babiker, N.A. Bashir, J.M. Abu-Ayshih. *Department of Biological, Sciences, AL al-Bayt University, Jordan*

The Prevalence of the different variants of G6PD was studied in a relatively ethnically pure Bedouin population in the north eastern semidesert of Jordan. The study comprised about 520 school children (age: 7–16 years) from 32 villages. Laboratory investigations included routine screening tests, activity measurement and Kinetic evaluations.

The B+ variant of G6PD showed the highest prevalence (87.8%) while the African variant expressed in only 0.57% of the population. Different Mediterranean-like variants contributed about 7.62% to the enzyme deficiency in the population. Two new low-activity variants (−1 and −2) were characterized with an activity of 3% and 6% of the normal enzyme. Variant (−1) showed a higher (110%) electrophoretic mobility than the normal enzyme. Both variants shared low Km for G6P (18.91 and 14.8 uM but whereas the value of Km for NADP was low (1.17 uM) for variant −1 was relatively high (120.82 uM) for the other variant (−2).

P-1192 THE STUDY OF PIRUVATE KINASE DEFICIENCY IN VENEZUELA DURING 20 YEARS

Aixa Müller, Gisela Méndez, Benita Ponte, Andres Soyano, Alberto E. Soyano. *Instituto de Oncología y Hematología MSAS-UCV, I.V.I.C, Clinica El Avila, Caracas, Venezuela*

The deficiency of PK produces congenital hemolytic anemia. This paper shows the hematological, biochemical and immunological STUDY of 35 CASES with PK deficiency detected among 2089 patients with congenital hemolytic anemia and the quantification of PK done to different Venezuelan population to try to get the origin of the PK deficiency in Venezuela. A comparison has been made between the human PK and the PK from dogs with PK deficiency.

Méthods: Hematology, indirect bilirubin, Iron levels, ferritin, quantification of glycolytic and G-6-PD RBC enzymes, purificatión and characterization of residual PK (Vmax, Km, pH, heat stability test), ATP, RBC Na^+ y K^+, detection of lymphoid antigens with monoclonal antibodies by flow cytometry (CD7, CD4, CD8, CD10, CD19, CD20), IgG, IGM, IgA, CH50, C3, C4.

Results: The PK deficiency was detected in 35 patients from 9 families. The patients had jaundice, splenomegaly, and 5 cases from 3 families showed leg ulcers. Hb: 7–10 g/dl, Hto: 23–25.5%, reticulocytes: 11–57%, indirect bilirubin: 4–7%, serum iron: 128–209.4 (VN: 60–160 µg/dl), ferritin: 323–1650 (VN: 6–10 ng/ml). The PK RBC levels in the deficient patients were 30–40% of normal levels. The residual PK from the leg ulcer patients showed an enzyme no allosteric with increased Km for ADP. The PK from the dog homozygous for PK deficiency showed 42% of the normal RBC levels and the residual dog PK showed heat instability, no allosteric, with increased Km for the ADP and PEP. The intraerythrocytic cation composition of our pts were Na: 15±1.9 meq/10^{13} RBC. (VN: 9±2.5), K: 119± 4.9 meq/10^{13} RBC. (VN: 97.2±7.7). The PK deficient patients showed normal levels of IgG, IgA, IgM, CH50, C3, C4, linphocytes B and T. Deficiency of PK was not found in Venezuelan indians or negroid population. It was detected in population of caucasian origin

Conclusión: Deficiency of PK in Venezuela is uncommon; only 35 patients from 9 families has been detected in 20 years. Three families had a residual PK with heat inestability, no allosteric, Km increased to ADP. It was classified as a variant of PK and it could be compared from a kinetic point of view with the residual PK found in dogs with PK deficiency. There was no changes in the humoral and cellular immunity parameters evaluated in this PK deficient patients..

P-1193 ON CONNECTION OF IRON, LIPID PEROXIDATION AND HEMOGLOBIN DERIVATIVES IN ANAEMIA PATIENTS

M.Y. Anoshina, O.V. Moshynsky, M.V. Yagovdick, N.N. Tretiak, A.F. Romanova. *Research Institute of Hematology & Blood Transfusion, Kiev, Ukraine*

It has been found out that serum iron (SI) and coefficient of transferrin saturation with iron decrease and blood serum total and latent ironbinding ability increase in patients with hemoglobin concentration below 110 g/l are accompanied by lipid peroxidation (LPO) processes activation. The activation is mainly due to neutral LPO both in erythrocytes and in blood plasma. Even in iron latent deficiency LPO processes, especially in erythrocytes ($r = -0.647$, $P = 0.002$), are disturbed significantly. Hemoglobin derivatives take part in enhancement of LPO processes. Methemoglobin activates mainly erythrocyte phospholipid peroxidation ($r = 0.586$, $P = 0.004$) and carboxyhemoglobin participates in activation of erythrocyte neutral LPO ($r = 0.438$, $P = 0.018$). We suggest that compensator mechanisms support balance of organism supply with energy production substrates at oxygen homeostasis damage at insignificant decreasing of SI concentration. Further decrease of SI concentration is accompanied by accumulation of LPO toxic molecular products. It is the proof of compensator mechanism disturbance, first of all blood antioxydant system.

P-1194 RED CELL GLUTATHIONE METABOLISM AND SERUM BILIRUBIN IN IDIOSYNCRASIES DUE TO OXIDATIVE STRESS

G. Pelissero, M. Buratti, F. Barraco, A. Zanella, A. Colombi. *Divisione di Ematologia, IRCCS Ospedale Maggiore, Milano; Occupational Health Institute, University of Milan; Istituti Clinici di Perfezionamento, Milano, Italy*

Chemical idiosyncrasy refers to an abnormal subjective reactivity to environmental chemicals or drugs. Individual intolerance to certain drugs may be explained by subject difference in the availability of glutathione (GSH), which is known as responsible for cellular protection against oxidative injury and detoxification of drugs. Actually decreased levels of red cell GSH content and/or increased levels of serum bilirubin (HBIL) has been observed in subjects with increased susceptibility to drugs or oxidative stress related diseases.

In order to understand the relationship between cellular GSH alteration and HBIL, blood GSH levels were measured, according to Elmann, in 94 subjects (31 hemolytic patients and 63 healthy blood donors), divided in 3 groups [patients with HBIL (Pt), n = 31 (Bil. = 3.41±1.9 mg/dL); HBIL blood donors (Dh), n = 21 (Bil. = 1.87±0.7 mg/dL); control donors (Dn), n = 42 (Bil. = 0.47±0.2 mg/dL)]. Moreover hematological and biochemical studies, including the most important enzymes of red cell energetic metabolism and GSH biosynthesis and the analysis of red cell membrane proteins, were performed on 4 patients of group (Pt) with hemolytic anemia of probable idiosyncratic nature.

Red cell GSH values, expressed as µg GSH/mg Hb, were 1.75±0.36 in group (Pt), 2.05±0.36 in group (Dh) and 2.30±0.25 in group (Dn). No correlation was found in any studied group between bilirubin and GSH. Ratios between subjects with abnormally low and normal red cell GSH content, in HBIL groups, were respectively 15/31 in group (Pt) and 5/21 in group (Dh). On the contrary no alterations of GSH values were found in subjects with normal bilirubinemia (Dn). In hemolytic patients (4 subjects) with suspected drug intolerance, GSH values were all below reference range. In one case the activity of glutathione synthetase was 0.21 UI/g Hb, a border line value at the lower reference range.

Because hyperbilirubinemia appears largely associated with red cell GSH deficiency and GSH measurement is not yet performed as a routine clinical test, bilirubin values could thus be profitably adopted in discriminating subjects which have to be screened for individual susceptibility to oxidative stress by means of the study of GSH metabolism.

P-1195 ALTERATIONS IN ENERGY METABOLISM OF RAT RED BLOOD CELLS INFLUENCED BY ISOSORB DINITRATE

S.D. Maletić, Lj.M. Dragićević-Đoković, M.M. Kostić. *Institute of Physiology, Faculty of Medicine, University of Kragujevac, Kragujevac, Yugoslavia*

Effects of isosorb dinitrate (ISDN), a nitric oxide (NO) donor, on energy metabolism of rat red blood cells (erythrocytes and reticulocytes) were studied. Therefore suspensions of rat erythrocytes, either reticulocyte rich red blood cell suspensions containing 60–90% of reticulocytes, were aerobically incubated without (control) or in the presence of different concentrations (0.1, 0.25, 0.5, 1.0 and 1.5 mmol/l) of ISDN.

Total, coupled and uncoupled oxygen consumption in reticulocyte rich suspensions amounted to 16.77±1.66, 14.65±0.93 and 3.09±0.56 µmol/h/ml reticulocytes, respectively. ISDN inhibited total ($p < 0.05$) and coupled ($p < 0.001$), while increased uncoupled ($p < 0.05$) oxygen consumption in dose-dependent manner. Reduction of coupled respiration was accompanied with stimulation of glycolytic rate for 2.3-fold ($p < 0.01$), as measured by lactate accumulation and glucose consumption. Stimulation of glycolysis was consequence of increased activity of HK-PFK and PK enzymatic system. ATP, ADP and AMP levels were not altering significantly under influence of ISDN ($p > 0.05$). Calculated mean ATP-turnover time was prolonged for 4-fold in the presence of 1.5 mmol/l ISDN, which indicates an inhibition of ATP-consuming processes in ISDN-treated reticulocytes.

In mature erythrocytes ISDN induced no significant alterations of glycolysis and adenine nucleotides levels ($p > 0.05$).

Metabolic effects of ISDN were not mimic by exogenous 8-Br-cGMP, except the slight, dose independent inhibition of OxP ($p < 0.05$ in the presence of 1.0 mmol/l 8-Br-cGMP) accompanied by no changes of glycolytic rate ($p > 0.05$). These data indicate that ISDN induced: a) inhibition of coupled respiration in reticulocytes and b) stimulation of glycolysis in reticulocytes are mediated by NO as an effector molecule, but not by cGMP.

P-1196 EFFECTS OF UBIQUINONE ON THE ANTIOXIDANT AND METABOLIC STATUS IN THE BLOOD OF THE CADMIUM EXPOSED RATS

Lj.M. Dragićević-Đoković, B.I. Ognjanović[1], S.Z. Pavlović[1], S.D. Maletić, A.Š. Štajn[1], R.V. Žikić[1], M.M. Kostić. *Institute of Physiology, Faculty of Medicine; [1]Institute of Biology, Faculty of Sciences, University of Kragujevac, Kragujevac, Yugoslavia*

To examine effects of acute exposure to exogenous cadmium (Cd) on the blood antioxidant defense system (AOS) and metabolic status, and the possible protective role of ubiquinone (UQ), 2 months old male Wistar rats were exposed to 20 mg/kg b.m. of UQ (i.m., 48 hours before the sacrificing), 0.4 mg/kg b.m. of Cd (i.p., 24 hours before the sacrificing), or to the same dosages of UQ (48 hours before the sacrificing) + Cd (i.p., 24 hours before the sacrificing) simultaneously.

Activities of superoxid dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) were significantly increased in red blood cells of Cd-and UQ-treated rats ($p < 0.05$ or less). In Cd + UQ-cotreated rats, red blood cells activities SOD and GR were the same as in controls, whereas CAT and GSH-Px activities were exceeded normal values ($p < 0.05$). Concentration of reduced glutathione was significantly increased in red blood cells of Cd-treated rats, while in UQ and Cd + UQ-treated rats was significantly decreased. These data indicate that UQ only partially improves the AOS.

Blood concentration of glucose (3.926±0.140 μmol/ml blood) was significantly decreased in Cd-treated rats ($p < 0.01$), significantly increased in UQ-treated rats ($p < 0.02$), while in Cd + UQ-cotreated rats was the same to control values ($p > 0.05$). Lactate level was not changed during treatment. ATP level (0.407±0.020 μmol/ml blood) significantly decreased in UQ-and Cd + UQ-cotreated rats ($p < 0.05$). This indicates that acute exposure of rats to Cd, UQ and Cd + UQ were only partially altered metabolic status of the blood.

P-1197 GILBERT'S SYNDROME AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY INTERACTION IS CRUCIAL FOR BILIRUBIN LEVELS

M.D. Cappellini, M. Sampietro, F. Martinez Di Montemuros, L. Lupica, D. Tavazzi, G. Fiorelli. *[1]Centro Anemie Congenite, Ospedale Maggiore Policlinico IRCCS, Dipartimento di Medicina Interna, Università di Milano, Italy*

The pathophysiology of neonatal jaundice in G6PD-deficient newborns as well as of different bilirubin levels in adult G6PD-deficient subjects carrying the same G6PD mutation was extensively debated involving the environment as a variable influencing the phenotype. Recently, Kaplan et al. demonstrated that the TA repeat promoter polymorphism in the gene of diphosphoglucuronate glucoronosyltransferase (UGT) associated with the mild form of Gilbert's syndrome coinherited with the Mediterranean G6PD deficiency is crucial to neonatal hyperbilirubinemia. A similar observation was reported by Sampietro et al. considering the bilirubin levels in G6PD adult deficient subjects carrying WHO class 2 variants (out of acute crisis) with different UGT1A genotypes. Here we present two subjects with G6PD Union (1360 C — T) one of whom was homozygous for the variant UGT1A allele and the other was normal homozygous, and one more subject with G6PD Tokyo (1246 G — A) homozygous for the UGT1A variant genotype. This subject has a steady-state bilirubin level of 6.0±1.5 mg/dl reaching values of 76.0 mg/dl during hemolytic crisis. G6PD molecular defect was identified by PCR amplification of exons 10–11 followed by endonuclease digestion with Fsp I. The variant promoter of UGT1A was detected by PCR amplification of a 71–73 bp sequence encompassing the TATA box followed by high-resolution polyacrilamide gel electrophoresis (PAGE). Data are summarized in Table 1.

A significant difference in bilirubin level between the two subjects with G6PD Union and different UGT1A genotypes was observed ($p < 0.001$). These observations furtherly support the hypothesis that interaction between benign genetic polymorphisms could be responsible for relevant clinical phenotypes.

Table 1

Case	G6PD variant	G6PD activity (% of N)	UGT1A genotype	Bilirubin (mg/dl)	Ret. count (%)
1	Union (1360C → T)	3.2	$(TA)_7/(TA)_7$	6.5±2.7 (3.0–10.6)	2.1±1.8
2	Union (1360C → T)	0.8	$(TA)_6/(TA)_6$	1.5±0.2 (1.4–1.7)	1.5±0.3
3	Tokyo (1246G → A)	3.3	$(TA)_7/(TA)_7$	6.0±1.5 (3.4–8.4)	1.0±0.1

P-1198 EFFECT OF "PHYSIOLOGICAL" CONDITIONS ON G6PD AND 6-PGD ACTIVITIES AND AFFINITIES FOR THEIR SUBSTRATES

A.A. Leite[1], O.C. de O. Barretto[2]. *[1]FCF-UNESP, Araraquara; [2]Faculdade Medicina USP, LIM-27, Sao Paulo, Brazil*

The standard methods used for G6PD and 6-PGD activity assay and affinity determination for their physiological substracts have been carried out in reagent systems which present 145 mOs at pH 8.0. The study aimed to assess their activities and affinities in nearly physiological conditions regarding osmolarity, cation concentrations, pH and temperature. The blood was collected in ACD, filtered in microgranular and alfa-cellulose columns; the red cells were lysed and the enzymatic assays performed according to standard methods (WHO, 1967; Beutler, 1968). The "physiological" reagent system was as follows: tris-HCl 250 mM, pH 7.34, 3 MM $MgCl_2$, 0.2 mM NADP, which together with hemolysate and substrate gave a final 290 mOs osmolarity with ionic strength I = 0.188, at 37°C.

	mOs	I	G6PD activity [1]	G6PD K_m for G6P [π]	6-PGD activity [1]	6-PGD K_m for 6-PG [π]
G6PD type B						
WHO	145	0.086	12.6± 1.32	47.6± 2.58*	8.8± 0.94	32.5± 5.65
"Physiological"	90	0.188	11.3± 1.19	95.1± 10.3**	8.4± 1.03	41.3± 5.44
G6PD type A						
WHO	145	0.086	1.4± 0.37	50.0± 1.33*	.8±0.94	28.0± 4.22
"Physiological"	290	0.188	1.1± 0.14	95.4± 10.5**	10.7± 1.09	43.2± 3.74

*25°C **37°C [1]UI.g Hb^{-1} [π] μM.L^{-1}.

Other authors have focused on the effect of isolated factors on G6PD activity and affinity (Luzzatto, 1973; Yoshida, 1980). The study was planned to investigate how they would work at near "physiological" conditions. According to our results, the G6PD B, the G6PD A^- and the 6-PGD activities as well as their affinities obtained at "physiological" conditions decreased significantly when compared to the standard conditions. G6PD Km increases ±100% and 6-PGD Km increases 30%. These findings suggest that the higher "physiological" ionic strength and temperature (37°C) in fact reflects the actual pentose shunt rate and probably the NADPH yield, giving us a better idea of what happens in the red cells if compared to the the standard methods.

P-1199 DIVALENT CATIONS CALCIUM AND MANGANESE ACTION UPON THE ERYTHROCYTE MEMBRANE JUNCTIONAL COMPLEX

T.M.D. Medeiros[1], O.C. de O. Barretto[2]. [1]*Universidade Federal do Rio Grande do Norte, Natal;* [2]*Faculdade de Medicina da U.S.P., LIM-27, Sao Paulo, Brazil*

It is known that calpain I (EC 3.4.22.17) is a red cell protease which acts selectively upon spectrins and protein 4.1, and is activated by 0.05 mM calcium. Although its action may be observed on purified protein 4.1, this activity is not found when the hemolysate containing the calpain I with 0.05 mM calcium is incubated with crude membrane preparations, hinting there is some protection against proteolysis probably carried out by surrounding proteins. Protein 4.1 is part of the erythrocyte membrane junctional complex which also comprises actin, aducin, band 4.9, besides spectrins and glycophorin C edges. Membrane preparations were obtained from erythrocytes cleared from leucocytes contamination by microgranular and alfa-celullose columns filtration, washed in buffered saline pH 7.4 at 4°C and lysed 1:20 in 5 mM phosphate buffer pH 7.4 thereafter. Two sets of experiments were done:

A. with calcium-aliquots of hemolysate containing ghosts in suspension were incubated with increasing Ca^{2+} chloride concentrations from 0.05 mM to 2.0 mM and 0.18 mM chloramphenicol for 1 hour at 37°C.

B. with manganese-aliquots of hemolysate containing ghosts in suspension were incubated with 0.05 mM Ca^{2+} chloride in all tubes (in order to activate calpain I) plus increasing Mn^{2+} chloride concentrations from 0.05 mM to 2.0 mM and 0.18 mM chloramphenicol for 1 hour at 37° C. Subsequently the ghosts were washed and solubilized according to standard methods and submitted to SDS-PAGE at 10% polyacrylamide.

It was observed that at high concentrations of both divalent cations, calpain I is able to degrade the protein 4.1. This may indicate that the divalent cations induced a disruption of the junctional complex structure by breaking intermolecular bonds, exposing the band 4.1 to the protease action. This work was supported by CNPq, Brazil.

P-1200 LOCALIZATION OF THE GENE FOR CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE I TO A LESS THAN 1cM ON CHROMOSOME 15q15.1–5.3

H. Tamary[1,2], L. Shalmon[2], H. Shalev[3], D. Dobrushin[2], M. Zoldan[1], P. Resnitzky[4], D. Shaft[4], M. Korostishevsky[5], B. Bonne-Tamir[5], R. Zaizov[1,2]. [1]*Ped. Hematol/Oncol, Schneider Children's Medical Center of Israel;* [2]*Felsenstein Research Institute, Petah Tikva;* [3]*Soroka Medical Center, Beer Sheva;* [4]*Efrati Research Institute, Kaplan Hospital, Rehovot;* [5]*Human Genetics, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel*

Congenital dyserythropoietic anemias (CDA) are a rare group of red cell disorders of unknown etiology. Since the genetic basis for CDA type I is not evident and as there is no known candidate gene for the disorder, we used homozygosity and linkage mapping to localize the genetic defect responsible for CDA type I in 25 Bedouins from 4 large consanguineous families. We report the linkage of this disease to markers on chromosome 15 (located at q15.1–q15.3). Fourteen markers (D15S129–D15S161) within a 12 cM interval were typed in the relevant family members. Nine of the markers yielded maximum load scores of 1.625 to 12.928 at $\theta = 0.00$. Haplotype analysis revealed 8 different haplotypes and highlighted the existence of a founder haplotype. Identification of historical crossover events further narrowed down the gene location to between D15S779 and D15S778. The data suggest localization of the CDA type I gene within a 0.5 cM interval. Sequence analysis of the coding region of protein 4.2 the only known erythroid specific gene in the locus, did not reveal any change in CDA type I patients. Further analysis of this locus may lead to the identification of a gene essential to normal erythropoiesis.

P-1201 CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE I IN COMBINATION WITH a-THALASSEMIA

M.H. Antonelou[1], I.S. Papassideri[1], F. Karababa[2], M. Gyparaki[2], A. Loutradi[2], L.H. Margaritis[1]. [1]*University of Athens, Department of Biology, Division of Cell Biology and Biophysics;* [2]*LAIKON General Hospital, Center of Thalassemias, Unit of Prenatal Diagnosis, Athens, Greece*

The case of a 36 years old woman is reported, who developed clinical symptoms of icterus and splenomegaly early in childhood, as well as severe anemia (Hct:19%) during pregnancy, treated with numerous transfusions. Hematological data were slightly below the normal values (Hb = 8.7–10.4%, Hct = 30–34%). After physical examination which revealed subicterus conjuctiva and palpable spleen (8 cm below the costal margin), the propositus has been splenectomized. CDA type I was diagnosed on the basis of the following features: binucleate erythroblasts (mostly basophilic and polychromatic) with internuclear chromatin bridges and spongy appearance of the nucleus. Based on the absence of "double membranes" and on the fact that the Ham's test was negative, CDA type II was ruled out. On the other hand, contrary to typical cases of CDA type I, protein sediments were detected in the erythroblast cytoplasm. The isolation and analysis of the red cell ghosts proteins with SDS-PAGE revealed deviations from the normal profile, which do not characterize a typical case of either CDA type. In particular, five abnormal proteins of high MW and several minor ones, which are not the products of exogenous proteolysis, were present and spectrin and band-3 (anionexchanger) were apparently decreased. In addition, a hemoglobin sediment was observed that trapped non-selectively quantities of all the membrane proteins, and therefore cannot account for the above mentioned diminished proteins. After separation of the membrane from its skeletal components, the abnormal high MW proteins were found in the membrane fraction. DNA analysis in the a-globin gene region revealed the A → G mutation in the poly-A site of the a2-glogin gene, in homozygosity (ATA AAG → ATA AGG), although b-chains tetramers (HbH) which are typical of this genotype, were not detected. The correlation of this specific genotype with the clinical manifestations of CDA and the abnormal features of the red blood cell membrane is studied, in the propositus and her relatives.

P-1202 TWO CASES OF ATYPICAL CONGENITAL DYSERYTHROPOIETIC ANEMIA (TYPE II) PRESENTING WITH THE LABORATORY FEATURES OF HEREDITARY SPHEROCYTOSIS

P. Bianchi, G. Pelissero, E. Bredi, M. Zappa, C. Vercellati, C. Boschetti, F. Morelati, A. Iurlo, D. Soligo, A. Zanella. *Divisione di Ematologia, Centro Trasfusionale and Centro Trapianti di Midollo, IRCCS Ospedale Maggiore di Milano, Milan, Italy*

Congenital dyserythropoietic anemias (CDA) are a heterogeneous group of disorders characterized by ineffective erythropoiesis with prominent dysplastic features of the erythroid precursors in the bone marrow. Three main subtypes have been described, but variants or atypical forms have been reported over the years.

This report describes the clinical, biochemical, and molecular data of two patients with atypical CDA type II with repeatedly negative acidified serum (Ham's) test, presenting with the laboratory features of hereditary spherocytosis (HS). The patients were referred to us with a diagnosis of HS for membrane biochemical studies. Both displayed moderate anemia, reticulocytosis consistent with the degree of anemia, increased unconjugated bilirubin, presence of spherocytes in peripheral blood in the absence of erythroblasts, and decreased osmotic fragility. Screening tests for abnormal or unstable hemoglobins and direct Coombs test were negative. The Ham's test was negative in two occasions, both using autologous serum and serum samples from 50 blood donors. The study of the red cell membrane proteins by SDS-PAGE was normal except for a deglycosylated, thinner and slighthly faster band 3 as commonly found in CDA II. The repeated negativity of Ham's test and the HS-like hematologic pattern prompted us to sequence the cDNA of band 3 and glycophorin A (which is known to be involved in the glycosylation of band 3) in the hypothesis of a new type of red cell membrane defect, but no mutations were found. Western blot analysis of the red cell membrane revealed the presence of bands corresponding to GRP78, PDI and calreticulin, recently described as markers for CDA II (Alloisio et al, Blood, 87 4433–4439, 1996). Transmission electron microscopy performed on peripheral blood showed that about 10% of red cells in both patients displayed "double" cytoplasmic membrane. Bone marrow studies, performed in one patient only, revealed that 30% of erythroblasts were bi- or multi-nucleated, and displayed "double" cytoplasmic membrane when examined by transmission electron microscopy.

In conclusion two Ham's negative variants of CDA II mimicing mild HS are reported. These cases are misclassified using the routine hematological tests and can be detected only by biochemical studies.

P-1203 INCREASED ENDOTHELIN-1 LEVELS THROUGH INTERACTION OF RED BLOOD CELLS WITH ENDOTHELIAL CELLS

P. Quehenberger, A. Bierhaus, P. Fasching, W. Speiser, P.P. Nawroth. *Clinical Institute for Medical and Chemical Laboratory Diagnostics, University of Vienna, Austria; Medical Clinic I, University of Heidelberg, Germany*

Recently it was shown that the membrane of red blood cells (RBCs) undergoes non-enzymatic glycation. This formation of advanced glycation endproducts (AGE) is enhanced in patients with diabetes mellitus. In this study we evaluated the influence of RBCs of patients with non insulin dependent diabetes mellitus (NIDDM) on the expression of the potent vasoconstrictor Endothelin-1 (ET-1). Endothelial cells were incubated with RBCs of patients with NIDDM and ET-1 was measured in cell culture supernatants. ET-1 induction was depending on the patients glucose metabolism as RBCs of patients with HbA1c levels higher than 8.0 induced ET-1 synthesis more than two-fold compared with RBCs of patients with HbA1c levels at normal ranges ($p < 0.05$). Induction was significantly reduced through preincubation of endothelial cells with 0.1 μM antisense oligonucleotides against the endothelial receptor of AGEs, RAGE ($p < 0.0001$). This indicates that the observed effect was at least in part due to the interaction of non-enzymatic glycated proteins of the red blood cells membranes with endothelial cells. AGEs are known to mediate their cellular effects at least partly through activation of the transcription factor NF-κB. Northern blot and Nuclear Run On experiments demonstrated that ET-1 can be induced through NF-κB p50/p65 in a time and dose dependent manner. Chimeric constructs containing the 5′-promotor region of the ET-1 gene linked to a reporter luciferase gene were transiently transfected into bovine aortic endothelial cells. Using these reporter constructs, we demonstrated that plasmids overexpressing NF-κB p50/p65 are able to induce ET-1 promotor dependent transcription.

P-1204 TIME-COURSE EXPRESSION OF HUMAN BLOOD GROUP ANTIGENS DURING ERYTHROID DIFFERENTIATION

V. Bony[1], P. Gane[1], Carl G. Gahmberg[2], J.-P. Cartron[1], P. Bailly[1]. [1]*INSERM U76, Institut National de la Transfusion Sanguine, Paris, France;* [2]*University of Helsinki, Finland*

Time course expression of blood group antigens, as detected by flow cytometry with specific monoclonal antibodies, was examined using a two-phase liquid culture system that supports the proliferation and maturation of human erythroid progenitors. In phase I, peripheral blood mononucleated cells were grown for 6 days in the absence of Epo. During phase II, non adherent cells were further grown for 14 in the presence of Epo and erythroid maturation proceeded to reticulocytes and red cells.

Blood group-associated proteins with potential adhesive properties like LW/ICAM-4 and Lutheran (Lu) were not expressed on early erythroid progenitors (phase I) but their appeared later during the differentiation, after GPC (carrier of Gerbich antigens) and GPA (carrier of MN antigens). OK^a (M6 leucocyte activation antigen) appeared during phase I, but its expression was down regulated as ICAM-1, -2, -3 and the α4 chain of the β1 integrin VLA-4.

Flow cytometric analysis revealed that at day 3 of phase I, about 50% of erythroid cells already expressed K2, A and Rh50 antigens, whereas GPC and Fy6 antigens were detected at day 5 (20% positive cells). During phase II, between days 8 and 9, the developmental expression of RhD, LW^{ab} and Rhc antigens was correlated with GPA expression, suggesting that these antigens, like GPC and GPA appear at the mature CFU-E and proerythroblast stage, respectively. Other blood group antigens developed during the late phase of the culture between days 10 and 14 of phase II (erythroblast stage), notably the RhC and Lu^b antigens as well as the proteins encoding well characterized membrane transporters like the Kidd/urea transporter (Jk^3) and Band 3 (Di^b antigens). These data add new informations on the biogenesis of membrane proteins and antigens during the differentiation and maturation of the erythroid lineage.

P-1205 STRUCTURE OF THE KIDD/UREA TRANSPORTER GENE AND MOLECULAR GENETIC BASIS OF THE Jk_{NULL} PHENOTYPE

N. Lucien[1], F. Sidoux-Walter[1], B. Olivès[1], J. Moulds[2], P.-Y. Le Pennec[1], J.-P. Cartron[1], P. Bailly[1]. [1]*INSERM U76, INTS, Paris, France;* [2]*University of Texas-Houston, USA*

The Kidd (JK) blood group is carried by an integral membrane glycoprotein which transports the urea through the red cell membrane. The red cells from Jk_{null} individuals exhibit a selective defect of urea transport capacity and lack the Kidd/urea transporter protein *(Olives et al., J. Biol. Chem. 1994, 1995).*

To analyse the molecular genetic basis of the Jk_{null} phenotype, the exon-intron structure of the human blood group Kidd/urea transporter gene has been determined. It is organized into 11 exons distributed over 30 kb of DNA. The mature protein is encoded by exons 4 to 11. The transcription initiation site was identified by 5′ RACE-PCR at 335 bp upstream the translation start point located in exon 4. The 5′ flanking region, from nucleotide −837 to −336, contains TATA and inverted CAAT-boxes as well as GATA-1/SP1 erythroid-specific cis-acting regulatory elements. Analysis of the 3′-UT region reveals that the two equally abundant erythroid transcripts of 4.4 and 2.0 kb arise from usage of different alternative polyadenylation signals.

No obvious abnormality of the Kidd/urea transporter gene, including the 5′ and 3′-UT regions, has been detected by Southern blot analysis of two unrelated Jk_{null} individuals (B.S. and L.P.) genotyped as homozygous for a "silent" Jk^b allele. Further analysis indicated that different splice site mutations occurred in each variants. The first mutation affected the invariant G residue of the 3′ acceptor splice site of intron 5 (variant B.S.) while the second mutation affected the invariant G residue of the 5′ donor splice site of intron 7 (variant L.P.). Expression studies in *Xenopus* oocytes demonstrated that the truncated proteins encoded by the spliced transcripts did not mediate a facilitated urea transport as compared to the wild type Kidd/urea transporter protein and were not expressed on the oocyte's plasma membrane. These findings provide a rationale explanation to the lack of Kidd/urea transporter protein and defect in urea transport in Jk_{null} cells.

P-1206 INCREASED TYROSINE PHOSPHORYLATION OF BAND 3 IN AUTOIMMUNE HEMOLYTIC ANEMIA

M.L. Barjas-Castro, M.F. Locatelli, M.J.A. Saad, C.R.O. Carvalho, S.T.O. Saad. *Hematology and Hemotherapy Center/Department of Internal Medicine, University of Campinas, São Paulo, Brazil*

Acquired lesions of the red cell membrane following autoantibody to antigen interactions play a relevant role for erythrocyte clearance. Biochemical studies of red cell proteins showed quantitative defects involving band 3, protein 4.1 and protein 4.2. With the aim to investigate the *in vivo* tyrosine phosphorilation of band 3, we performed immunoblotting of intact red cell using anti-phosphotyrosine antibody in five patients with clinical autoimmune hemolytic anemia (AIHA) and five controls. Immunohematological investigation screening was carried out by direct antiglobulin reaction with polyspecific and monospecific reagents (performed using Gel test), elution by chloroform, glicine-EDTA cell treatment and panel of red cell. Four patients presented nonspecific IgG autoantibodies and one presented anti-e autoantibody. Fresh blood was collected for phosphorylation study without anticoagulant and immediately kept on boiling water with Laemmli solution. Samples were resolved by SDS-PAGE on 8% gels, transferred to nitrocellulose membrane, blocked 16 h in non fat dry milk and incubated overnight at 4°C with anti-phosphotyrosine antibodies. After washing, the membranes were incubated with ^{125}I-protein A and analyzed by autoradiography. Patients with AIHA presented between 2 and 3 folds tyrosine phosphorylation increase when compared to controls. The modifications of the erythrocyte membrane by autoantibody are an important theme in the attempt to understand the pathophysiology of membrane damage in AIHA. Senescent cell antigen, generated by the degradation of protein band 3, might play a role in the removal of red cells in clinical AIHA and, in this situation, band 3 might be modified to maintain functions. Probably no specific autoantibodies are responsible for the stimulation of band 3 tyrosine phosphorylation. In summary our results showed an increase of tyrosine phosphorilation of band 3 in AIHA.

P-1207 β-SPECTRIN S^{ta} BARBARA: A NOVEL FRAMESHIFT MUTATION OF THE β-SPECTRIN GENE ASSOCIATED WITH HEREDITARY SPHEROCYTOSIS

D.S. Bassères, D.L. Vicentim, S. Bordin, M.B. Melo, F.F. Costa, S.T.O. Saad. *Hemocentro, Unicamp, Campinas, Brazil*

Hereditary spherocytosis (HS) is a common inherited anemia characterized by the presence of spheroidal red cells and increased osmotic fragility of erythrocytes. We have studied a brazilian family with HS inherited in an autosomal dominant fashion. The propositus was a 25-year-old white woman of Italian origin, who presented moderate HS and a regular number of acanthocytes in the blood smear. Her hematological profile was: Hb 11.0 g/dl, RBC 3.48 $\times$ 10^{12}/l, MCV 88fl, MCHC 35.9 g/dl, reticulocyte count 696 $\times$ 10^{9}/l (20%). Densitometric scans of Coomassie blue stained SDS-PAGE of the propositus showed 11% reduction in spectrin content. Her brother, who also presented the defect and had already undergone splenectomy, presented 24% of spectrin deficiency and 17% of ankyrin deficiency. These findings pointed the β-spectrin gene as a likely candidate for being the mutated gene. In order to confirm this supposition we performed PCR amplification of all exons of the β-spectrin gene and performed, subsequently, SSCP analysis of all amplified products. Exon 14 showed a band shift when compared to a control. Direct sequencing of this exon showed a double band pattern in the patient compared to the control, indicating that the patient should be heterozygous for a frameshift mutation in this region. After cloning the patient's amplification product of exon 14, two sequences were recovered: one identical to the normal β-spectrin allele and one presenting a C deletion at codon 638 (B-spectrin S^{ta} Barbara). This deletion leads to premature termination after 31 aminoacids. In an attempt to detect the truncated protein encoded by this allele, we performed Western Blotting analysis of the proteins present in the membrane and cytoplasmic fractions of the patients' erythrocyte. No truncated proteins were detected using policlonal anti-β-spectrin antibodies in both fractions, leading to the hypothesis that this protein is not stable under physiological conditions or that the mutated mRNA is not stable. In either case the mutated allele is not expressed, leading to β-spectrin deficiency. However, the amount of β-spectrin deficiency observed is less than expected, suggesting that there may be a change in gene expression regulation in order to favor the translation from the normal allele.

P-1208 CONSERVATION OF LIPID ASYMMETRY IN CIRCULATING OLD RED CELLS

F.L.A. Willekens[1], H.J. Bos[2], B. Roerdinkholder[2], Y.A.M. Groenen[1], C. Reutelingsperger[3], G.C.V.D. Plas[2], J.M. Werre[2]. [1]*Rijnstate Hospital and* [2]*R.C. Blood Bank Arnhem;* [3]*Dept. of Biochemistry, University of Limburg, The Netherlands*

Introduction: Loss of lipid asymmetry in the membranes of nucleated cells by exposure of phosphatidylserine (PS) is thought to be a crucial part of the process of apoptosis of those cells. Red cells also lose the phospholipid asymmetry of their plasma membrane during incubation in vitro.

Aim of the Study: Is lipid asymmetry also lost in RBC's during their life span in vivo?

Methods: Red cells were separated in five fractions of different cell age (I–V) by a combination of percoll gradient- and counterflow centrifugation. FITC-labelled annexin V was used to detect PS in the outer layer of the red cell membranes by means of a flowcytometric technique. As marker of RBC age the percentage of HbA1c was used.

Results: In fractions I to V: 1) The HbA1c increased from 3.7±0.54% to 6.7±0.59% ($p < 0.016$), while in whole blood it was 5.2±0.35%; 2) The percentages of annexin V positive cells were respectively 0.26±0.17, 0.14±0.04, 0.15±0.08, 0.12±0.05 and 0.12±0.03 (n.s.), while in whole blood it was 0.11±0.04.

Conclusion: As in RBC fractions of different cell age the same small amount (±0.1%) of cells with loss of lipid asymmetry can be found, this loss apparently plays no role in the clearance of old red cells from the circulation. However, the possibility remains that other red cells with lipid asymmetry are so effectively removed from the circulation that they can not be detected by the methods used.

P-1209 LOSS OF ANTICOMPLEMENT ACTIVITY FROM CIRCULATING OLD RED CELLS

F.L.A. Willekens[1], H.J. Bos[2], B. Roerdinkholder[2], Y.A.M. Groenen[1], G.C.V.D. Plas[2], J.M. Werre[2]. [1]*Dept. Clin. Chem., Rijnstate Hospital;* [2]*Blood Bank, Arnhem, The Netherlands*

Introduction: Autoantibodies bound to altered band 3 of old red cells (RBC) are thought to play an important role in the clearance of these cells from the circulation. Enhancement of this process e.g. by a decrease of anticomplement activity is probably essential, as the absence of the anticomplement proteins CD55 and CD59 in PNH and the genetic deficiency of CD59 lead to complement haemolysis. Also these proteins are lost from the normal RBC by vesiculation. Therefore the Aim of the study was: Do old circulating RBC show a decrease in the number of the anticomplement proteins CD55 and CD59?

Methods: Red cells of six normal individuals were separated in five fractions of different cell age (I–V) by a combination of percoll gradient- and counterflow centrifugation. A triple colour flowcytometric technique was used to determine the mean fluorescence intensity (MFI) of CD55 and CD59, and of glycophorin A (GPA) as specific RBC marker. As marker of RBC age the HbA1c % was used. MCV and mean surface area (MSA) were measured by means of a Sysmex SE9000 and the rouleaux technique respectively.

Results: In fractions I to V: 1) The HbA1c increased from 3.7±0.54% to 6.7±0.59% ($p < 0.016$); 2) The MFI of CD55 and CD59 decreased by 11% and 8% (from 100% to 89±1.8% and 100% to 92±2.8%; $p < 0.016$) resp. while the MFI of GPA did not decrease; 3) The MCV decreased by 25% (from 96±2.4 to 73±0.4 fl; $p < 0.016$); the MSA decreased by 15% (from 156±3.8 to 132±5.5 mm^2; $p < 0.016$).

Conclusion: During red cell ageing the number of CD55 and CD59 molecules per cell decreases, but their surface density increases. Therefore, loss of anticomplement activity from red cells per se does not facilitate their future death.

P-1210 CLINICAL AND MOLECULAR EVALUATION OF NON-DOMINANT HEREDITARY SPHEROCYTOSIS (HS)

E. Miraglia Del Giudice, B. Nobili, G. D'Urzo, R. Ilardo, M. Francese, M.L. Conte, S.W. Eber, A. Iolascon, S. Perrotta. *The Departments of Pediatrics, Naples and Bari, Italy; Kinderklinic, Gottingen, Germany*

Seventy-five unrelated Italian HS children with normal parents have been investigated. Classification of the HS clinical forms (mild, moderate, moderately severe and severe) of the disease was carried out following well established criteria. Particularly, mild HS is associated with compensated hemolysis without anemia, whereas hemolysis is incompletely compensated in moderate HS. Patients with moderately severe disease have a more severe anemia with hemoglobin levels of 60 to 80 gm/L and reticulocytes 10% or higher, whereas those with severe HS show life-threatening anemia and are transfusion dependent until splenectomy. Irrespectively of the disease severity and of the type of membrane protein alteration these patients were screened for the presence of a-spectrin^{LEPRA}, a synthetically partially deficient a-spectrin allele, as well as for the −108 T → C mutation of the ankyrin promoter. They were also screened for the occurrence of ankyrin or β-spectrin *de novo* mutations.

Distribution of the clinical phenotypes was nearly similar to that observed in the group of the HS dominantly transmitted, with certain exception concerning severe form (i.e., 10% vs. 5%). In about one half of the patients we were able to detect the molecular alterations underlying HS and consequently to identify the true manner of inheritance. Five patients showed a genuinely recessive pattern of inheritance (i.e. 4 a-spectrin^{LEPRA} and one ankyrin −108 T → C mutation). *De novo* inactivation of one ankyrin allele was found in 23 patients (31%) and the *de novo* inactivation of one β-spectrin allele was found in 10 HS subjects (13%). Comparing clinical phenotypes with the relative genotypes we observed that patients with the lack of expression of one ankyrin or β-spectrin allele had HS ranging from mild to moderately severe, probably due to modulating factors occurring *in trans*. Four out of 8 patients with severe HS on the contrary showed a recessive pattern of inheritance.

P-1211 ARRO-I: A NEW PARTIAL D PHENOTYPE INVOLVING EXON 4 AND 5

M. Hemker[1,2], P.C. Ligthart[2], B.H.W. Faas[2], A.E.G.Kr. Von dem Borne[2], C.E. Van der Schoot[2], D.J. Van Rhenen[1], P.A. Maaskant-van Wijk[1]. [1]*Bloodbank Rotterdam, Rotterdam;* [2]*CLB, Amsterdam, The Netherlands*

The Rh blood group system, the most polymorphic system on red blood cells, is genetically controlled by two different but highly homologous genes on chromosome 1. *RHCE* encodes the different RhCcEe polypeptides and *RHD* codes for the D polypeptide. In the present study a new partial D antigen is identified and called ARRO -I. Serological analysis with monoclonal anti-D reagents showed the absence of epD 1, 2, 4 and 8 (nine-epitope model). Furthermore, red cells were C-E-c+e+ VS− and V+.

The molecular basis of this new partial D phenotype was studied using polymerase chain reaction (PCR) and partial sequence analysis of *RH* transcripts. By PCR analysis with *RHD*-specific multiplex PCR [1] the *RHD*-exons 3, 6, 7 and 9 could be amplified, but not the RHD exons 4 and 5. Furthermore, the amplification of intron 4 revealed the same sized products as from DNA of a normal D-donor, indicating the presence of *RHD* intron 4. So far cDNA sequencing showed only replacement of two *RHD*-specific nucleotides by *RHCE*-specific ones: C602G (T → R) and T667G (F → V) in exons 4 and 5.

The changes in exons 4 and 5 are probably based on point mutations, as intron 4 seemed to be derived from *RHD*. This could also be concluded from hybrid-PCR's and restriction analysis. Because *RHD* has only been sequenced from nt 480 till nt 748, we cannot exclude the presence of additional mutations.

(1) Maaskant-van Wijk et al. Blood, 1997; 90 Suppl.: 2099 p472a.

P-1212 THE DIFFERENTIAL DIAGNOSIS OF MEMBRANOPATHY AND ENZYME DEFICIENCY ANEMIAS BY LASER SCATTERING FLOW CYTOMETRY SYSTEMS

J.M. Jou, R. Brugués, E. Hernández, J. Seuma, M. Kinder, R. García, M. Corbella. *Servei d'Hemostàsia i Hemoteràpia, Hospital Clinic i Provincial, Universitat de Barcelona, Spain*

The hematology systems which work by laser scattering flow cytometry as Technicon H series (Bayer Diagnostics) provide a direct measurement of individual RBC hemoglobin concentration (dMCHC) and its standard deviation (HDW) which are determined by laser-beam measurement. Since Hb and MCV distribution curves have fixed thresholds, the percentage (%) of microcytic (MICRO, <60 fL), macrocytic (MACRO, >120 fL), hypochromic (HYPO, <280 g/L) and hyperchromic (HYPER, >400 g/L) RBCs are automatically calculated. Automated reticulocyte counting were performed by Sysmex R-1000 (TOA Instruments). In the present study we have identified and quantified abnormal RBC populations (MICRO, MACRO, HYPO and HYPER) in 216 patients: 70 hereditary spherocytosis (HS), 19 hereditary eliptocytosis (HE), 20 congenital xerocytosis (CX), 33 G6PD enzyme deficiency in men and 45 in women and 18 homozygous and 11 heterozygous PK deficiency. A control group of 75 healthy subjects was also included in the study. The most important findings were: 1.- In HS, increasing hyperchromic-normocytic RBCs (HYPER/NORMO) (12.4%) and MICRO (7.6%). Children showed a MVC lower (79 fL) than adults (87 fL). 2.- In HE and CX, increasing HYPER/NORMO (5.2 and 5.9%, respectively). The RDW, HDW and HYPER were more increased in HS than in CX. The HDW was increased in HE with normal reticulocyte counts. 3.- The heterozygous G6PD and homozygous PK deficiencies showed increasing MACRO/HYPO because of high reticulocyte counts. 4.- In G6PD deficiency in women and in heterozygous PK deficiency, reticulocyte counts showed good correlation (>0.77) with MACRO and HYPO but the same correlation was very bad (r > 0.20) in HS and CX.

In conclusion, the hyperchromic RBCs are normocytic in HS, CX and HE. The HS have more hyperchromic RBCs than CX and HE which have the same percentage. The macrocytic-hypochromic RBCs population seems to be reticulocytes in enzyme deficiency anemias and not in HS and CX.

P-1213 PHENOTYPIC EXPRESSION IN HEREDITARY SPHEROCYTOSIS AGGRAVATED BY COMBINED BAND 3 MUTATIONS (G714R, K56E & P854L/G130R)

Y. Yawata, A. Yawata, A. Kanzaki, M. Kaku, M. Takezono, T. Inoue, N. Okamoto. *Kawasaki Medical School, Kurashiki City, Japan*

It has been known that a patient with autosomal dominantly inherited hereditary spherocytosis (HS) with band 3 (B3) mutation has become extremely severe by the presence of additional B3 mutation(s). We have recently described such a trait (band 3 Okinawa) with a compound mutation (G714R, K56E & P854L/G130R) (A. Kanzaki, S. Hayette, et al: Brit J Haematol 99: 522–530, 1997). The proband showed blood transfusion-dependent severe hemolytic anemia. Although the migration and levels of spectrin (Sp), ankyrin (Ank), protein 4.1 (P4.1) and actin were normal on the SDS-PAGE gels, band 3 (B3) copies were moderately diminished (approx. 40% reduction on the SDS-PAGE gels, and 49.8% by cytofluorometry with eosine-5-maleimide). The most striking feature was that protein 4.2 (P4.2) was completely missing. Electron microscopy with the freeze fracture method demonstrated −33.8% reduction of the number of the intramembrane particles (IMPs: mostly B3) and marked oligomerization of the IMPs. The results were confirmed by the increased B3 oligomerization detected by HPLC analysis. The fluorescence recovery after photobleaching (FRAP) method also detected markedly increased lateral mobility and enhanced oligomerization, exactly mimicking those which we previously observed in the complete protein 4.2 deficiencies (P4.2 Nippon, P4.2 Shiga & P4.2 Komatsu). The rebinding assay demonstrated the impaired binding of normal P4.2 protein to mutated B3 in the patient. Electron microscopy demonstrated extremely marked disruption of cytoskeletal network with nearly no normal basic units. Therefore, in this patient, the major pathogenesis appears to reside in the moderately decreased B3 in addition to the total absence of P 4.2, which was induced by band 3 mutations. It should be noted that P4.2 has a critical role for maintaining normal cellular functions.

P-1214 ERYTHROCYTE CYTOSKELETON PROTEIN 4.2 DOES NOT SEEM TO BE A "HOUSE-KEEPING" PROTEIN

E.M. Guerra-Shinohara[1], O.C. de O. Barretto[2]. [1]*Instituto, AdolfoLutz;* [2]*Faculdade Medicina USP, LIM-27, Sao Paulo, Brazil*

In the course of an extensive investigation of membrane proteins of selected mammalian representatives, we focused on the protein 4.2, which has been considered to play an important role in cytoskeleton anchorage to the integral protein 3, together with ankyrin (band 2.1). The red cells were collected in ACD, washed in buffered saline, lysed in cold 5 mM phosphate buffer pH 8.0. The ghosts were washed, solubilized according to standard methods and applyed in SDS-PAGE, in 10% polyacrylamide and in exponential 3–17% polyacrylamide as well.

It was observed that the Rodentia representatives: *Cavia porcellus* (guinea pig) (n:14), *Myocaster coypus* (Southern Brazilian swamp big rat) (n:2), *Dasyprocta sp* (cutia) (n:4), as well as Perissodactyla *Equus caballus* (horse) (n:13) do not exhibit the protein 4.2. Interestingly, these studied animals do present high ankyrin concentration but the *Equus caballus*, which does not exhibit a sharp band although exhibit a difuse bunch of minor components between proteins 2 and 3, which could account for an ankyrin family.

Besides these findings, these Rodentia species do present the band 6, which does not occur in the other common Rodentia as *Raffus norvegicus* (white rat) (n:9) and *Mus musculus* (mouse) (n:12).

All these data together point to the suggestion that protein 4.2 is not a "housekeeping" protein, as its absence does not disrupt the cytoskeleton membrane. Physiologically its absence may be compensated by the. higher ankyrin concentrations observed. This work was supported by CNPq, Brazil.

P-1215 HEREDITARY ELLIPTOCYTOSIS ASSOCIATED WITH α AND β SPECTRIN DEFECTS: A BIOCHEMICAL STUDY OF 25 CASES

P. Caprari[1], A. Tarzia[1], P. Cianciulli[2], F. Sorrentino[2], M.P. Caforio[1], D. Maffi[1], S. Amadori[2], A.M. Salvati[1]. [1]*Laboratory of Clinical Biochemistry, Istituto Superiore di Sanità, Rome;* [2]*Division of Haematology, S. Eugenio Hospital, Rome, Italy*

Hereditary Elliptocytosis (HE) is a heterogeneous group of red blood cell disorders associated with abnormalities of the skeletal membrane proteins. The most common functional defect is a diminished self-association of spectrin dimers to form tetramers due to a variety of α or β spectrin mutations.

In this work a clinical and biochemical characterization of 25 cases of HE in Italy was performed. In 7 cases HE was combined with another red cell disorder (thalassaemia, hereditary spherocytosis, G6PD deficiency). Haematological parameters, erythrocyte morphology, the acidified glycerol lysis test, erythrocyte pyruvate kinase activity and reduced glutathione were assessed. The biochemical characterization of spectrin defects was performed by evaluating 1) the membrane proteins patterns on SDS-PAGE, 2) the spectrin dimer-tetramer equilibrium in crude spectrin extract, 3) the peptide map of partial tryptic digests of spectrin.

Haemolytic anaemia was evident in 50% of cases, particularly in the presence of combined erythrocyte genetic defects. In 7 cases the spectrin content was decreased (73–86% of normal values) with a relevant increase in spectrin dimers (up to 70%). The cases with normal spectrin content also showed an increased value of spectrin dimers (10–25%) but to a lesser extent. Sp $\alpha^{V/41}$ polymorphism was detected in 48% of cases. The most frequent defects were detected in α spectrin (17 cases) and the abnormal peptides 74 kd, 78 kd, 68 kd, 54 kd and 46 kd were observed. In one case truncated β spectrin was associated with a protein 4.1 abnormality. In the remaining cases a shortened β spectrin was demonstrated. In Italy β spectrin defects seem to be more frequent than in other European countries.

P-1216 AUTO-IMMUNE HAEMOLYTIC ANAEMIA: CLINIC, BIOLOGICAL AND ETIOLOGICAL STUDY

M.A. Bekadja, S. Zouani, D. Mouhtadi, F. Saadi, H. Touhami. *Division of Hematology, Oran University Hospital Oran, Algeria*

Fourteen adults patients (18 males and 22 females, average 38 years), with autoimmune anemia were studied at the time of diagnosis at Oran University Hospital, between ten years period (1986–1996). All patients were characterized by clinical and laboratorial examination, which included cytology of peripheral blood, reticulocyte count, and bilirubin. Immunohematology investigation screenings were: direct antiglobulin reaction with polyspecific and monospecific reagents (Coombs test) - anti-IgG, anti-IgM, anti-Complement. Whenever an antibody was found on screening, its specificity was determined using a suitable panel of red cells. Clinic signs to the diagnosis are dominated by the paleness coetaneous mucous (98%), the icterus (95%), the splenomegaly (60%) and the hepatomegaly (20%). The biological viewpoint, the anemia (Hb < 6 g) is found in 60% of cases, the erythromyelemia in 35%. *Our results of the immunohaematological evaluation of the patients showed* that: The direct Coomb's test (DTC) is positive in 98% of cases. At 9 patients, the analysis serologic puts in obviousness a IgG + C (33%), an alone IgG (33%), an alone complement (22%), and a IgG + M (11%). The DCT is found negative in 03% of cases. The etiological inquiry, watch that 40% of AIHA are secondary, to a malignant haemopathy in 56% of cases and the AIHA is idiopathic in 25% of cases. To the therapeutic plan, the majority of patients (39/40) are processed by corticoids whose 08 case associated to a chemotherapy and 02 case to the immunosuppressive.

To the advanced viewpoint, among 40 patients, 13 are died, 13 are alive and 14 lost of view.

P-1217 NATURAL HISTORY OF WARM-TYPE AUTOIMMUNE HEMOLYTIC ANEMIA

M. Omine[1], H. Harada[1], S. Miwa[2], H. Uchino[2], T. Maekawa[1], T. Nomura[2], H. Mizoguchi[2]. [1]*Division of Hematology, Internal Medicine, Showa University Fujigaoka Hospital, Yokohama, Japan;* [2]*The Research Committee for Idiopathic Hemopoietic Disorders, Japan*

As stated by Dacie and Worlledge, AIHA is a disorder with heterogeneous etiology, and runs an unpredictable course, but may be controlled by judicious use of corticosteroids. To delineate long-term natural history of this disease, 2 cohorts of patients with warm-type AIHA have been followed for approximately 20 and 10 years, respectively by the Research Committee. Cohort 1 (retrospectively collected) consisted of 185 patients, and Cohort 2 (prospectively collected) 223 patients of all ages. Majority was classified as idiopathic variety in both Cohorts. An interim report of this study was presented at 1996 ISH Congress at Singapore. Survival estimates of the idiopathic AIHA in Cohort 2 at 2, 5, and 10 years from diagnosis were 95%, 80%, and 74%, respectively. Causes of death in 33 idiopathic AIHA was attributed to the side effects/complications of treatment in 50%, and to unrelated diseases or causes in 39%. The likelihood of DAT reversion to negative was 35%, 48%, and 55% at the same respective years. DAT reversion rates at 5 years were 40% for IgG + C type, and 70% for IgG only. Survival and reversion were significantly inferior in the elderly population. "Cure" was observed in 30% after 7 years. Cure rate in children was 40%, while it was 18% in adults. Seventy % of surviving patients were pursuing a nearly normal daily life at the last observation. Thus, the repeated long-term follow-up study provides meaningful informations on the overall clinical pictures of warm-type AIHA.

P-1218 HAEMOLYTIC UREMIC SYNDROME FOLLOWING PNEUMOCOCCICAL PNEUMONIA AND SEPSIS IN THE 3-YEAR-OLD GIRL

S. Čulić, D. Kuljiš, V. Čulić, M. Saraga. *Clinical Hospital Split, Split, Croatia*

Objective: The haemolytic uremic syndrome (HUS) is most common in children under the age of 4. Microangiopatic anaemia results from mechanical damage to the red blood cells as they pass through the altered vasculature.

Case Report: We review the case of a 3-year-old girl presenting in extremely bad condition; pallor, adinamic, confused, dispnoic, dehydrated with peripheral circulatory failure. The physical findings: tubular breath sounds with moist rales on the affected side of the lung. Liver enlargement 5 cm, spleen 3 cm. Initial laboratory findings were WBC 98, 6 × 10^9/l, haemoglobin 5, 1 g/dl, haematocrit 15, 0%, platelet count 175 × 10^9/l AST 110 U/l, ALT 69 U/l, LDH 2247 U/l, urea 44.4 ml/l, kreatinin 116 umol/l, acidum uricum 1014 umol/l, fibrin degradation products (FDP) 256 ug/ml, feritin 2686 ng/ml, haptoglobin 0, 113 g/l. Ultrasound showed enlargement of the kidneys with hyperechogenic changings in parenchyma. Streptococcus pneumoniae was found in blood culture. She was treated with high doses (10 mg/kg) of corticosteroides for nine days with subsequent tapering, fresh frozen plasma, antibiotics and 5S human immunoglobulin, hydration and supportive therapy. After 36 days she reached complete stabile remission.

Conclusion: We confirmed that streptococcus pneumoniae was in our case the trigger of HUS. High doses of corticosteroides, fresh frozen plasma, antibiotics, and 5S human immunoglobulin may be a successful treatment.

P-1219 APLASTIC ANEMIA: EXPERIENCE OF TEN YEARS

M. Gutiérrez, F. Ramírez, R. García, M. Borrego, D. Chiquín, C. Mendoza. *Hospital Domingo Luciani, IVSS, Caracas, Venezuela*

Between April 1987 and May 1997, 34 patients with aplastic anemia (A.A.) were treated. They were divided in two groups in relation with the age: 20 children <18 y. (mean 8.5±4.3 SD), 14 adults >18 y. (mean 39.2± antiinflamatory drugs, antithyroid and phenytoin; and Constitutional A.A. (CAA) 4/34 (12%): 2 Fanconi and 2 Diamond Blackfand. We excluded CAA for all statistical analysis.

According to severity of disease were classified in: non severe (NSAA) 8/30 (27%), severe (SAA) 15/30 (50%) and very severe (vSAA) 7/30 (23%). The treatment was: steroids 5/30, steroids plus androgens 6/30, Cyclosporine (CsA) plus steroids 11/30, CsA plus Antithymocyte globulin (ATG) 6/30 and BMT 2/30. The evolution to clonal disease was: AML 1 (3%), Myelodisplasia 4 (13%), PNH 3 (10%) at 6, 22 and 38 months after diagnosis. Survival analysis: O.S. at 40 m. was: children 78%, adults 57%. O.S. at 20 m. depending on severity was: 87% NSAA, 76% SAA and 28% vSAA. Significant difference was found in favor the group NSAA in relation with the vSAA ($p = .018$). Comparing the patients who received inmunosupression (IS): CsA or CsA plus ATG with the other group without IS therapy was not significance difference ($p = .060$) although the first group are living longer 72 m. v/s 41 m.

Conclusions: 1- Idiopathic AA was the most frequent etiology in the present study. 2- 73% of patients had severe disease. 3- The benefits of IS in the survival of SAA and vSAA could not be demonstrate due to small size of the sample.

P-1220 APLASTIC ANEMIA — SURVIVAL ANALYSIS OF 33 PATIENTS

R. Milosevic[1], N. Antonijevic[1], D. Babic[2], G. Jankovic[1], M. Colovic[1]. [1]*Institute of Hematology;* [2]*Clinical Centre of Serbia, Belgrade Institute of Statistics, School of Medicine, Belgrade*

Objective: The aim was to study survival of patients with aplastic anemia related to sex, etiology, illness duration, degree of bone marrow hypoplasia and kinds of treatment.

Design and Methods: We analyzed 33 aplastic anemia patients treated from 1988. to 1995. Diagnosis was made on criteria taken from International Agranulocytosis and Aplastic anemia study - 1987. For the survival analysis we used Kaplan-Meier model.

Results: There weren't statistical significance between sex ($p = 0.789$); patients with secondary disease have longer survival related to those ones with idiopathic form ($p = 0.044$); in patients where illness last longer than 12 months survival was longer ($p = 0.000$); patients with severe bone marrow hypoplasia have less chance for longer survival related to ones with moderate and mild hypoplasia ($p = 0.026$). Related to use of antilymphocyte globulin, glucocorticosteroids and androgens, cyclosporine; alogenic bone marrow transplantation has statistical advantage ($p = 0.006$).

Conclusions: Favorable prognostic factors are: secondary disease, disease duration over 12 months, mild and moderate bone marrow hypoplasia. Sex didn't have influence on survival. Best method of treatment is alogenic bone marrow transplantation.

P-1221 APLASTIC ANAEMIA IN CHILDHOOD: Report of 28 cases

S. Polychronopoulou, J.P. Panagiotou, V. Mikraki, S. Kostaridou, M. Antoniadis, S. Haldas. *Dept. of Paediatric Haematology-Oncology "Aghia Sophia" Children's Hospital, Athens, Hellas*

Aplastic anaemia (AA) of childhood encompasses diverse aetiologies, both acquired or constitutional, although it remains idiopathic in the majority of instances. Because of its rarity in the paediatric age any clinical experience

reported may add valuable information. We report retrospectively 28 patients, 19 boys and 9 girls, with median age of 7 years (range: 5 mo–14 yrs) with A.A., whom we have followed for the last 20 years. Our patients were diagnosed and classified according to the existing standard clinical and laboratory criteria (Camitta et al. criteria for SAA). Eighteen out of the 28 patients were diagnosed as having idiopathic A.A., 7/28 Fanconi's anaemia, 1 A.A. post hepatitis B, 1 after benzene exposure while 1 patient suffered from dyskeratosis congenita. Among these patients 23 were fulfilling the SAA criteria and 5 presented with A.A. of moderate severity. Eleven patients from the SAA group (11/23) who were diagnosed before 1986 (group A) were treated with combinations of corticosteroids and anabolic drugs, while the 12/23 patients who were diagnosed after 1986 (group B), were treated with immunosuppressive treatment (ATG + PDN±cyclosporin) (8), and with allogeneic BMT (4). The group C of 5 patients with non SAA, were treated either with corticosteroids alone or combined with anabolic drugs or cyclosporine. In group A patients, only 2 are alive and in complete remission for 20 and 9 years respectively. On the contrary 7/12 patients of group B are alive (5/8 with immunosuppressive treatment, 2/4 with BMT) with a follow up ranging from 6 months to 11 years (median time = 5.5 years) being all of them in complete remission. All patients of group C are alive and transfusion independent for a median of 11.5 years (range: 3–17 years).

As indicated from our experience, therapeutic approach of A.A. of childhood, with immunosuppressive treatment or BMT, results in a satisfactory long term survival. It is noteworthy that none of the long-term survivors of the immunosuppressive treatment arm, at least during our follow-up period, evolved into a malignant haemopoietic disease (MDS or AML).

P-1222 APPLICATION OF HUMAN RECOMBINANT GRANULOCYTE COLONI-STIMULATING FACTOR (rHu-G-CSF) ONTO PATIENTS WITH MEDICA-MENTALLY AND RADIATELY PROVOKED NEUTROPENIA AND APLASTIC ANAEMIA

L. Gercheva[1,2], P. Lazarova[1,2], G. Kyuchukov[1], Kr. Metodiev[1]. *[1]Med. University, Varna, Bulgaria; [2]Hadassah, Jerusalem, Israel*

The effect of rHu-G-CSF on neutrophile restoration in 85 patients was studied. The patients were:29 with acute lymphoblastic leucemia (ALL), 6 with acute non-lymphoblastic leucemia (ANLL), 1 with chronic myelogeneic leucemia in haematologic malignancies, whereas the patient with Kostmann's syndrome was treated with higher dose, up to 20 mcg/kg, due to lack of effect at the end of first week. A dose of 10 mcg/kg was applied to patients with aplastic anaemia and post-radiation neutropenia. The mean background level of leucocytes just before treatment was 0.73 + 0.13 × 10/l and that one of neutrophiles was 0.17 + 0.09 × 10/l. Meanwhile the therapy the authors registered constantly: leucocyte and granulocyte quantity, some enzymes, body temperature and clinical status of the patients. The treatment was discontinued when the absolute neutrophile spectre reached over 1.0 × 10/l. Shortest application was reported for the carcinoma cases: 4.3 days averagely, followed by ALL: 5.8 days, NHL: 6.3 days, ANLL - 7.4 days, PRN: 10.3 days, AA: 14.5 days. One (with ALL) of treated 75 patients had a lethal issue meanwhile the therapy, another one (with ALL too) shew a recidival manifestation; 68 of the rest 73 patients demonstrated a total recovery and 5 - a particular one (increase of ANC with over 100%). Only 5 patients of the group registered rapidly-progressive side effects: 2 with muscular-skeletal pain, 2 with headaches and 1 with vomitting. The nosologic cases were thoroughly analysed which proved that rHu-G-CSF shortened considerably the time for recovery from leucopenia (opportunity for the so called "antibiotic umbrella" in neutropenic patients), thus allowing effective results from the treatment of clinically manifested infectious complications and intensification of chemotherapy.

P-1223 INTENSIVE IMMUNOSUPRESSION WITH ANTILYPHOCYTE GLOBULIN (ALG), CYCLOSPORINE A (CyA) AND GRANULOCYTE-COLONY STIMULATING FACTOR (G-CSF) AS TREATMENT FOR ACQUIRED SEVERE APLASTIC ANEMIA (SAA)

G. Dinçol, M. Nalçacı, A.S. Yavuz, K. Dinçol, M. Aktan, Ö. Doğan, M. Ağan. *Division of Hematology, Department of Internal Medicine, Istanbul Medical School, University of Istanbul, Çapa, Istanbul, Turkey*

Between May 1995 and January 1998, nine consecutive patients (median age 47 years; range, 20–72 years) with newly diagnosed SAA received horse ALG 15 mg/kg/d (1 to 5), methylprednisolone 2 mg/kg/d (1 to 5 and than tapered), G-CSF 5 μg/kg/d (1 to 90) and CyA 5 mg/kg/d (1 to 120). Overall treatment was well tolerated. Six patients responded, 4 with complete and 2 with partial remission. Post-treatment peripheral median blood counts were as follows: neutrophils 2.3 × × 10^9/L-174 × 10^9/L) in 4 patients with complete remission. Two patients with partial remission received CyA for an additional 6 months which resulted in complete remission, the other still being treated. Of the 3 non-responders, 2 died of bleeding and one was lost to follow-up. Although observed in only 9 patients, the effect of G-CSF in responders and non-responders seems to be of prognostic significance due to the fact that patients whose neutrophil levels gradually increased during the first week of treatment with G-CSF and remained stabile during the first 3 months would respond to treatment, whereas those patients with no such increases would, most probably, be non-responders. This seems to be a reliable criterium in predicting the response early during the course of treatment. We are also of the opinion that in patients with partial remission, a prolonged treatment, preferably 12 months, with Cy A should be tried.

P-1224 SERUM IMMUNOGLOBULINS IN APLASTIC ANEMIA — With special reference to IgG subclasses

M. Hasegawa, T. Yagi, H. Ohyabu, S. Tsumoto, T. Kageyama. *Dept. Int. Med., Osaka Med. College, Takatsuki, Osaka, Japan*

studies describe elevated blood levels of r-globulin. Under these circumstances, determining serum immunoglobulins, particularly IgG subclasses, in AA patients would be useful. A total of 31 AA patients, consisting of 14 with mild, 12 with between AA patients and healthy controls in serum IgG1, serum IgG2, IgG3 and IgG4 were all significantly lower in AA patients than in healthy controls. These results facilitate elucidating the true pathophysiology of AA, and thereby clarify the immune mechanism underlying the disease.

P-1225 TREATMENT OF APLASTIC ANAEMIA WITH THYMOGLOBULINE AND CYCLOSPORIN

P.C. Apostolov, V.A. Markov, D.N. Popova, R.G. Oucheva. *Alexander's Hospital, Sofia, Bulgaria*

Thirteen patients with aplastic anaemia, diagnosed and treated in the Haematological Clinic of Alexander's Hospital-Sofia, in the period 1986–1997 were included in the present study. Median age was 34 (range 16–53). Nine of them were male and 4 - female. Four of the patients (31%) died. The median survival found was was 61 months. A treatment response was achieved in 5 (38%) of the patients and lasted for 4 to 72 months (median 34). The peripheral blood lymphocyte subpopulations were estimated in some of the patients before and after the immunosuppressive treatment.

P-1226 THE POSSIBLE SIGNIFICANCE OF DECREASED ACTIVITY OF THE MULTIDRUG RESISTANCE P-GLYCOPROTEIN IN APLASTIC ANAEMIA

R.T. Calado, A.B. Garcia, R.P. Falcão. *Department of Clinical Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil*

Acquired aplastic anaemia (AA) is the consequence of a variety of aetiologies, and, in some cases, may be induced by the exposure of a susceptible host to certain drugs or chemical substances. However, the reason for this idiosyncratic susceptibility remains elusive. Metabolic defects have been implicated, but the complexity of biochemical pathways precludes accurate studies. P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP) are related to multidrug resistance (MDR) in cancer cells, and are also expressed by the haematopoietic tissue where it is speculated that they play a protective role against xenobiotics. Their functions are easily evaluated. To investigate a possible altered activity of P-gp and MRP in peripheral blood lymphocytes (PB) from patients with AA, the functions of both P-gp and MRP were respectively assessed by means of rhodamine (Rh123) and daunorubicin (DNR) efflux, by flow cytometry. PB was obtained from 9 patients, 7 with severe and 2 with non-severe AA (mean, 28.7 years; range, 16–38), and 13 healthy controls (25.3 years; 23–42). In four patients, an association was found with insecticide exposure, while no attributable cause was detected in any other. Three had not been previously treated at the time of the study, while six had relapsed or had failed to respond to immunosupressive therapy. A significantly decreased proportion of both CD4+ and CD8+ T cells effluxed Rh123 in patients with AA, relative to controls (CD4+: mean±SEM, 19.9%±2.6% vs. 27.8%±1.9%, $p < 0.03$; CD8+: 52.4%±5.9% vs. 68.3%± 2.3%, $p < 0.02$). In contrast, Rh123 efflux in CD19+ B and CD16+/CD56+ NK cells, and DNR efflux in all PB lymphocytes did not differ between patients with AA and controls. These data indicate that the percentage of T cells bearing P-gp activity is decreased in AA, suggesting that reduced P-gp activity may contribute to drug-induced injury to haematopoietic cells in some cases of AA, by increasing the proportion of susceptible cells.

P-1227 A CASE OF THYMOMA WITH PURE RED CELL APLASIA, MYASTHENIA GRAVIS AND SYSTEMIC LUPUS ERYTHEMATOSOUS

N. Laoutaris, T. Hatzis, J. Marsh, C. Belesi, E. Samaridis, S. Alexia, E. Manioudaki. *Department of Haematology, General State Hospital of Nikea, Piraeus, Greece; Department of Haematology, St. George's Hospital Medical School, London, UK*

Thymomas can occur together with diseases of autoimmune origin. The aim of this work was the study of the haematological and immunological characteristics of a unique case of thymoma, together with diseases which are considered to have autoimmune origin, like pure red cellaplasia (PRCA), myasthenia gravis (MG) and systemic lupus erythematosous (SLE). A 62 years old female, with severe anaemia and intense weakness, was admitted for investigations. Haematological parameters were measured using an automated counter. In addition several other laboratory tests were done: blood and urine chemistry, short- (STCs) and long-term bone marrow cultures (LTCs), peripheral blood lymphocytes immunophenotype by flow cytometry, CT scan of thorax, bone marrow aspirate examination, reticulocytes, ESP, immunofixation and quantative estimation of immunoglobulins, anti-thyroid, anti-TPO, anti-viral, anti-nuclear and anti-DNA antibodies and histology of an excised mass from the anterior mesothorax. The patient was managed with immunosuppression, plasmapheresis and thymectomy but showed no improvement. She finally died from myasthenia gravis.

Results: Normochromic-normocellular anaemia, Hb 5.3 gr/dl, reticulocytes: rare, bone marrow aspirate: PRCA, CT scan of thorax: compatible with thymoma and pericardial infusion, ESR 155 nun, histology: thymoma, anti-nuclear and anti-DNA antibodies positive. STCs (colonies/10^5 mononuclear cells): CFU-Mix 6.7 CFU-GM 16.20, CFU-G 52.64, CFU-M 6.6, BFU-E 58.40, normal, CFU-E no development. LTCs normal. Peripheral blood lymphocytes: increased absolute numbers and percentage, 11% (normal 1%), of double positive CD19+CD5.

Conclusions: 1) a study of a case of thymoma with PRCA, MG and SLE was done, because of the rarity of these are seen together 2) the cell cultures showed that PRCA was due to differentiation block from the BFU-E stage and thereafter 3) the increased number of double positive CD5+CD19 (B CD5 +) lymphocytes, which are related with autoimmunity and autoantibody production, is possibly due to the coexistence and activity of many autoimmune diseases 4) the patient died from MG, despite immunosuppression, plasmapheresis and thymectomy. The significant increase of CD5+CD 19 lymphocytes, which posssibly indicates the presence and activity of many autoimmune diseases, is a bad prognostic marker.

P-1228 "PNH-LIKE" RED CELL POPULATIONS IN ACUTE LEUKEMIA AND MYELOPROLIFERATIVE SYNDROMES

E. Terpos, E. Michali, M. Samarkos, Chr. Meletis, A. Tsimberidou, G. Kanellopoulou, A. Mougiou, E. Variami, K. Konstantopoulos, J. Meletis. *1st Dept. of Internal Medicine, University of Athens School of Medicine, Laiko Hospital, Athens, Greece*

PNH is a rare acquired stem cell disorder resulting from a somatic mutation in the PIG-A gene leading to a deficient expression of GPI anchored proteins red cell membrane, including CD55 (DAF) and/or CD59 (MIRL). "PNH-like" red cell populations have been associated with various hematological disorders. We investigated the CD55 or CD59 defective red cells using the sephacryl gel microtyping system in normal subjects, acute leukemias and myeloproliferative syndromes. Fifty microliters of erythrocyte suspension in low strength buffer were added on the top of the sephacryl gel microtubes containing antimouse immunoglobulin. Fifty microliters of anti-CD55 and anti-CD59 were added and after incubation the microtubes were centrifuged at 126 g. Cells that lack CD55 or CD59 do not agglutinate and pellet at the bottom of the microtube. Table gives patients with deficient red cell populations for every hematological disorder respectively.

Diagnosis	CD55 (−)/CD59 (+)	CD55 (+)/CD59 (−)	CD55 (−)/CD59 (−)
Normal subjects (n.121)	1/121 (0.8%)	2/121 (1.6%)	2/121 (1.6%)
ANLL (n.49)	6/49 (12.2%)	3/49 **(6.1%)**	11/49 **(22.4%)**
ALL (n.17)	0	0	2/17 (11.7%)
Polycythemia Vera (n.39)	7/39 (17.9%)	0	5/39 (12.8%)
E. Thombocythemia (n.65)	16/65 **(24.6%)**	1/65 (1.5%)	13/65 **(20%)**
Myelofibrosis (n.26)	6/26 **(23/1%)**	2/26 **(7.7%)**	2/26 (7.7%)
CML (n.26)	10/26 **(38.4%)**	0	3/26 (11.5%)

Our findings supports the existence of "PNH-like" phenotype in a a variety of hematological disorder, more frequently in ANLL and essential thrombocythemia, but also in apparently normal subjects. These populations may represent a single stem cell mutation leading to another clonal disorder. The most likely explanation for these event is that in the multi-step pathogenesis of PNH, apart from PIG-A gene mutation, another factor (like bone marrow failure for the case of genuine PNH) is required.

P-1229 DIELECTRIC MEASUREMENTS OF ERYTHROCYTE AGGREGATION IN DIABETES MELLITUS

D. Zilberman-Kravits[1], A. Pribush[1,2], I. Harman[3], D. Meyerstein[2,4], N. Meyerstein[1]. [1]*The Dr. J. Kaufmann Hematology Laboratory, Physiology Dept., Faculty of Health Sciences;* [2]*Chemistry Dept., Faculty of Natural Sciences;* [3]*Diabetes Unit, Dept. of Medicine, the Soroka University Medical Center, Ben-Gurion University of the Negev, Beer-Sheva;* [4]*the College of Judea and Samaria, Ariel, Israel*

Increased red blood cell (RBC) aggregation is recognized as one of the main reasons for hemorheological abnormalities in diabetes mellitus. This RBC feature was quantified by measuring the blood admittance (Y) at a frequency of 0.5 MHz. The values of (Y_{120}/Y_5)-1 and Y_5 served as quantitative measures of the steady-state size of the aggregates and the aggregation rate (subscripts denote the time of measurements in sec, after the end of shearing). Increased Y_5 indicates faster kinetics of the aggregation whereas a decrease in (Y_{120}/Y_5)-1 reflects the bigger steady-state size of the aggregates. The enhanced aggregation was manifested in increased size of the aggregates in blood of NIDDM patients only. No correlation was found between the clinical severity of this disease and (Y_{120}/Y_5)-1 values. To clarify reason(s) for the enhanced aggregation in NIDDM, levels of HbA1c and plasma glucose, cholesterol and triglycerides were assayed for both types of diabetes. Levels of cholesterol and triglycerides are higher in NIDDM than those in IDDM whereas concentrations of the two others are similar. It was shown that low molecular weight dextran (M.W. = 9.600) effectively inhibits the enhanced aggregation. In the presence of 2.7 mM dextran, the (Y_{120}/Y_5)-1 value increases from 5.0±3.1

to 27.0±13.4% (n = 6) while Y_5 values remain unchanged. Further increase in the dextran concentration has no effect on the aggregation. Thus, the low dextran concentration completely reverts the enhanced RBC aggregation in diabetes mellitus.

aggregation index	control* n = 30	IDDM; n = 10	NIDDM*; n = 61
(Y_{250}/Y_5)-1, %	25.0±5.1	18.3±9.3	9.5±8.5
Y_5, μSm	50.0±12.2	61.9±24.2	56.0±19.6

Mean±S.D.; * the (Y_{120}/Y_5)-1 values are statistically different (p = 0.02).

P-1230 CLINICAL AND HEMATOLOGICAL STUDY OF 11 PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

M.N. Yenerel, M. Nalĉaca, T. Atamer, Y. Pekĉelon. *Istanbul University, Istanbul Medical School, Department of Internal Medicine, Division of Hematology, Ĉapa, Istanbul, Turkey*

In this study, presenting symptoms, laboratory findings and clinical course of eleven patients with paroxysmal nocturnal hemoglobinuria (PNH) were studied. The median age was 33 (range, 17 to 65; 5 females and 6 males). Six patients had been diagnosed as aplastic anemia (AA) before PNH developed. In these patients diagnosis of PNH was established with emerging evidence of hemolysis during their follow up periods, 4 to 174 months (median 30 months), after the diagnosis of AA. Four of the 6 patient had been treated with immunosuppressive agents for AA. The characteristics of the patients at diagnosis are given in Table 1. All the patients had pancytopenia. GPI anchor defects of the granulocyte and lymphocyte were found to be the most sensitive index for the diagnosis of the PNH.

Table 1. The characteristics of the patients at diagnosis

	Case (n = 11)	%
PNH followed by AA	6	55
Pancytopenia	11	100
Absolute Reticulocytosis (>%3)	8	73
Bone marrow aspiration: Hypocellularity	2	18
Hypocellularity	9	82
Elevated LDH (>5 times normal)	10	91
Acid hemolysis test positivity	8	73
Sucrose lysis test positivity	6	54
Hemosiderinuria	5	46
Iron deficiency	8	73
GPI anchor defect*	6	100

** Performed only in six patient.*

Treatment: All the patients were given folic acid replacement therapy. Methylprednisolone was used in 8 of them. One received oxymetholone. Iron was administered intermittently in 8 patients in whom iron deficiency developed.

Complication: Two patients (18%) complicated with thrombosis during their follow up periods. Recurrent v.saphena magna thrombosis developed in one and sinus sagittalis thrombosis in the other. Bone marrow aplasia occurred in 2 of the patients, 120 and 150 months after the diagnosis of PNH was established.

In conclusion: It is important to bear in mind that PNH may develop in patients with AA particularly treated with immunosuppressive agents. Reticulocyte counts, serum LDH levels, acid hemolysis test and flow cytometric analysis of GPI anchored molecules should be performed during follow up period.

P-1231 IMPROVEMENT OF ENHANCED ERYTHROCYTE AGGREGATION BY LOW MOLECULAR WEIGHT DEXTRAN

A. Pribush[1,2], E. Katorza[1], D. Mankuta[3], H.J. Meiselman[4], D. Meyerstein[2,5], N. Meyerstein[1]. [1]*The Dr. J. Kaufmann Hematology Laboratory, Physiology Dept., Faculty of Health Sciences;* [2]*Chemistry Dept., Faculty of Natural Sciences;* [3]*Dept. of Gynecology and Obstetrics, the Soroka University Medical Center, Ben-Gurion University of the Negev, Beer-Sheva;* [5]*the College of Judea and Samaria, Ariel, Israel;* [4]*Dept. of Physiology and Biophysics, School of Medicine, University of Southern California, USA*

Enhanced red blood cell (RBC) aggregation causes serious disturbances in blood rheology. To improve the abnormal RBC aggregation, we investigated the *in vitro* effect of low molecular weight dextran (M.W. = 9,600) to inhibit the enhanced RBC aggregation in normal and preeclamptic pregnancy, multiple myeloma, iron deficiency and β-thalassemia. The data show that dextran affects both the kinetics of erythrocyte aggregation and the steady-state size of the aggregates by a dose dependent manner. RBC aggregation reverts to normal at dextran concentration of 2.7 mM. Microscopic observations also demonstrate that dextran reduces erythrocyte aggregation. The dextran-induced decrease in RBC aggregation was also observed for control erythrocytes suspended in artificial aggregating media and autologous plasma. These findings indicate that the dextran effect does not depend critically on either plasma composition or membrane state. It is assumed that the competitive adsorption of dextran to the erythrocyte membrane decreases the surface concentration of plasma bridging molecules (mostly fibrinogen). This mechanism predicts that the dextran inhibition effect should be observed regardless of whether the enhanced RBC aggregation is caused by transformations of the RBC membrane or alterations in plasma composition. Thus, the experimental results obtained in this study indicate that treatment by a non-toxic substance as dextran may be helpful in many pathological situations.

P-1232 MOLECULAR GLUCOSE-6-PHOSPHATE DEHYDROGENASE VARIANTS IN LAZIO

D. Maffi[1], F. Martinez Di Montemuros[2], M.T. Pasquino[1], P. Caprari[1], M.P. Caforio[1], P. Cianciulli[3], F. Sorrentino[3], S. Amadori[3], M.D. Cappellini[2], G. Fiorelli[2], A.M. Salvati[1]. [1]*Laboratorio di Biochimica Clinica, Istituto Superiore di Sanità, Roma;* [2]*Centro Anemie Congenite, Ospedale Maggiore IRCCS, Università di Milano;* [3]*Cattedra e Divisione Ematologia, Università "Tor Vergata", Ospedale S. Eugenio, Roma, Italy*

In order to explore the molecular basis of G6PD deficiency in Lazio, a region of central Italy, 113 G6PD deficient subjects (24 family groups and 44 singles) residing in this region were studied. Since the Mediterranean variant has a high prevalence in Italy, molecular screening of this variant was performed by using Amplification Refractory Mutation Systems (ARMS) in all the subjects examined; for this purpose two allele specific primers were synthesised: ARMS1 to amplify the mutant allele and ARMS 2 to amplify the normal one. All the samples negative for the Mediterranean G6PD mutation were submitted to polymerase chain reaction and restriction enzyme analysis (PCR-RE) and polymerase chain reaction and single strand conformational polymorphism analysis (PCR-SSCP). We found the G6PD Mediterranean genotype in roughly 56%, G6PD Seattle in 13% and G6PD A^- in 4% of cases. The remaining 27% is still under investigation. To date no case of CNSHA was detected. These data are in agreement with the previously reported frequencies in northern Italy. It is noteworthy that the immigration is partially responsible for the frequencies of the A^- variant. Indeed two patients carrying the A^- variant were Africans, while the remaining two were from Sicily. The statistical analysis of the haematological and biochemical data pointed out that G6PD deficient people carrying the Mediterranean variant have the lowest enzyme activity values and a significant decrease of blood haemoglobin and erythrocyte GSH compared either to normal or to G6PD deficient subjects carrying other enzyme variants.

Chronic myeloproliferative disorders

P-1233 ANAGRELIDE FOR TREATMENT OF HIGH PLATELET COUNT IN PATIENTS WITH POLYCYTHEMIA VERA

M.E. Petrone, D.S. Tierney, M.P. Mahoney, R.M. Petitt, M.N. Silverstein. *Roberts Pharmaceutical Corporation, Eatontown, New Jersey; Mayo Clinic, Division of Hematology, Rochester,, Minnesota*

Anagrelide hydrochloride (HCl), an orally administered drug (member of the imidazo 2, 1-b quinazolin-2-one compounds) selectively reduces platelet counts (PC) and has been shown to be efficacious for the treatment of thrombocytosis in patients with essential thrombocythemia. We report on the efficacy and safety of anagrelide use in reducing high PC in 117 patients with polycythemia vera (PV). Patients were diagnosed according to criteria established by the PV Study Group. The mean PC prior to anagrelide therapy was approximately 1,000,000/μL.

The primary efficacy parameter was rate and time to complete or partial response to Anagrelide. Complete response was defined as a reduction in PC to ≤600,000/μL or ≥50% from baseline value for ≥4 weeks; partial response as a 20% to <50% reduction in PC.

For all PV subjects, the mean age was 61 years, 62% were female, and 96% were white. More than half (64/117; 55%) of all PV subjects were ≥61 years of age.

Hydroxyurea was the most common prior therapy for thrombocythemia (84/117; 72%), followed by Aspirin (35/117; 30%) of all PV subjects. Ten PV subjects received no prior therapy for thrombocythemia, and four PV subjects had a prior therapy status that was unknown.

Of those 117, 99 received at least 4 weeks of anagrelide treatment, making them eligible for efficacy analysis (EA). 66% of the EA subjects were classified as complete responders, and 12% of the EA subjects were classified as partial responders. Thus, 78% of the EA subjects had a satisfactory response to anagrelide treatment. On average, complete response was seen approximately 36 days after the first dose of a continuous 4 week period of treatment. The mean dose of anagrelide required for response was approximately 2.4 mg/day.

The most frequently reported AEs were headache, diarrhea, palpitations, asthenia, and edema. There were no deaths attributed to anagrelide. In summary, anagrelide is a new therapeutic option for rapidly and specifically reducing PC's in patients with PV. In addition, anagrelide has an excellent safety profile.

P-1234 PREDICTION OF OUTCOME IN IDIOPATHIC THROMBOCYTOSIS OR ERYTHROCYTOSIS BY ENDOGENOUS ERYTHROID COLONY ASSAY

L.-Y. Shih, C.-T. Lee. *Chang Gung Memorial Hospital, Taipei, Taiwan*

Patients with polycythemia vera (PV) often have marked thrombocytosis; however, bleeding and/or iron deficiency may mask polycythemia and lead to misdiagnosis of essential thrombocythemia. Some patients with idiopathic pure erythrocytosis may later have a PV evolution. As management and outcome of these patients may be different; it is clinically important to define these patients at their initial diagnosis. We studied the in vitro growth of erythroid progenitors of bone marrow and blood from 55 patients with idiopathic thrombocytosis (IT: platelets >1000 × 10^9/l, Hb < 13 gm/dl or normal RBC mass) and 24 idiopathic erythrocytosis (IE: increased RBC mass but did not fulfill the diagnostic criteria for PV) at diagnosis in order to characterize these patients. They had no secondary thrombocytosis or erythrocytosis. Of the 55 IT patients, 25 had endogenous erythroid colonies (EEC), their initial Hb levels ranged between 6.4 gm/dl and 12.9 gm/dl. Seventeen of the 25 EEC(+) IT patients had PV evolution 2 to 45 months after presentation. Of the 8 EEC(+) IT patients who did not develop PV, 5 received chemotherapy soon after presentation due to presence of vascular complications, 4 had renal insufficiency, and 3 patients had some degrees of myelofibrosis with splenomegaly and possible hypersplenism; which might delay or preclude the evolution of PV. Five of the 24 patients with IE formed EEC, all of them developed PV in 4 years. None of EEC(−) IT and IE patients had PV evolution in a median follow-up of more than 3 years. EEC(+) patients had a higher rate of thrombosis/bleeding than EEC(−) IT (80% vs 30%, p = 0.00035) and IE (60% vs 16%, p = 0.078). Our study showed that the assessment of EEC is helpful in early identification of IT or IE patients who will have PV evolution and in predicting the occurrence of vascular complications.

P-1235 DIAGNOSTIC SIGNIFICANCE OF SPONTANEOUS MEGAKARYOCYTIC (s-CFU-Mk) AND ERYTHROID (s-BFU-E) COLONIES IN ESSENTIAL THROMBOCYTHEMIA (ET) AND IDENTIFICATION OF A NEW, MIXED MEGAKARYOCYTIC/ERYTHROID HEMATOPOIETIC PROGENITOR

P.R. Scalzulli, A. Morelli, M. Nobile, F.S. Mantuano, R. Matera, P. Musto, M. Carotenuto. *Division of Hematology, IRCCS "Casa Sollievo della Sofferenza" Hospital, S. Giovanni Rotondo, Italy*

ET is a neoplastic chronic myeloproliferative disorder (CMD) characterized by an abnormal, clonal proliferation of megakaryocytes in bone marrow (BM), resulting in high levels of platelets in peripheral blood (PB). Recently, in vitro clonogenic assays have demonstrated in BM and PB of ET patients the spontaneous proliferation of s-CFU-Mk and s-BFU-E, without addition of specific growth factors. Since the diagnosis of ET is usually based upon exclusion criteria, at the present time, this represents the only positive test for distinguishing ET from other CMD or secondary thrombocytosis (ST).

With the aim of evaluating specificity and sensitivity of spontaneous colonies in diagnosing ET, we evaluated BM and/or PB s-CFU-Mk and s-BFU-E in 97 subjects with thrombocytosis and 30 normal controls. According to PVSG criteria, 80 patients had ET, 5 polycythemia vera (PV) and 12 ST. Clonogenic assays performed in absence of growth factors showed the presence of s-CFU-Mk in BM and/or PB from 70 (87.5%) out of ET patients. In 61% of these patients s-BFU-E were also observed. In patients with PV only s-BFU-E were found. The spontaneous growth of colonies was never seen in subjects with ST or in normal controls. Interestingly, in 4 patients with ET, in addition to s-CFU-Mk, spontaneous mixed CFU-Mk/BFU-E colonies were also identified. This new hematopoietic progenitor is a putative common precursor of both megakaryocytic and erythroid lineages.

Our results indicate that BM or SP detection of s-CFU-MK has 100% of specificity and 87.5% of sensitivity in diagnosing ET. Though frequently also found in ET, s-BFU-E appear to be rather associated with diagnosis of PV. Finally, the evidence of a new mixed megakaryocytic/erythroid precursor in ET patients further confirms the possible neoplastic involvement of a pluripotent-stem cell in this CMD.

P-1236 IDENTIFICATION OF NOVEL GENETIC REARRANGEMENTS ASSOCIATED WITH THE MYELOPROLIFERATIVE DISORDERS

H.L. Gilbert, R.S. Flora, R. Todd, B.M. Sawyer, J. Acharya, F.E. Cotter, T.C. Pearson. *Department of Haematology, UMDS, St. Thomas' Campus and Institute of Child Health, London, UK*

The myeloproliferative disorders (MPD) are characterised by the excessive proliferation of one or more cell types of the myeloid lineage. Polycythaemia vera (PV) and primary thrombocythaemia (PT) are subtypes of the MPD and are thought to arise due to a somatic mutation in a multipotent progenitor cell. The majority of PV and PT patients do not demonstrate karyotypic changes so we undertook a study to seek genetic rearrangements which may not be detectable by high resolution chromosome analysis. Inter-Alu DNA fingerprinting utilises primers which are specific for Alu repeat sequences. PCR amplification of tumour DNA and comparative analysis with non-tumour DNA from the same patient, can be used to identify genetic lesions that may be associated with the activation of oncogenes or the inactivation of tumour suppressor genes. Peripheral blood samples were obtained from 35 PV and 40 PT patients from which T-lymphocyte and granulocyte DNA was extracted. Inter-Alu DNA fingerprints of T-lymphocyte and granulocyte DNA were generated and scanned to identify quantitative or qualitative differences between the myeloid and lymphoid lineages which could be of relevance to the malignant process in these patients. The primary PCR screen identified differences in 9/75 patients. Further analysis of 2 PV patients confirmed the presence of a 206bp fragment in T-lymphocyte DNA which was absent from the granulocyte DNA. Sequence analysis showed the 2 fragments to be identical and primers were designed to either end of the fragment to allow screening of a PAC library by PCR. All 21 primary PAC pools were positive indicating that the fragment contained repetitive sequences. We are currently extending this sequence by walking into uncloned genomic DNA using vectorette PCR. This should enable single copy sequence to be isolated to re-screen the PAC library. The identification of hybridising clones will enable the chromosomal origin of the fragments to be identified and the role of these genetic lesions in PV can be determined.

P-1237 ANAGRELIDE, A NOVEL PLATELET REDUCING AGENT: THE EUROPEAN EXPERIENCE IN TREATMENT OF 60 PATIENTS

WITH ESSENTIAL THROMBOCYTHEMIA OR CHRONIC MYELOID LEUKEMIA

P.E. Petrides, M.K. Beykirch, O.M. Trapp. *Department of Medicine III, Munich University School of Medicine Großhadern, Munich, Germany*

We have treated over a period of more than 7 years 80 patients with primary thrombocytoses with anagrelide. Inclusion criteria were: age >18 years, diagnosis of ET, CML, OMF or PV, platelet counts >600000/μl with symptoms or >900000/μl without symptoms, written consent. A detailed analysis of the treatment of 60 of our patients has now been completed.

Of the *48 ET-patients* (age 19 to 79) 16 were previously untreated, 15 pretreated with hydroxyurea and 17 had multiple pretreatments [Petrides et al, submitted]. 41/48 had microvascular, thromboembolic or bleeding complications (platelet counts between 850.000 and 3.1000.000/μl). 87% of our patients were complete responders, the others either partial or treatment failures. 40% of our patients had short term side effects (less than three weeks) such as headache (most frequent), palpitations, tachycardia, nausea or fluid retention. However, only in 5 patients these adverse effects were such as pronounced that they required discontinuation of therapy. We have treated now two patients more than 7 years. The median maintenance dosage is 2.5 mg/day.

The *12 CML patients* [Trapp et al., Blood Cells, Mol & Dis 24.9 (1998)] had been pretreated with hydroxyurea, interferon-α, melphalan or busulfan. Their refractory platelet counts prior to therapy were between 970.000 and 3.6000.000/μl. 7 patients had thrombohemorrhagic complications. All 12 patients (median age 58 years) were responders. Their median platelet count was then 340.000/μl, the median anagrelide dosage 1.9 mg. In all patients disease thrombohemorrhagic complications disapperaed or did not recurr.

Conclusions: In patients with essential thrombocythemia anagrelide is a new efficacious and safe therapeutical option, in patients with chronic myeloid leukemia and a high platelet count it is an important adjunct, when thrombocytosis cannot be controlled with traditional drugs.

P-1238 THE ITALIAN CONSENSUS CONFERENCE ON DIAGNOSTIC CRITERIA FOR MYELOFIBROSIS WITH MYELOID METAPLASIA

G. Barosi. *for the ICSG on MMM (Italian Cooperative Study Group on Myelofibrosis with Myeloid Metaplasia), Laboratory of Medical Informatics. IRCCS Policlinico S. Matteo, Pavia, Italy*

The purpose of this work was to develop a definition of myelofibrosis with myeloid metaplasia (MMM) using diagnostic criteria that would remain valid within the set of patients with chronic myeloproliferative disorders or myelodysplastic syndromes, and to promote this definition as a standard for patient inclusion in clinical studies. A list of 37 diagnostic criteria (23 positive and 14 negative) with their diagnostic performance were extracted from the literature or were newly proposed by an 18-member Advisory Council. A Consensus Panel of 12 Italian experts was created. They were offered the results of the literature review and through a questionnaire they ranked the necessary and optional diagnostic criteria according their preferences in order to identify a core set of criteria. To establish the definition of the disease, the Panel was convened (Bologna, May 1997) and, using consensus formation techniques, it was asked to score the diagnosis of 46 patient profiles as appropriate or not appropriate for MMM. Using the experts' consensus as the gold standard, the chi-square, sensitivity and specificity were calculated for each of the 90 possible definitions of the disease obtained through the core set. Definitions with sensitivity or specificity of <80% were eliminated. The remaining definitions were ranked by kappa statistics. Necessary criteria consisted of "diffuse bone marrow fibrosis" and "absence of Philadelphia chromosome or BCR-ABL rearrangement in peripheral blood cells". The six optional criteria in the core set consisted of: splenomegaly of any grade; anisopoikilocytosis with tear-drop erythrocytes; the presence of circulating immature myeloid cells; the presence of circulating erythroblasts; the presence of clusters of megakaryoblasts and anomalous megakaryocytes in bone marrow sections; myeloid metaplasia. The definition of the disease with the highest final score was as follows: diffuse bone marrow fibrosis necessarily present and Philadelphia chromosome or BCR-ABL rearrangement in peripheral blood cells necessarily absent; any other two of the core set criteria present when splenomegaly is present; any other four of the core set criteria present when splenomegaly is absent. In conclusion, we propose a definition of MMM whose use will help standardize the conduct and reporting of clinical studies and should help practitioners decide whether a patient with a myeloproliferative disorder or a myelodysplastic syndrome has MMM.

P-1239 PROGNOSTIC VALUE OF BONE MARROW BIOPSY IN ESSENTIAL THROMBOCYTHEMIA

G. Lambertenghi Deliliers, C. Annaloro, A. Oriani, F. Radaelli, D. Lambertenghi Deliliers, E. Pozzoli. *Centro Trapianti di Midollo, Ospedale Maggiore I.R.C.C.S. and University of Milan, Italy*

Although the diagnostic and prognostic potential of bone marrow biopsy (BMB) in chronic myeloproliferative disorders (CMPD) has been estensively investigated, no histological parameter is reliably predictive in specific diseases. The routine BMBs taken at diagnosis between 1977 and 1994 from 93 essential thrombocythemia (ET) patients (pts) (35 male, 58 female: age range 10–86, median 54.5 years) were reviewed to look for clinicopathological and prognostic correlations. The value of the histological findings was also tested using cluster analysis. As of December 1997, median follow-up was 102 months (range 44–233); 9 pts had died and 23 had suffered major clinical events: 10 life-threatening thromboembolic episodes, 8 myelofibroses, 2 blastic crises, and 3 secondary malignancies. No relationships could be found between individual histological findings and the common clinical parameters; none of the histological or clinical variables were prognostically relevant in terms of overall survival (OS) or event-free survival (EFS). Cluster analysis identified two groups of pts. Group 1 (40 pts) significantly differed from Group 2 (53 pts) as follows: more frequent reticulum abnormalities ($p < 0.000001$), an increase in myeloid precursors ($p < 0.005$), dysmyelopoiesis ($p < 0.005$), frequent aggregates of immature precursors ($p < 0.005$), abnormal myeloid series maturation polarity ($p < 0.05$), a decrease in erythroid precursors ($p < 0.05$), a predominance of small megakaryocytes ($p < 0.0005$), the presence of trapped megakaryocytes ($p < 0.0001$). Group 1 had a significant worse EFS ($p = 0.0377$) (mainly due to an excess of thromboembolic events: $p = 0.0325$), and OS (p) = 0.0162). According to these histological findings, Group 2 showed the common features of CMPD, whereas the abnormalities in Group 1 were reminiscent of myelodysplasia. The diagnosis of ET therefore encompasses a histological spectrum ranging from a classical myeloproliferative to a variant "dysplastic" extreme. More interestingly, this distinction also has prognostic implications: in fact, the demonstration of dysplastic features in ET can be considered as a suitable parameter for defining a prognostic score in order to optimize therapeutic strategies.

P-1240 ESSENTIAL THROMBOCYTHEMIA (ET) AND PREGNANCIES OUTCOME

M.L. Randi, C. Rossi, G. Luzzatto, L. Menapace, A. Girolami. *Inst of Medical Semeiotics, II Chair of Internal Medicine, University of Padua Medical School, Padua, Italy*

Since the advent of automatic evaluations of platelet count, several young asymptomatic patients with ET have been incidentally discovered. ET can be diagnosed in young women who may become pregnant. Recurrent abortion or fetal growth retardation have been described in ET women, whereas some Authors have seen normal pregnancies in such patients. Over the last 20 years, we have prospectively followed 16 pregnancies in 13 females out of a total of 44 women in reproductive age (mean age 30.3±5.2 years) with ET diagnosed in agreement with the Polycythemia Vera Study Group. In 8 cases the first observation of an increased platelet count was made during pregnancy. The mean platelet count was $941.1 \pm 404.1 \times 10^9$/L at diagnosis and $876.7 \pm 421.8 \times 10^9$/L during pregnancy. The outcome of pregnancies were: 3 premature deliveries (18.7%), 4 abortions (25%) and 9 at term deliveries of normal babies (56.2%). 2 out of the 4 abortions occurred in a woman with antiphospholipid autoantibodies (APA); her second abortion occurred in spite of subcutaneous heparin treatment. 3 patients received aspirin (100 mg/day) during pregnancy and the outcomes were 1 abortion and 2 regular deliveries. No complication occurred in both women and infants of normal and/or premature deliveries.

According to the literature, the outcome of pregnancies of ET women are normal in about 45% of cases and our experience seems to be similar or slightly better. However, it is remarkable that the abortions seem to be due to problems different from ET (APA syndrome). The question about the use of therapy in young ET women is so far unanswered. However, our experience seems to indicate that, in the absence of additional risk factors for abortion, treatement of ET is not warrated.

P-1241 THROMBOTIC RISK IN ESSENTIAL THROMBOCYTHEMIA: RESULTS FROM A PROSPECTIVE, CONTROLLED STUDY

M. Ruggeri, G. Finazzi, A. Tosetto, S. Riva, F. Rodeghiero, T. Barbui. *Department of Haematology, San Bortolo Hospital, Vicenza and Department of Haematology, Ospedali Riuniti, Bergamo, Italy*

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder characterised by the occurrence of thromboembolic episodes, in particular in patients over 60 years and with a previous history of thrombosis, and by haemorrhages in patients with platelet counts over 1.500×10^9/L. In these subgroups of patients the use of cytoreductive therapy is beneficial in terms risk/benefit ratio. No data are available on the thrombotic risk and survival in young ET patients, asymptomatic and with a platelet count below 1.500×10^9/L. Thus, the optimal management of these low risk patients is unknown. To assess the incidence of thrombosis and the survival in this group of patients, we planned a prospective, observational study in a cohort of 65 patients with "low risk" ET (age below 60 years, no history of thrombosis in the past and at diagnosis, platelet counts below 1.500×10^9/L) and 65 age and sex matched controls. Patients were not treated with cytoreductive therapy, until occurrence of thrombosis or haemorrhages. Thrombotic events were objectively documented in cases and controls.

The median of follow-up was 4.1 years, with an incidence of thrombosis in patients of 1.88% patient/years and 1.48% patient/year in the control group ($p = 0.368$). Pregnancy and surgery do not act as a trigger condition in these patients.

We conclude that the thrombotic risk in young ET *patients, with no a past* history of thrombosis and with a platelet count lower than 1.500×10^6/L is not appreciably increased and a conservative therapeutic approach should therefore be considered in these patients.

P-1242 TPO, C-MPL AND MEGAKARYOCYTES IN IDIOPATHIC MYELOFIBROSIS

M. Souyri[1], L. Cocault, M. Flon, D. Clay, M.C. Martyré, C. Challier, B. Dupriez, J.L. Demory, M.C. Le Bousse-Kerdilès[2]. [1]*INSERM U363, ICGM, Hôpital Cochin, Paris;* [2]*INSERM U268, Hôpital Paul Brousse, Villejuif, France*

Implication of megakaryocytic proliferation in the pathogenesis of idiopathic *myelofibrosis is now well established. Physiological role of Thrombopoietin* (TPO) and MPL, its specific receptor, in proliferation and differentiation of cells from megakaryocytic lineage has been extensively documented. Moreover, it has been recently shown that in vivo overexpression of TPO, led to myeloproliferation and myelofibrosis in mice, with clinical characteristics similar to human idiopathic myelofibrosis. We thus thought that it would be important to study the potential implication of TPO and its receptor in the myeloproliferation that characterized idiopathic myelofibrosis.

First, we compared c-mpl molecular expression in peripheral blood mononuclear cells (PBMC), CD34+ cells and megakaryocytes purified from patients and healthy donors. We used a semi-quantitative RT-PCR analysis, with primers which allowed the amplification of a 280 bp fragment in the extracellular domain of mpl: for each sample, an aliquot of the PCR reaction was analyzed after 24, 27 and 30 cycles of amplification. Actin gene was used as an internal control. Our first results showed that in IMF patients, c-mpl expression is significantly increased in $CD34^+$ progenitors, whereas it appears to be reduced in PBMC. In megakaryocytic cells, this expression is heterogeneous. Given the role of Mpl in hematopoietic progenitors proliferation, it is reasonable to make the assumption that its overexpression in $CD34^+$ cells could be part of the mechanisms at the origin of their expansion in IMF.

In a second approach, we analyzed TPO level in patients sera by two techniques: a proliferation assay performed on a TPO dependent cell line engineered in our laboratory (UT7 mmpl cells), and an Elisa assay. This allowed us to show an increase of TPO level in the sera of 21 out of 27 patients. The mean increase is 3 fold, which is in agreement with the results recently reported by Wang et al. in this hemopathy. These data show that in IMF, TPO increase is moderate, which is in contrast with the results obtained in murine experimental models of myelofibrosis.

P-1243 SEVERE VASCULAR COMPLICATIONS IN ESSENTIAL THROMBOCYTHEMIA (ET): A STUDY OF THE PREDICTIVE FACTORS IN A SERIES OF 148 PATIENTS

F. Cervantes, C. Besses[1], A. Pereira, L. Florensa[1], F. Solé[1], J.C. Hernández-Boluda, S. Woessner[1], J. Sans-Sabrafén[1], C. Rozman, E. Montserrat. *Postgraduate School of Hematology Farreras-Valenti, Hematology Department, IDIBAPS, Hospital Clinic;* [1]*Unitat d'Hematologia 1973, Hospital del Mar, Barcelona, Spain*

To determine the clinicohematological factors predictive for the appearance! of major vascular complications (MVC) in ET patients, 148 patients consecutively diagnosed with ET within a 15-year period were retrospectively assessed for the development of MVC during a median follow-up of 58.5 months.

Seventy-seven patients had vascular risk factors, and 37 a history of MVC at ET diagnosis. Forty-nine MVC were registered in 33 patients during the follow-up period. The actuarial probability of MVC was 27% at 6 years in the whole series, 35.6% for patients above 60 years, and 21.4% for patients younger than 60 years, whereas only one of the 36 patients younger than 45 years had MVC. At multivariate analysis, age >60 years, history of major ischemic events (actuarial probability of MVC at 6 years: 42.6%) and hypercholesterolemia (actuarial probability of MVC at 6 years: 59.5%) were the variables associated with an increased risk of MVC.

The above results suggest that in ET patients over the age of 60 years treatment should be recommended, whereas in younger patients treatment decisions should be primarily based on the existence of a previous history of severe ischemic events. In addition, the existence of hypercholesterolemia should be routinely assessed in ET patients.

P-1244 MYELOPROLIFERATIVE DISORDERS (MPDs) AND VENOUS THROMBOSIS: SIGNIFICANCE OF HEREDITARY OR ACQUIRED CLOTTING DEFECTS

M.L. Randi, L. Menapace, C. Rossi, P. Zerbinati, P. Simioni, A. Girolami. *Inst of Medical Semeiotics, II Chair of Internal Medicine, University of Padua Medical School, Padua, Italy*

Most thrombotic complications in MPDs with increased platelet count interest arterial district. Less frequently, venous thrombosis may occur in these patients, in a ratio 1:5 to other thrombotic events. During the last 20 years, in our Department, we have diagnosed in agreement with Polycythemia Vera Study Group criteria, 209 patients with essential thrombocythemia (ET) and 180 with polycythemia vera (PV). 53 (13.6%) of them (27 ET and 26 PV) had thrombosis of the venous system and 158 (47.5%) of the arterial vessels. Since january 1997, we started to evaluate antithrombin (activity), protein S (activity, free and total antigen), protein C (activity and antigen), activated protein C resistence (aPCR)(ratio) and anti-phospholipids antibodies (APA)(ELISA assay) in consecutive MPDs patients with a positive history, objectively documented, of vein thrombosis. Out of the 22 patients included, 15 had vein thrombosis (7 deep vein thrombosis, 1 longitudinal cerebral sinus, 5 portal and 2 hepatic veins thrombosis) whereas 7 patients (controls) had no history of thrombosis. Among the 15 thrombosis 2 patients had protein C defect, 1 protein S defect, 1 aPCR and 1 APA syndrome (33.3%), while only 1 case (16.6%) in the control group had aPCR. Due to the small number of subjects evaluated it is not possible to drow a conclusion. Our data seem to confirm the coexistence of an inherited thrombophilia condition or aPCR and increase platelet count in about 1/3 of MPDs with a history of venous thrombosis. The relation between thrombophilia defects or aPCR and deep vein thrombosis have been clearly demonstrated. Whether the concomitant presence of increased platelet count may further increase the risk of vein thrombosis in MPDs patients or may influence the site in which thrombosis occurs remain to be clarified. In contrast, in some MPDs patients, thrombosis of venous system seems to be due to the increased platelet count alone. It is likely that in patients with thrombosis in unusual sites i.e. cerebral sinuses, portal or sovra-hepatic veins, thrombocytosis might have played an important role

P-1245 EFFICACY OF ASPIRIN (ASA) IN ESSENTIAL THROMBOCYTHEMIA (ET) WITH ADDITIONAL THROMBOTIC RISK FACTORS

M.L. Randi, F. Fabris, C. Rossi, A. Girolami. *Inst of Medical Semeiotics, II Chair of Internal Medicine, University of Padua Medical School, Padua, Italy*

The indications and controindications for the use of ASA or other antiplatelet drugs have not yet been clearly established. Most physicians administer ASA both for primary and secondary prevention of thrombosis in ET, but the dosage adopted is variable. We report our experience on 58 patients (20 males, 38 females) (mean age 50 ± 16 years) with ET diagnosed in agreement with Polycythemia Vera Study Group criteria and treated with ASA 100 mg/day. The indications to the use of ASA were 1) the presence of atherosclerotic risk factors (smoking, hyperlipemia, diabetes or hypertension) in asymptomatic

patients younger than 45 or 2) major thrombosis or microvascular disturbances (MVD). Most investigators agree in considering the advanced age a risk factor for thrombosis in ET. At time of diagnosis 15 out of our 58 patients (25.8%) had a major thrombotic complication (6 TIA, 2 strokes, 4 MI, 2 portal and 1 giugular thrombosis) and 21 (36.2%) referred MVD (headache, dizziness, eritromelalgia and vertigo). The mean follow-up under ASA therapy was 80±52 (range 12–125 months). During the follow-up, 4 patients had major thrombosis (2 strokes, 1 myocardial infarction and 1 pulmonary embolism) and 10 patients had MVD which represent a significative reduction of both major thrombosis ($p = 0.012$) and MVD ($p = 0.035$). Two patients died for major thrombosis (1 stroke and 1 pulmonary embolism). In only 3 out of 58 patients (5.7%) minor bleedings have been observed (epistaxis or dental bruisings). While the efficacy of ASA has been convincingly demonstrated in MVD of ET patients, an unacceptable rate of serious hemorrhages have been recorded in these patients treated with high doses of ASA. However, low dosages of ASA are able to reduce thromboxane B_2 production from platelets even in patients with increased platelet count. ET patients with thrombotic risk factors may be considered more prone to thrombosis than other patients with increased platelet count alone. However, the risk associated with the use of myelosuppressive drugs in subjects without sure risk may be azardous. In our experience, the use of low doses of ASA seems to be effective in reducing both major thrombotic complications and MVD frequency without an unacceptable bleeding increase in ET patients with additional risk factors for thrombotic complications.

P-1246 PLASMATIC LEVELS OF CYTOKINES AND GROWTH FACTORS IN ESSENTIAL THROMBOCYTHEMIA

R.F. Marta, P. Vassallu, L.I. Kornblihtt, F.C. Molinas. *Instituto de Investigaciones Médicas Alfredo Lanari, Facultad de Medicina, Universidad de Buenos Aires, Argentina*

Essential thrombocythemia (ET) is a bcr/abl negative chronic myeloproliferative disorder, associated with thrombotic, hemorrhagic and/or vasomotor manifestations. Its clonal origin was shown in heterozygous females by the presence in blood cells of only one G6PD isoenzyme or the non-random inactivation of HUMARA gene. It is not yet clear which are the abnormalities leading to megakaryocyte disregulation in ET. The aim of our study was to know if there is a peripheral expression of the intramedullar increase of cytokines and growth factors. Thirty two untreated patients with ET diagnosed according to PVSG criteria were included in the study, median age 46 years (range, 21-82). Sixteen patients had symptoms: hemorrhages 3, thrombosis 4, TIA's 4, erythromelalgia 4 and acroparesthesia 1. Median platelet count was, 954x1000/µl (586-2312). Plasmatic levels of cytokines were assayed by commercial ELISA technique (R&D). Raised levels of IL-6sR were found in 16/31 patients, 41 pg/ml (16-64.8) (Normal Value, 15-40); IL-3, 6/30, 7.4 pg/ml (7.4-187) (NV < 31); IL-11, 1/29, 8 pg/ml (8-85.5) (NV < 31.2); Tpo, 4/32, 32 pg/ml (32-85) (NV < 62.5); SCF, 3/32, 1586 pg/ml (714-2075) (NV1000-1790); Epo, 4/13, 13 IU/ml (1.8-64) (NV 3.1-16). Values were normal for IL-6 and GM-SCF. Correlation between Tpo and platelet count was r:-0.41, $p < 0.025$. No association was found between clinical manifestations and cytokine increase. These results show the plasmatic expression of cytokines in some patients with ET, while IL-6sR was raised in 51% of them.

P-1247 THE EFFECT OF CARDIOVASCULAR RISK FACTORS ON THE RISK OF THROMBOTIC COMPLICATIONS IN ESSENTIAL THROMBOCYTHAEMIA (ET)

R. Jantunen[1,4], E. Juvonen[1], E. Ikkala[1], K. Oksanen[1], P. Anttila[3], T. Ruutu[1]. [1]*Department of Medicine, Helsinki University Central Hospital, Helsinki;* [2]*Kanta-Häme Central Hospital, Hämeenlinna;* [3]*Helsinki Municipal Hospital, Helsinki;* [4]*Jorvi Hospital, Espoo, Finland*

The correlation of smoking, hypertension, hypercholesterolaemia and diabetes mellitus with thrombotic complications was analysed in 170 patients with ET. The median follow-up time was 69 months (range 11-313). 64/150 patients (43%) had a thrombotic complication. 58 patients had 111 arterial thrombotic episodes and 16 patients 28 venous thrombotic episodes. Smoking history could be recorded in 150 patients and the other risk factors in 132 patients. No risk factor was manifested in 74/132 patients (56%) while 58/132 patients (44%) had at least one factor, and 15 patients showed several of them. 32/150 patients smoked, 21/132 had hypercholesterolaemia, 19/132 hypertension and 4/132 diabetes. 31/58 patients (53%) with at least one risk factor and 22/74 patients (30%) without any of them showed an arterial thrombotic complication ($p < 0.01$). Smoking increased the risk for arterial thrombosis considerably (19/32 smokers versus 39/118 non-smokers, $p < 0.01$). In general, men (38/68, 56%) had arterial thrombosis significantly more often than women (20/82, 24%). The presence or absence of risk factors had less impact on the risk of thrombotic complications in men than in women. 53% of men without and 62% with risk factors had thrombotic complications while the numbers in women were 8% and 45% respectively. Conclusion: The presence of vascular risk factors, independent of ET, increased the risk of thrombotic complications in ET. Especially in women an intervention to these factors may decrease markedly the risk of complications.

P-1248 STUDY OF ERYTHROPOIESIS IN POLYGYTHEMIA VERA. RETROSPECTIVE ANALYSIS AND SENSIBILITY TO INF ALFA-2A

N. Gonzalez, Ma.P. Navascues, E. Gómez Sanz[1], J. Cesar, I. Fdez Fuertes, C. Burgaleta. *S. Hematology. Ramon y Cajal and Gregorio Marañon[1] Hospitals Madrid, Spain*

INF-α 2a is a citoquine effective in some chronic myeloproliferative syndromes. Indications of INF-α in polycythemia vera (PV) need to be determined. We have analyzed the erythropoietic response in PV, in relation with the time of evolution, treatment, and the "in vitro" effect of INF-α, in order to evaluate possible indications of IFN-α 2a in PV. The study was performed in 28 patients confirmed as PV. All patients presented spontaneous erythroid colonies (EEC) in culture. 14 patients were treated with hydroxiurea (HU) and 14 were controlled by phlebotomy. Erythropoyetic precursors (BFU-E), in peripheral blood cultures were determined in presence of Erythropoietin (1 U/ml) and without Erythropoietin (EPO). Effect of INF-α 2a (1000 and 5000 U/ml) was tested in 16 patients. Table 1 shows the results obtained in patients without HU (group A), before and after 2 years of evolution, and in patients, with more than 2 years, under treatment with HU (group B). Hemoglobin (Hb) and hematocrit (Htc) values were controlled in both groups. Clonal proliferation of erythroid precursors remain abnormal in all patients.

Table 1.

	Group A		Group B
	Evolution <24 m	Evolution >24 m	Evolution >24 m
Htc %	61±5 $p < 0.05$	51±6	49±5
Hb g/l	205±20 $p < 0.05$	171±12	160±12
BFU-E/2x10^5	27±22	21±20	39±37

Inhibition of EEC and EPO dependent colonies by INF-α2a was low and variable with 1000 U/ml, and very effective with 5000 U/ml in group A. In patients under treatment with HU. INF-α 2a have inhibitory effect on abnormal erythroid colonies, without significant difference between both doses (Table 2).

Table 2.

	Group A		Group B	
% Inhibition of INF	BFU-E/EEC	BFU-E/Epo	BFU-E/EEC	BFU-E/Epo
1000 U/ml	0-20	43±30	44±38	64±32
5000 U/ml	95±5	74±23	53±36	69±16

Those results shown persistence of abnormal erythropoiesis in PV after normalization of Hb and Hto values by treatment. IFN alfa-2a show inhibitory effect of abnormal erythroid colonies at therapeutic or high doses in non treated patients, and synergetic effect with low doses in patients treated with Hydroxiurea.

P-1249 ACUTE PHASE REACTANTS IN THE DIFFERENTIAL DIAGNOSIS BETWEEN CLONAL AND REACTIVE THROMBOCYTOSIS

P. Custodi[1], A. Cerutti[2], M. Duranti[2], C. Balduini[3]. [1]*Divisione di Medicina Interna;* [2]*Laboratorio Analisi, Ospedale "S. Biagio", Domodossola;* [3]*Istituto di Medicina Interna e Oncologia, IRCCS "S. Matteo" Pavia, Italy*

Objective: To study the usefulness of some simple laboratory tests in the differential diagnosis between primitive and secondary thrombocytosis.

Design and Methods: During a period of about 2 years we have recruited among the patients of a 1st level center, 103 individuals with platelet count $\geq 600 \times 10^9$/L, on whom we determined some parameters already indicated by the literature as potentially usefull for the differential diagnosis between primitive and secondary thrombocytosis: MPV, PDW, LDH, fibrinogen (FBG), C-reactive protein (CRP), interleukin-6 (IL-6) and the erythrocyte sedimentation rate (ESR). Patients were separated into 3 groups according to the etiology of there thrombocytosis: reactive (75 patients); associated with a myeloproliferative disorder (16 patients); mixed (12 patients affected at the same time by a myeloproliferative disease and by a disorder known to be associated with reactive thrombocytosis).

Results: Our results (Mann-Whitney U test) demonstrate the usefulness, in the differential diagnosis between primitive and secondary thrombocytosis, of the acute phase reactants. First of all the ESR ($p < 0.000001$), whose levels don't show any overlap between the 2 first groups of patients, followed by CPR, IL-6 and FBG. When the first and the third group are compared, the ESP, on the contrary of the other 2 acute phase reactants, yet allows some discrimination ($p < 0.00001$).

Conclusion: When a patient has an unexplained high platelet count we can find one of the following situation: a) acute phase reactants, first of all the ESR, in the normal range, that allows a diagnosis (almost sure) of primitive thrombocytosis; b) acute phase reactants above the normal range, that is indicative of a reactive form; c) high levels of CRP and fibrinogen accompanied by normal values of ESR, that is indicative of a mixed form.

P-1250 THE VALUE OF HEMATOCRIT IN THE DIAGNOSIS OF P. VERA[1]

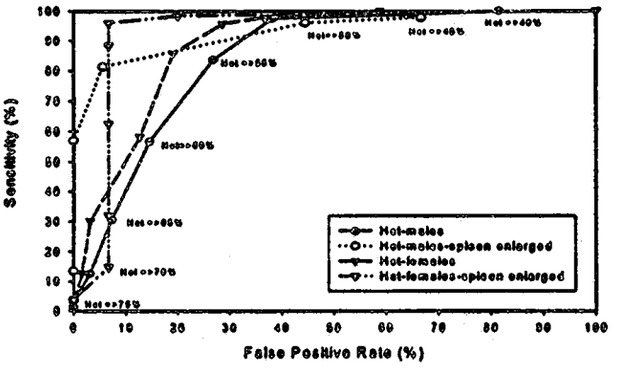
B. Djulbegovic[1], I. Hozo[2], A. Najman[3]. [1]*University of Louisville Division of Medical Oncology/Hematology, Louisville, KY;* [2]*Indiana University NorthWest, Department of Mathematics, Garry, IN, USA;* [3]*Centre Hospitalier et Universitaire Saint-Antoine, Paris, France*

Increased red blood cell mass is *conditio sine qua non* for the diagnosis of Polycythemia Rubra Vera (PV) (Semin Hematol 1975;12:339). However, the determination of red blood cell mass (RM) is time consuming, expensive and requires the use of radioactive isotope Cr^{51}. The previous work demonstrated a positive, although imperfect correlation between RM as determined by Cr^{51} method and hematocrit (Hct), a simple, cheep, and widely available method to assess RM. We, therefore, set to estimate the value of Hct in the diagnosis of P. Vera by calculating its sensitivity (S) and specificity (Sp). We also report S and Sp in patients with P. Vera with and without palpable spleen. Data from 595 patients were used for the analysis (431 from the original Polycythemia Vera Study Group cohort and 164 patients who were deemed not to have PV but had clinical features similar to PV). Two methods were used to calculate sensitivity and specificity: 1) "gold standard" (GS) method assuming a correct classification of PV cases using PVSG diagnostic criteria, 2) latent class analysis (LCA) which assumes the absence of a true GS. The latter method was used only for patients with splenomegaly.

In patients with palpable spleen, for a cut-off point of Hct $\geq$ 60% for males, and Hct $\geq$ 55% for females (which is equal to a probability of 99% that RM is truly increased when measured by Cr^{51}) (Semin Hematol 1975;12:339), GS method gave S = 57% [95% CI 49–64%], Sp = 100% [82–100%] for males, and S = 88% [81–93%], Sp = 94% [66–99%] for females, respectively. Using same cut off points, but combining them in one analysis, LCA method resulted in S = 85% [80–90%] and Sp = 100% [97–100].

The graph shows receiver-operating characteristic (ROC) curve for other cut off points (False positive rate = 1-Sp). As it can be seen, at high values specificity of hematocrit is very high, virtually ruling-in PV even in the absence of the other criteria for the diagnosis of PV. We conclude that at high values hematocrit may substitute for measurement of RM by Cr^{51}.

[1]The authors want to thank to all members of PVSG without whose remarkable contributions and generous data sharing this analysis would not be possible.



P-1251 EVALUATION OF BONE MARROW PATTERN BY MAGNETIC RESONANCE IMAGING IN PATIENTS WITH SYSTEMIC MASTOCYTOSIS ON THERAPY WITH α-INTERFERON

P. Giraldo, A. Arroyo, M. Roca[1], J.A. García-Erce, J.A. Marín-Cárdenas[1], P. Mayayo, D. Rubio-Félix, M. Giralt. *Haematology and* [1]*Radiology Department Miguel Servet Hospital, Zaragoza, Spain*

Purpose: To evaluate the changes in bone marrow pattern by magnetic resonance imaging (MRI) in patients with systemic mastocytosis (SM) receiving α-Interferon (α-IFN) therapy.

Patients and Methods: A prospective, descriptive study was performed in 6 patients diagnosed of SM between 01/91 and 12/96. Clinical classification was aggresive lymphadenopathic mastocytosis with eosinophilia (2 cases) and indolent SM with severe and reiterative episodes of immediate-type hypersensitivity reactions (4 cases). α-IFN was started, if no response to anti H1, H2, ketotifen or prednisone. The mean dose of α-IFN was 9 mU/sc/weekly. Clinical response was evaluated monthly.

Type of MRI Study: Signal intensity sequences in T1 (TR600 ms TE:20) in coronal imaging located in vertebra, pelvis, humeral and femoral bones. The imaging analysis was performed in sequence SET1 on L3 and L4 vertebra. MRI was performed before starting therapy and one year later; and in 5 cases after two years on therapy. Bone marrow infiltration (BMI) patterns: 1) normal (N), 2) non-homogeneous (NH), 3) homogeneous (H). The NH pattern being subclassified in three different subtypes: reticular (NHR), mottled (NHM) and diffuse (NHD). MRI pattern scoring system was establish, assessing progressive values (4-H, 3-NHD, 2-NHM, 1-NHR, 0-N).

Results: Mean age 42.6 (range 28–77), M/F 3/3. Histological features: paratrabecular nodular BMI 5 cases; diffuse infiltration 1 case (range 30–70%). All cases showed reticular fibrosis. MRI analysis showed involvement in spine, pelvis and humerus in all cases. MRI patterns were: spine (5H, 1NHD), pelvis (2H, 4NHD) and humerus (1NHD, 4NHM, 1NHR); femur was involved in 4 cases (1H, 1NHD, 2NHM). Scoring system values: 15, 13, 11 (2 cases) and 8 (2 cases). After a year on therapy, improvement of symptoms, skin lessions, BMI, and scoring were observed.

Conclusions: MRI is an effective non invasive procedure to evaluate bone marrow replacement and to determine the extent of disease in SM. In our experience, MRI seems a very useful method to evaluate the response to therapy.

P-1252 MEGAKARYOPOIETIC AND INFLAMMATORY CYTOKINE LEVELS IN PRIMARY (PT) AND REACTIVE THROMBOCYTOSIS (RT)

H.C. Hsu, M.L. Jiang, W.H. Tsai, M.L. Hsu, C.H. Ho, S.Y. Wang. *Veterans General Hospital-Taipei, Taipei, Taiwan R.O.C.*

Many cytokines are involved in the platelet production and/or reactive conditions. A simple laboratory test to reliably distinguish RT from PT would be useful in clinical hematology practice. We analyzed by sandwich ELISA the serum level of IL-3, -6, -11, -8, soluble IL-6 receptor, TPO, SCF and transforming growth factor (TGF)-β1 (R & D Systems. MN) in normal human controls (NC), RT and PT. PT included patients with essential thrombocythemia, chronic myeloid leukemia and polycythemia vera.

	NC (⊥ SE; N = 38)	PT (± SE; N = 42)	RT (± SE; N = 11)
TPO (pg/ml)	28±3	126±31	167±75
IL-8 (pg/ml)	8±1	48±4	39±19
IL-6 (pg/ml)	1±1	6±3	133±65
sIL-6 R (ng/ml)	41±2	52±3	38±10
TGF-β1 (ng/ml)	3±1	89±5	63±14
IL-3 (pg/ml)	1.2±0.7	14.3±6.0	0±0
IL-11 (pg/ml)	12±2	7±2	51±34
SCF (pg/ml)	2013±71	2515±126	1886±497

Statistically significant difference between NC, PT and RT (p value <0.05; Kruskal-Walllis ANOVA test) was observed for TPO, IL-8, IL-6, sIL-6 R, TGF-β1, IL-11 and SCF. However, only IL-6 had significant difference between PT and RT (p = 0.0006, Whitney U test). These results suggest that serum levels of IL-6 may help in the differential diagnosis of thrombocytosis.

P-1253 INTERFERON-ALPHA AND HYDROXYUREA IN THE THERAPY OF HYPERTHROMBOCYTOSIS, ASSOCIATED WITH MYELOPROLIFERATIVE DISORDERS

N.D. Khoroshko, N.V. Tsvetaeva, A.G. Turkina, V.S. Zhuravlev, E.A. Lukina, E.A. Semenova. *Haematological Research Centre, Moscow, Russia*

Twenty patients with chronic myeloproliferative disorders [2 had polycytemia vera (PV), 7 - essential thrombocytemia (ET), 11 - proliferative phase of idiopathic myelofibrosis (MF)], associated with hyperthrombocytosis, were treated with interferon-alpha (IFN-α) preparations (Intron-A, "Shering Plough"; Reaferon, Russia). The disease duration in 18 patients was 3–6 months, in 2–84 (PV) and 48 (ET) months; median age was - 45 years, there were 4 males and 16 females. At the beginning of IFN-α therapy the platelets level was 600–2450 × 10^9/L; the majority of patients had headaches, vertigo, vision disorders, pains in distal parts of extremities. Bone marrow biopsies showed both quantitative and qualitative megacaryocytic disorders: large dysplastic megacaryocytes, forming big clusters. Myelofibrosis was minimal, probably due to early diagnosis. Treatment with low doses of IFN-α (3 ME daily, then 2–3 times a week) and hydroxyurea (500–1000 mg/d) was associated with obvious releif of symptons and gradual lowering of platelet count (to < 450 × 10^9/L) in 3–6 months. Median folow-up is 12 months with the maximal of 72 months. The elevation of daily or weekly dose of IFN-α significantly worsens the life quality (alopecia, insomnia, depression), especially in elderly patients (>55 years). In 5 patients the clinical effectiveness was confirmed by positive shifts in sequential bone marrow biopsies. Therefore, IFN-α can be used as the first-line therapy in myeloproliferative disorders, presenting with hyperthrombocytosis.

P-1254 PLATELET AGGREGATION ABNORMALITIES IN ESSENTIAL THROMBOCYTHEMIA

A. Galanopoulos[1], A. Tsakri[1], V. Grigoraki[1], S. Hantziara[2], A. Mylonakis[1], N.I. Anagnostopoulos[1]. *[1]Dept. of Haematology, District General Hospital of Athens; [2] Blood Transfusion Center, District General Hospital of Athens, Greece*

The aim of the study is to evaluate the correlation of platelet aggregation with the clinical course of patients with essential thrombocythemia (ET). This retrospective study included 66 patients (pts) diagnosed with ET between 1989 and 1997 from a single Haematologic Unit. Diagnostic criteria for ET were those specified by the Polycythemia Vera Study Group. In 66 ET pts, 31 M and 35 F, M/F: 0.88, median age 69 years, thromboembolic complications of the large vessels were seen in 14%, microvascular disturbances in 12% and hemorrhages in 100%. Platelet aggregation was tested in 37 pts. Reduced platelet responses to ADP, collagen, epinephrine and arachidonic acid occured in 23/31 (83%) and loss of responsiveness only to epinephrine in 5/31 (16%). *In vitro* spontaneous aggregation (SPA) was observed in eight of the 24 tested pts (33%). Thromboembolic complications were seen in 14/31 pts (45%) with platelet aggregation abnormalities (PAA), in 3/6 pts (50%) with no PAA and in 4/8 pts (50%) with *in vitro* SPA. There was no statistically significant difference in the thromboembolic events between patients with and without PAA [1]. Also, there was no significant difference in the thrombotic episodes in patients with and without *in vitro* SPA. Bleeding disorders were only seen in 3% of pts with hypoaggregation. We did not find, a higher incidence of bleeding disorders in pts with more than 1 × 10^{12}/L platelets (4/31, 12%). In conclusion there is a high incidence of PAA and even *in vitro* SPA in ET patients but thrombotic or bleeding disorders are not correlated with them.

(1) Cancer 1990; 66:549–556.

P-1255 EXPRESSION OF CD71/GLY A AND CD71/CD33 IN PERIPHERICAL BLOOD SAMPLES IN ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA

N. Gonzalez, MªC. Mendoza, E. Gomez Sanz[1], J. Cesar, E. Sanz, C. Burgaleta. *Hematology Service. Ramón y Cajal and Gregorio Marañón[1] Hospitals. Madrid., Spain.*

Chronic myeloproliferative syndromes may show spontaneous proliferation of hematopoietic precursors in peripheral blood culture, as a manifestation of the stem cell proliferation. Identification of proliferative markers in peripherical blood may help to the differential diagnosis in polycythemia and essential thrombocytemia. Flow cytometry is useful to follow different stage of maturation in erythopoietic cells.

We study the expression of erythroid markers, CD71 (transferrin receptor) and glycophorin A (Gly A), and expression of CD71 and CD33 in 11 patients with ET, 5 patients with PV and 5 healthy donors, as control group.

Cell surface antigens were measured by flow cytometry using direct inmunofluorescence label of mononuclear cells from peripherical blood with monoclonal antibodies: CD71 (FITC)/Gly A (PE) and CD71 (FITC)/CD33 (PE) and analyzed in LYSYS II program. Blast region was selected in FSC/SSC dot-plot, and percentage of expression was analyzed in FL1/FL2 dot-plot. Autoflorescence was avoided using a negative control.

The percentage of cells CD71+ was statistically different (p < 0.05) respect to the control group (29±24%) in both PV (77±17%) and ET (80±10%) patients. The expression of CD71 measured as Mean fluorescence channel was greater (p < 0.05) in PV patients (533±66) than in ET patients (419±26) and in control group (475±63). The percentage of cells CD71+/Gly A+ and CD71+/CD33+ are represented in the table.

	% cells CD71+/GlyA+	% cells CD71+/CD33+
Controls	27±22	27±14
PV	59±26 (p < 0.1)	50±23 (p < 0.1)
ET	63±17 (p < 0.01)	55±18 (p < 0.01)

Patient s with PV and ET presented greater number of inmature cells of erythroid lineage in peripherical blood, than control group.

P-1256 EXPRESSION OF CD34/C-KIT IN PERIPHERICAL BLOOD IN ESSENTIAL THROMBOCYTHEMIA

C. Burgaleta, MªC. Mendoza, N. González, J. Cesar, E. Gomez Sanz[1], I. Fdez Fuertes. *Service Hematology. Ramón y Cajal and Gregorio Marañón[1] Hospitals. Madrid., Spain.*

Expression of CD34/CD117 (c-kit) is increased in bone marrow cells from Chronic myeloproliferative syndromes. Essential Thrombocythemia (ET) is difficult to asses by clinical parameters and identification of proliferative markers in peripherical blood may be of clinical utility.

Expression of the stem cell receptor (c-kit) and CD34 performed in peripherical blood in 24 patients with thrombocytosis. 21 were confirm as ET and 3 remain as reactive thrombocytosis. Results were compare with 10 healthy donors as control group.

Expression of CD34 and c-kit was measured by direct label in mononuclear cells from peripherical blood with monoclonal antibodies by flow cytometry and analyzed in LYSYS II program. Cells were selected in a gate by SSC/FSC and percentage of expression in FL1 and FL2 histograms. Autofluorescence was avoided by a negative control.

CD34/c-kit expression was significative superior in the group of ET patients. Patients without treatment (4) showed higher expression than patients under treatment with hydroxiurea.

Independent analysis of c-kit and CD34 showed increased expression of c-kit that was affected by previous treatment and slight increment of expression of CD34.

Those results shown the increased expression of proliferative markers in peripherical blood and the utility of c-kit in diagnosis of ET.

P-1257 ROMANIAN EXPERIENCENCE IN ESSENTIAL THROMBOCYTHEMIA DIAGNOSIS AND TREATMENT (THE NEW METHODS USE)

A.D. Moicean, S. Nicoara, A. Colita, E. Niculescu-Mizil, E. Butoianu, I. Popescu, D. Colita. *Fundeni Clinical Hospital, Bucuresti,, Romania*

We tested the Iland's logistic regression algorithm in order to differentiate the essential thrombocythemia (ET) from polycytemia vera with marked thrombocytosis (PVt). For ET treatment we tested the new agents such as interferon alfa and anagrelide. For Budd-Chiari syndrome, a major thrombotic complication of ET, we performed a porto-cave anastomosis. Between 1976-1996 in the Clinic of Hematology Fundeni Hospital, Bucuresti, Romania, 116 patients with thrombocytosis were hospitalised. 64 of them were diagnosed with ET according to the PVSG criteria. For 30 of them, we had applied the Iland's logistic regression algorithm (1987) to differentiate ET from PVt. For ET management we have been using the conventional cytotoxic chemotherapy (hydroxyurea, busulfan) and since 1990 we use the nonmutagenic and non-leukemogenic alternative therapy with interferon alfa and anagrelide. For a 35 year old female with Budd-Chiari syndrome and ET we performed a porto-cave anastomosis. 16 patients out of 30 were diagnosed with ET appling the Iland's logistic regression algorithm. 26 patients were submitted to hydroxyurea treatment; the level of platelets was 1364.148+/-409.544 x 10^9/l before treatment and 553.462+/-276.578 x 10^9/l after. 18 patients were submitted to busulfan therapy; the platelets' level was 1407.778+/-616.943 x 10^9/l before treatment and 495.294+/-241.929 x 10^9/l after. Three of our patients were submitted to recombinant interferon alfa and one to anagrelide. All these four patients had good results. The female with ET and a porto-cave anastomosis for Budd-Chiari syndrome is doing well, 18 months after the treatment. The Iland's logistic regression algorithm is very helpful to differentiate ET from PVt when the red blood cells mass assay is not conclusive. The vascular anastomosis saved our case with Budd-Chiari syndrome as complication in ET. For our patients the conventional cytotoxic chemotherapy with hidroxyurea and busulfan assured a good control of platelets level and complications. We still have a little experience with interferon and anagrelide in ET treatment.

Autoimmunity, immuno-hematology

P-1258 INCREASED CD5+/CD19+ B LYMPHOCYTE SUBSET IN AUTOIMMUNE DISORDERS

M.L. Smith, D.S. Richardson, L. Hendry, I.M.H. Chalmers, T. Milne, J.D. Cavenagh, A.C. Newland. *Department of Haematology, Royal London Hospital, London, UK*

CD5+/CD19+ lymphocytes (B-1 B cells) constitute a sub-population of normal B-lymphocytes. Several studies have suggested an increase in this population in both haematological and non-haematological autoimmune conditions such as rheumatoid arthritis and Grave's disease. Chronic lymphocytic leukaemia may represent a malignant expansion of these cells and this disease is associated with a significant incidence of autoimmune complications.

We have performed immunophenotypic analysis on the peripheral blood of 38 patients with autoimmune cytopenias, between April and December 1997 and report 7 patients (in 9 phenotyping episodes) who have an expansion of the CD5+/CD19+ lymphocyte population by dual immunofluorescence. Of these 5 had autoimmune thrombocytopenic purpura (ATP), 1 had autoimmune neutropenia (AIN) and 1 had autoimmune haemolytic anaemia (AIHA). Patients were aged between 13–76 years (median 36 years), six were male and five female. Seven patients had κ:λ light chain expression analysed and none showed a clonal pattern. The normal range for CD5+/CD19+ lymphocytes determined by our laboratory is 2.9% +/- 1.7%, with an absolute CD5+/CD19+ count of 6.4 +/- 4.4 x 10^7/l. Patients results ranged between 6.1–21.4% (median 11%), with absolute counts ranging from 15.8–99.1 x 10^7/l (median 24.8 x 10^7/l).

We conclude that increases in the CD5+/CD19+ lymphocyte population are more common than has been previously thought and that further studies are required to determine the prognostic and clinical significance of this finding.

P-1259 CROSS-LINKING OF THE MONOCLONAL IgM ANTI-Sia-Ib (ANTI-Gd) COLD AGGLUTININ GAS TO SIALYLATED ANTIGENS INDUCES ACTIVATION OF RESPIRATORY BURST IN GRANULOCYTES

A. Serra, A. Pereira, C. Sanz, T. Gallart. *Hospital Clinic. Barcelona, Spain*

Introduction: Cold agglutinins (CAs) directed against sialylated antigens (Ag) are infrequent, but their interest is increasing because of the relationship of these Ag to adhesion molecules. In our Department, we have characterized the monoclonal IgM anti-Sia-Ib (anti-Gd) CA GAS (Blood 1994; 83:1310 and Blood 1997; 90: 1576) which recognizes carbohydrate structures similar to selectin ligands sialylLe^x and sialylLe^a. Since sialyl-Le^x has recently been recognized as a cell activation receptor, we have investigated the ability of IgM GAS to activate respiratory burst in peripheral blood phagocytes.

Objetive: Investigating if the Ag recognized by the monoclonal IgM CA GAS is involved in phagocyte activation.

Methods: Monoclonal IgM GAS and a polyclonal IgM control were purified from sera by absortion-elution over red blood cells and gel-filtration chromatography (Sephacryl S300). Normal granulocytes and monocytes were isolated by Ficoll density gradient centrifugation and elutriation, respectively. Respiratory burst activation was measured by lucigenin-enhanced chemiluminiscence.

Results: IgM GAS activated the respiratory burst of peripheral blood granulocytes, but not monocytes, only upon cross-linking with anti-human IgM. This effect was inhibited in a dose-dependent manner when IgMGAS was previously incubated with sialyl-lactose.

Conclusion: Sialylated Ag recognized by the monoclonal IgM CA GAS are cell activation receptors in peripheral blood granulocytes.

P-1260 HEMOPOIETIC AND IMMUNOLOGICAL ABNORMALITIES IN TWO PATIENTS WITH LYSINURIC PROTEIN INTOLERANCE

C. Van den Bos[1,4], T.J. De Koning[2], M.B. Bierings[1], B.T. Poll-The[2], B.J.M. Zegers[3], G.T. Rijkers[3], T. Révész[1]. [4]*Dutch Cancer Society (NKB-KWF);* [1]*Departments of Hematology;* [2]*Metabolic Diseases;* [3]*Immunology, Wilhelmina Kinderziekenhuis/University Hospital Utrecht, The Netherlands*

Lysinuric protein intolerance (LPI) is an autosomal recessive defect in the transport of the dibasic amino acids lysine, arginine and ornithine at the cell membrane. Clinically, patients may present with an aversion to a protein rich diet, postprandial episodes of hyperammonemia, failure to thrive, poor growth, hepatosplenomegaly, osteoporosis and a variable degree of mental retardation. Both peripheral blood and bone marrow (BM) abnormalities have been reported. In BM hemophagocytosis was observed by both mature histiocytes and granulocyte precursors. Peripheral blood abnormalities include a recurrent mild hypochromic (hemolytic) anemia, and leuko- and/or thrombocytopenia. Immunological abnormalities such as poor vaccination responses have also been described. In our hospital two LPI-patients were evaluated both hematologically and immunologically. One patient of mediterranean origin has a recurrent mild anemia with clear hemophagocytosis in BM. On vaccination this patient showed an abnormal response to pneumococcal polysaccharide vaccine, and a normal response to tetanus and diphtheria booster vaccination. Cellular analysis showed a relative and absolute lymphocytopenia of both CD4+ and CD8+ T-cells. B-cell numbers (CD20+) were in the normal range, as were NK-cells (CD16-57+). The second patient of Dutch origin has so far not shown any peripheral blood abnormalities. Adequate responses to pneumococcal polysaccharide vaccine and Haemophilus influenza type b conjugate vaccine were observed. Analysis of peripheral blood lymphocytes showed T- and B-cells in the lower normal range, NK-cells in the upper normal range. BM-aspiration surprisingly revealed intramedullary hemophagocytosis.

On the basis of these observations the following can be concluded: i) intramedullary hemophagocytosis is a constant feature of LPI, ii) intramedullary hemophagocytosis can be observed in the absence of peripheral blood abnormalities, iii) in contrast to hemophagocytic lymphohistiocytosis normal NK-cell numbers were observed, iv) the abnormal vaccination responses observed by others and in one of our patients require in depth analysis of T- and B-cell function.

P-1261 BIRDSHOT RETINOPATHY SIMULATING AN OCULAR LEUKEMIC RELAPSE

Ph. Rousselot, S. Roman, A.L. Taksin, M.T. Daniel, M. Ullern, J.M. Miclea, H. Dombret, L. Degos, L. Jablon. *Hôpital Saint-Louis, Hôpital des Quinze-vingts, Paris, France*

Background: Ocular manifestation in leukemic patients raise the question of a specific localization of the leukemic process. We report the case of the association of an inflammatory birdshot retinopathy and acute myeloblastic leukemia (AML), simulating an ocular leukemic relapse.

Case report: A 49-year-old woman was admitted in February 1996 for AML1 (in the FAB classification), with a WBC at 61 000/mm^3 and cervical lymph nodes infiltration. Complete remission was achieved after the induction

course followed by two consolidation courses. CNS prophylaxis consisted of 6 intrathecal MTX-AraC injections and a 12 Gy irradiation. In May 1997 she started to complain about blurred vision and floaters in both eyes. The eyes were quiet, white and painless. Visual acuity was RE 0.6 and LE 0.8. Slit-lamp observation showed a few cells in the anterior chamber, lenses were clear and numerous cells were found in anterior and posterior vitreous without exsudate over the *pars plana*. Maculae seemed normal and optic nerve heads had edematous appearance. The fundus examination showed no evidence of birdshot spots. Fluorescent angiography revealed some hypopigmented spots deep into the retina, retinal vessels vascularitis and papillary edema. A vitreous biopsy was performed by vitrectomy to formally eliminate a specific leukemic ocular relapse. Cytological examination revealed a polymorphous infiltration of lymphocytes, monocytes and polymorphonuclear cells without evidence of blasts. The presence of HLA A29 associated with the clinical features confirmed the diagnosis of birdshot retinopathy. The bone marrow aspiration noted the persistence of the complete remission and cerebrospinal fluid examination was normal.

Conclusion: Birdshot retinopathy is a rare autoimmune ocular disease directed against the retinal S antigen which is associated with the HLA A29 allele of the major histocompatibility complex. Recommended therapy include immunosuppressive treatment or intravenous immunoglobulin. To our knowledge, the association between birdshot retinopathy and AML has not been previously described. Invasive procedure to obtain a cytological diagnosis in leukemic patients with ocular manifestation simulating a leukemic relapse is justified.

P-1262 DETECTION OF PLATELET ANTIBODIES (PIAb) BY SOLID PHASE RED CELL ADHERENCE (SPRCA) TEST IN VARIOUS DISEASES

A. Kaperonis, A. Antypas, E. Koukou, N. Sakarellou. *Elpis State General Hospital of Athens, Greece*

SPRCA (Immucor Capture P and Modified Capture P) was used in 38 adult patients with low platelet counts (PC < 150 K/UL) to detect IgG-allo and auto PIAb. The allo-PIAb detected were either HLA and/or platelet specific. 16 patients with idiopathic Thrombocytopenic Purpura (ITP) (fem. 7 pregnant),6 with Myelodysplastic Syndrome (MDS) (male 1, fem. 5), 3 multitransfused patients MT (male 2, fem. 1) and 13 with various hematologic malignancies (HM) (male 10, fem. 3) were examined.

In ITP, 5/16 (31,3%) had allo-PIAb (PC < 80 K/UL) and 6/16 (37,5%) had auto - PIAb (PC in 4 >80 K/UL). In MDS, only 1/16 (16,7%) (PC < 40 K/UL and multitransfused) had detectable allo-PIAb.

In the MT,1,3 (33,3%), a patient with Cooley's Anemia developed allo-PIAb despite leukocyte depleted transfusions and a high PC (>400 K/UL). None of the HM had detectable PIAb despite PC < 80 K/UL in 3/13 (23%)

In conclusion, SPRCA is able to detect PIAb in 11/16 (68,8%). ITP patients, 16.7% MDS patients and 33,3% of the MT. Moreover patients with HM may have very low PC without detectable PIAb. In order to minimize sensitization and alloimmunization, all multitransfused patients should adhere to leukocyte poor and preferably apheresis (one donor) blood products.

P-1263 IMMUNOPHENOTYPICAL CHARACTERIZATION OF BLOOD AND SPLENIC CELLS IN AUTOIMMUNE HEMOLYTIC ANEMIA

Y.L. Yevstakhevich, I.Y. Yevstakhevich, O.M. Tsiapka, G.B. Lebid, V.L. Novak, V.F. Indenko, V.O. Loginsky. *Research Institute of Hematology & Blood Transfusion, Lviv, Ukraine*

Spleen lymphoid system is involved in disease cours of autoimmune hemolytic anemia (AIHA) what is confirmed by benefit of splenectomy. To reveal its role in the disorder, we carried out the immunophenotypical investigations of blood and splenic cells obtained from 11 patients splenectomized for AIHA. Immunophenotype was assayed by immunofluorescent technique using panel of monoclonal antibodies. In peripheral blood no marked abnormalities were found in percent of B cells expressed the main B-linear antigens (CD20, CD21, CD22, also HLA Dr). However, the expression of DC37, marker of B cells originated from germinal centres, was significantly decreased. The peripheral B cells were activated and demonstrated an exceeded positivity for certain activation markers (CD25R, CD30, CD11b). Lymphoid cells from removed AIHA spleens did not differ apparently as regard to expression of B cell antigens including CD37 from cells of healthy spleen. Only HLA Dr expression on splenic lymphoid cells from AIHA was elevated. The cells lacked increased expression of the activation markers. A significant decrease of T cells expressed CD3 and CD5 was observed in blood as well as in spleen of the AIHA patients. In both cases CD4+ cell count appeared to by markedly decreased, especially in spleen, and CD8+ cell percent raised significantly over normal values. This resulted in a diminished CD4+/CD8+ ratio to 0.46±0.06 in blood and to 0.14±0.02 in spleen. CD16 cell level did not differ considerably from normal values. Thus, the results indicate on dependence of the observed changes in blood and spleenic lymphoid cell subsets from autoimmune process in AIHA.

P-1264 BASIC RESEARCH ON THE EFFECTS OF DOING EXERCISE ON THE IMMUNE RESPONSE

M. Masuhara, S. Takise[1], X.R. Lee[2], I. Tsuda, N. Tatsumi[3]. *[1]Osaka University of Health and Sport Sciences, Laboratory of Exercise Physiology; [3]Osaka City University, School of Medicine, Laboratory of Clinical Medicine, Japan; [2]Xi'an Institute of Physical Education, China*

The purpose of this investigation is to examine effects of doing exercise on the immune response. On this study, I used some animal models. Thirty male rats were divided into six groups. They performed exercise training from the 4th old - week through the 47th old - week. This training-term was made up of two stages.

The results may be summarized as folows:

1. The exercise training of long term caused the significant changes on the counts of leukocytes, neutrophiles, and lymphocytes and its subsets (CD4, CD8, CD61, CD11 and RT1A etc.). On the above mentioned parameters, exercise training group were significantly lower than non exercise group, but one group that do heavy exercise from the 4th old-week to the 27th old-week and stop the doing exercise from the 27th old-week to the 46th old-week was slightly higher than non exercise group.
2. The serum cortisol concentration of the group that do heavy exercise from the 4th old-week to the 27th old-week and do the moderate exercise from the 27th old-week to the 46th old-week was significantly higher than the other group. And also on the serum adrenaline and on the noradrenaline concentration, moderate trainning groups were slightly higher than the other group, but the cortizol concentration of these groups were lowest than of the other groups.
3. All these changes were dependent on the intensity and time of doing exercise, and especially the period of performing exercise is very important for the effects of doing exercise on the immune response.

P-1265 THE EFFECT OF TRANSFER FACTOR ON GRANULOCYTE-MACROPHAGUE COLONY FORMING UNITS IN ACQUIRED APLASTIC ANEMIA

V.E. Ferrer, R. González, F. Massó, L. Díaz de León, E. Miranda, K. Willms. *National University of México and México General Hostipal. Mexico Cuty, Mexico*

We studied the effect of an immunemodulator, transfer factor (TF), on colony forming units (CFU-C) in the blood of patients with AAA to see if it corrected the suppression of hemopoiesis in immune-mediated acquired aplastic anemia (AAA). TF was prepared following Lawrence's technique from leukoconcentrates of normal blood donors. Mononuclear cells from 5 patients were incubated with(+) or without(−) TF in RPMI at a 5:1 ratio of TF:patient's lymphocytes during 36 hours at 37°C with a CO2 flow of 5%. Normal controls + and - TF were also incubated. The cells were washed with RPMI and cultured in double layer soft agar. Lectures were done on day 14. Besides, normal cells were cultured with the supernatants (SN) of the incubated AAA patient's cells.

Results: while the cells of the 5 patients incubated without TF developed 0–7 colonies (x– = 2.4), those treated with TF developed 12–52 (x– = 30.8). Normal controls showed no changes + or - TF. The SN TF- of 2 patients lowered the No. of CFU-c of a normal control from 142 to 14 and 4 while the No. of CFU-c with SN + TF was 51 and 29. These results seem to support the hypothesis that TF might correct the suppression of hemopoiesis in immune-mediated AAA.

P-1266 AUTOIMMUNE HEMOLYTIC ANEMIA IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

I. Tijanic, M. Bakic, J. Tasic, L. Macukanovic-Golubovic, T. Vukicevic, M. Milenovic. *Clinic of Hematology and Clinical Immunology, Clinical Center Nis Serbia, Yugoslavia*

By definition autoimmune hemolytic anemia (AIHA) is due to increased destruction of red cells by autoantibodies. Autoimmune hemolytic anemia is a frequent complication of chronic lymphocytic leukemia (CLL). AIHA occurs in 10–20% of the patients with CLL. The aim of this study was to investigate: the severity of AIHA in CLL patients, the influence of particular parameters upon AIHA, as well as the efficiency of corticosteroid therapy.

We have investigated 27 CLL patients with AIHA, while he also had a control group of 28 patients with idiopathic AIHA. Both groups showed similar anemia degrees. The direct antiglobulin test (DAT) was positive in great percentage of both groups (90,00% and 92.86%). The greatest number of CLL patients (62.96%) with AIHA were in 4th clinical stage (Rai), yet it appeared that the clinical stage had no influence upon the anemia degree. In CLL patients, the severity of anemia was not correlated with the lymphocyte count. The degree of DAT positivity and the spleen size had no impact on the AIHA severity in both groups of patients. The corticosteroid therapy proved to be satisfactory in most of the AIHA patients treated.

In summary, the course of AIHA in CLL is quite variable and unpredictable.

Bone marrow transplantation: clinical

P-1267 SUCCESSFUL REGRESSION OF HTLV-1 IN A PATIENT WITH ADULT T-CELL LEUKEMIA TREATED BY ALLOGENEIC BONE MARROW TRANSPLANTATION

Kenichirou Tajima, Kazutaka Uehira, Masakazu Hanada, Hiroyuki Kitajima, Yuji Kishimoto, Shiro Fukuhara. *1st Dept Int Med, Kansai Med Univ, Moriguchi Osaka 570, Japan*

Adult T-cell leukemia (ATL) associated with HTLV-1 is a refractory disease of highly poor prognosis. We described here a patient with ATL treated by allogeneic bone marrow transplantation (BMT), who showed successful regression of HTLV-1. The patient was 47-year-old man who was referred to our hospital because of increasing in number of abnormal T-lymphoid cells in peripheral blood. He was positive for anti-HTLV-1 antibody, and a diagnosis of acute ATL involving gastro-intestinal tract was made. He received several courses of combination chemotherapy including CHOP regimen, however, he failed to achieve any remission. Following a conditioning of myeloablation with cyclophosphamide, MCNU (ranimustine) and total body irradiation, he received a treatment of allogeneic BMT from his HTLV-1 negative HLA-identical sister. He showed stable engraftment with complete chimerism, and he developed acute GVHD affecting the skin at grade II, which responded well to corticosteroids and cyclosporine A. Shortly after that, the patient was clinically and hematologically evaluated to have a complete remission. HTLV-1 proviral DNA was decreased below the detectable level of the competitive PCR tested, and the titer of anti-HTLV-1 antibody was also decreased but minimally detectable at six months after BMT. Our clinical experience suggests that allogeneic BMT could be a curable oriented treatment for ATL, and the donor derived immunity is expected to account for eradication of the HTLV-1 harboring cells.

P-1268 TRANSPLANTS FOR HAEMATOLOGICAL MALIGNANCIES IN PATIENTS ABOVE THE AGE OF 50 YEAR

S. Kulkarni, R. Powles, J. Mehta, S. Singhal, C. Horton, J. Treleaven. *Royal Marsden NHS Trust, Sutton, UK*

Prospectively maintained database at this hospital was searched to identify 22 patients above 50 yr. age undergoing 24 allografts (M:13, F11; median age 51 yr. Range 50–55) The diagnosis were AML (n = 6), CML (n = 7), Myeloma (n = 4), CLL (n = 2), MDS (n = 3) ALL (n = 1) and MD (n = 1). Conditioning therapy was Mel/TBI (n = 16), BuCy (n = 6), and Bu/Mel (n = 1) and 1 received DLI without conditioning. CsA/MTX (n = 21), CSA (n = 2) or MTX (n = 1) was used as GVH prophylaxis. Median days to ANC > 0.5 × 109/L and platelets >50 × 109/L were 21 d (7–32) and 27 d (7–143). Two patients had graft failure. 13 died due to transplant toxicity and 2 died due to leukaemia. Probability of toxic death was 60% at 1 yr. (95% CI 37.9–79.7). 3 yr. probability of relapse and event free survival (EFS) was 9.1% (−7.9%–26.1%) and 32% (12.2–52%).

37 autografts/syngeneic grafts were done (M:19, F:18; median age 53 yr, range 50–62) for AML (n = 20), ALL (n = 6), MDS (n = 5), T-PLL (n = 2), myeloma (n = 3, syngeneic) and CML (n = 1). Conditioning was done with mel/TBI (n = 18), Mel (n = 14), Bu-Mel (n = 2), BuCy (n = 1) and others (n = 2). The median days to ANC > 0.5 × 109/L and platelet >50 × 109/L was 21.5 d (13–102) and 39.5 d (15–98). 5 patients died due to treatment toxicity. 12 patients relapsed. The probability of toxic death was 21.1% (95% CI 5.4–36.9). The 3 yr. EFS and relapse probability was 18.4% (1.6–35.2) and 45% (19.1–70.7) and relapse was not influenced by addition of TBI to conditioning. We conclude that both auto-and allograft are associated with significantly higher mortality in this age group but allograft can induce long term success in significant number of patients. Proper selection of patients and reduced intensity of conditioning may improve the results.

P-1269 INTENSIVE CHEMOTHERAPY (CHT), ALLOGENEIC (ALLO) OR AUTOLOGOUS (AUTO) HEMATOPOIETIC CELLS TRANSPLANTATION (HCT) FOR HIGH-RISK ALL (HRALL). RESULTS OF THE ONGOING PROTOCOL PETHEMA ALL-93

J.M. Ribera, J.J. Ortega, A. Oriol, M. Fontanillas, J. Maldonado, R. Parody, C. Rivas, J.M. Hernandez-Rivas, E. Del Potro, P. Bastida, M.E. González, C. Calvo, J.M. Rodríguez, E. Feliu, J.F. San Miguel. *PETHEMA Group; Spanish Society of Hematology, Hospital Germans Trias i Pujol, Badalona, Spain*

Objective: To analyse the preliminary results of the ongoing multicenter prospective randomized protocol PETHEMA ALL-93 for HRALL patients.

Design: Induction (5 wk): vincristine (VCR), daunorubicin, prednisone, asparaginase (ASP), cyclophosphamide (CFM). Intensification: 3 cycles of monthly intensive chemotherapy including VCR, dexamethasone, mitoxantrone, CFM, high-dose ASP, methotrexate (MTX) and cytarabine, as well as teniposide and mercaptopurine (MP). Patients with HLA-identical sibling received ALLO-HCT, and the remaining were randomised to AUTO-HCT or to the same intensive chemotherapy followed by maintenance treatment (MP, MTX) for 2 yr.

Characteristics of the Series: 22 hospitals, 110 evaluable pts, 66 males, age (x±SD) 25±13 yr (range 1–50), WBC (x 10^9/L) 106±151 (0.5–822), ALL1 38, ALL2 72, early pre-B 30, common+pre-B 40, T 40, MyALL 50. Cytogenetics (n = 99): normal 34, no metaphases 22, hypodipoidy 6, pseudodiploidy 32 (Ph+18).

Response to therapy (January 1998): early death 1 pt, no response 14 (14%), CR 95 (85%), BM blast cells >10% at day 14 37/110 (34%). Major toxicity during intensification 6/86 pts (2 death, 4 exclusions from the trial). Toxic death rate in ALLO-HCT 15%; no deaths in AUTO-HCT and in CHT arms. 4-yr EFS and OS probabilities were:

	N	Median EFS	4-yr EFS	Median OS	4-yr OS
Overall	110	12 (8–16)	23 (13–33)	22 (14–29)	27 (15–39)
ALLO-HCT	40*	18 (0–47)	32 (18–46)	32 (16–49)	41 (29–53)
AUTO-HCT	23*	11 (6–16)	41 (19–63)	NR	51 (27–75)
CHT	21*	17 (2–32)	24 (6–42)	26 (14–38)	15 (0–41)

* Intention-to-treat analysis. In brackets, 95% CI. Medians expressed in months.

Conclusions: The preliminary results and the toxicity of the ongoing PETHEMA ALL-93 trial are acceptable. Up to now there are no differences in EFS and OS for CHT, ALLO and AUTO-HCT.

Supported by grants FIS 97/1049 and FIJC 97-PTH, FIJC 97 P-EF.

P-1270 OUTCOME OF ALLOGENEIC BLOOD STEM CELL TRANSPLANTATION FOR ADVANCED HAEMATOLOGICAL MALIGNANCY

G. Miflin, J.L. Byrne, C.J. Stainer, A.P. Haynes, N.H. Russell. *Nottingham City Hospital, Nottingham, UK*

Since 1993 we have undertaken 33 transplants using G-CSF mobilised PBSC. This analysis is of 22 patients either solid tumour (n = 2) or advanced haematological malignancy (n = 20) (myeloma 6, lymphoma 8, CML 1, ALL 2, AML 3) including 4 patients who had undergone previous autologous transplantation and 2 patients transplanted from 1 antigen mismatched donors. The median age of the recipients was 43 yr (23–57 yr) and 14 patients were >40 years. Only 2/22 patients developed > grade II acute GVHD and 6 out of 15 evaluable patients have developed chronic GVHD, all patients engrafted.

Compared with a group of patients undergoing allogeneic BMT both haematopoietic and immune reconstitution has been more rapid with a significantly accelerated recovery of CD4+ cells in patients with fully matched donors. Full donor chimerism was achieved earlier at a median of day +8 following PBSCT compared to day +18 post BMT ($p < 0.05$). In a similar group of patients with advanced disease or 1 antigen mismatched transplants (n = 5) undergoing allogeneic BMT (n = 35, median age 46 yr, 15–58 yr) (myeloma 13, MDS 5, CML 3, ALL 7, AML 4, lymphoma 3) 6/32 developed > grade II GVHD and 6/18 evaluable patients have developed chronic GVHD, 1 mismatched donor failed to engraft with BM and was rescued with PBSC. The disease free outcome has been improved following allogeneic PBSCT with a disease free survival at 2 yr of 72% compared to 35% for patients undergoing BMT ($p = 0.07$). This improvement is mainly due to a reduction in both early and late non-relapse mortality from 40% to 21% and reduced risk of relapse. These results suggest that more rapid haematopoietic and immune reconstitution may translate into a reduction of TRM and reduced relapse in patients undergoing allogeneic transplantation for advanced haematological malignancies.

P-1271 BONE MARROW TRANSPLANTATION FOR THERAPY RELATED MYLODYSPLASIA (t-MDS) AND ACUTE LEUKEMIA (t-AL): EXPERIENCE OF SAINT LOUIS HOSPITAL (PARIS)

I. Yakoub-Agha, P. Ribaud, A. Devergie, F. Garnier, G. Damaj, V. Rocha, L. Garderet, F. Selimi, P. Guardiola, G. Socié, H. Esperou, E. Gluckman. *Department of bone marrow transplantation, Saint-Louis hospital, Paris, France*

Between June 1980 and November 1997, thirteen patients underwent allogeneic bone marrow transplantation for t-MDS and t-AL defined as MDS or AML following prior cytotoxic exposure for cancers (2 Non Hodgkin Lymphomas, 4 Hodgkin diseases, 3 Breast cancers, 1 Choriocarcinoma, 1 Osteosarcoma, 1 Medulloblastoma and 1 Teratocarcinoma). Patients were treated by chemotherapy alone (4/13) and chemotherapy + radiotherapy (9/13) including two hematopoietic stem cell autografts. Seven patients developed secondary AML, one developed RAEBt and five developed RAEB, one from the latter group had progressed to AML at time of transplantation. The median time from diagnosis of the first cancer to the secondary hemopathy was four years (range: 6 months–11 years). Four AML patients were treated by induction chemotherapy before BMT. The median age at transplantation was 30 years (16–50 y). Patients were transplanted in first complete remission. (n = 3), untreated AML (n = 4), refractory AML (n = 1), RAEB (n = 4) and in RAEBt (n = 1). Donors were identical sibling, (n = 9), syngeneic (n = 1), unrelated bone marrow (n = 2) and unrelated cord blood (n = 1; one locus mismatched). The preparative regimens used were total body irradiation (TBI) and Cyclophosphamide (CY) for two patients, Misulban (BU) + CY + Etoposide (VP16) for five patients, BU + CY for three patients, TBI + Cytosine Arabinoside + CY for one patient, BU + CY + Thiotepa for one patient and CY + Melphalan for one patient. The three unrelated transplanted patients had received, in addition, Anti-Thymoglobuline. Two patients died at d13 and d62 without neutrophil engraftment. All remaining patients reached neutrophil count of 0.5 $\times 10^9$/l at a median of d20 (range 12–35 d). One patient rejected the graft at three months. Two patients developed acute grades 3–4 GVHD at d18 and d64 and five patients developed acute 0–2 grades GVHD between d14 and d30. Eleven of the thirteen patients died between d13 and d366 (median d135). Four patients died from relapse, three from Aspergillus infection, two from GVHD, and two from graft failure. One patient is alive in continuous complete remission (follow up 10 years) and one is alive in relapse (follow up 8 months).

Conclusion: The outcome of BMT in therapy related myelodysplasia and leukemia is very poor (median of survival is 5 months after BMT). This might be due to the disease status before BMT. These results suggest the need of delineating indications and performing prospective protocols to improve results.

P-1272 SURVIVAL AFTER ALLOGENOUS BONE MARROW TRANSPLANTATION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) IS NOT INFLUENCED BY PRETRANSPLANT INTERFERON α THERAPY

R. Hehlmann, A. Hochhaus, U. Berger, A. Muth, H.J. Kolb, M. Pfirrmann, J. Hasford. *The German CML Study Group; III. Medizinische Klinik, Klinikum Mannheim, Universität Heidelberg, Mannheim, Germany*

Conflicting results exist on the influence of pretransplant interferon-α (IFN) treatment on the outcome of allogenous bone marrow transplantation (BMT) in CML. Therefore, we analyzed 193 transplanted CML patients (135 m., 58 f.) representing 23% of a total of 856 randomized Ph+ or BCR-ABL+ patients of the German CML trials I (IFN vs. hydroxyurea [HU] vs. busulfan, recruitment 1983–91) and II (IFN + HU vs. HU, recruitment 1991–94). Median age at BMT was 35.4 (range 9.1–58.3) years. Median follow up after transplantation was 1.3 years for all patients and 3.4 years for living patients. Of 161 patients transplanted in first chronic phase 82 were pretreated with IFN, 52 with HU and 27 with busulfan. 44 patients (27%) received transplants from matched unrelated donors. 37 patients (23%) were transplanted within the first year after diagnosis (early BMT, median time to transplantation 8 months), 124 patients after this time (late BMT, median time to transplantation 23 months). There was no significant difference for prognostic parameters between pretransplant treatment groups except donor status. By 2/98 70 of the 161 chronic phase patients have died. The outcome of patients receiving related vs. unrelated transplants was not significantly different ($p > 0.6$). 5 years survival after transplantation is 55% for all patients (55% for IFN, 55% for HU, 57% for busulfan patients). Neither in the early ($p > 0.8$) nor in the late transplantation group ($p > 0.7$) an influence of pre-transplant treatment on survival is recognizable. In patients pretreated with IFN survival after BMT is not different for patients with early vs. late transplantation. We conclude on the basis of randomized trials that pretransplant treatment with IFN is not disadvantageous for subsequent BMT. IFN patients sent for BMT should be monitored for negative selection, since CML patients responding well to IFN and achieving cytogenetic remissions may be less likely sent to transplantation than IFN non-responders.

P-1273 TREATMENT OF CHRONIC MYELOID LEUKEMIA RELAPSES AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

H. Klamová, M. Lukášová, A. Vítek, J. Sajdová, J. Hrabánek, J. Moravcová, C. Haškovec, J. Břziková, K. Michalová[1], Z. Zemanová[1]. *Institute of Hematology and Blood Transfusion, Prague; [1]3rd Medical Department of 1st Medical Faculty, Prague, Czech Republic*

Relapses of chronic meyloid leukemia (CML) after allogeneic bone marrow transplantation (BMT) appear in 30–40% patients. Careful monitoring of the early signs of the relapse and timely treatment may increase the chance of survival of the relapsed patients.

The chance for the complete cure of CML by allogeneic bone marrow transplantation is affected by the conditioning regimen and by an anti-leukemic effect of lymphocytes from the original donor.

In the Institute of Hematology and Blood Transfusion in Prague we performed allogeneic BMT in 52 patients with CML between 1986 and 1997. Minimal residual disease after BMT was monitored in 29 patients by reverse transcription-polymerase chain reaction (RT-PCR). The results were compared with cytogenetics and fluorescence in situ hybridisation with the aim of relapse prediction.

Molecular relapse was demonstrated in 7/29 patients (27%), 6 of these patients had a cytogenetic relapse, and hematologic relapse appeared in 1 patient. The 7 patients with the signs of molecular relapse were treated with donor lymphocyte infusions (DLI) and/or interferon-alpha. 5 patients achieved the complete cytogenetic remission including the absence of molecular signs of the disease (RT-PCR negative status). 1 patient died in the hematologic relapse, one patient is only one month after DLI.

Quantitative RT-PCR for the bcr/abl transcript proved to be an important signal of the imminent relapse. The treatment with DLI and interferon started in the early stage of the relapse when the number of malignant cells is still low has a higher probability of success.

P-1274 PROGNOSTIC FACTORS INFLUENCING EARLY TRANSPLANT RELATED MORTALITY (TRM) AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION WITH HLA-IDENTICAL SIBLING DONORS FOR CHRONIC MYELOID LEUKAEMIA (CML)

D. Capelli[1], R.M. Szydlo[1], D.G. Savage[2], J.F. Apperley[1], J.M. Goldman[1]. *[1]ICSM at Hammersmith Hospital, London, UK; [2]Division of Medical Oncology, Columbia-Presbyterian Medical Center, New York, USA*

Allogeneic bone marrow transplantation (alloBMT) is a curative procedure for CML. However, one of the main causes of failure is early TRM. We have therefore looked at prognostic factors for non-leukaemic deaths within the first year after alloBMT in 254 consecutive patients receiving transplant from HLA-identical sibling donors between 1979 and 1996 (151 1st chronic phase, 103 advanced phase). Conditioning consisted of cyclophosphamide±busulphan, total body irradiation, splenic irradiation, daunorubicin or additional immunosuppression. Graft-versus-host disease (GVHD) prophylaxis was provided by the use of *ex-vivo* or *in-vivo* T-cell depletion with Campath 1M/1H (n = 78), or cyclosporin ± methotrexate (n = 176). The probabilities of TRM at 1 yr for chronic and advanced phases were 19.4% and 39.6% respectively (p = 0.0002). Thirty-two patient, disease and transplant factors were considered as prognostic variables in univariate analyses and stratified for disease stage; those achieving a level of significance p < 0.20 were entered into a proportional hazards regression analyses using a backward stepping approach. For chronic phase 4 factors were found to be associated with early death: use of more than two drugs as pre-transplant chemotherapy (RR = 1.67, p = 0.03), non caucasian patient ethnic origin (RR = 1.67, p = 0.01), ≥2% basophils in the pre-transplant bone marrow (RR = 1.49, p = 0.05) and severe (grade III–IV) acute GVHD (AGVHD) (RR = 1.78, p = 0.005). For advanced phase disease, only the severity of AGVHD was found to be significant (RR: 2.44, p < 0.0001). In conclusion, methods to predict and prevent acute GVHD need further attention since its occurrence is the major risk factor for mortality early after alloBMT.

P-1275 DIFFERENT TREATMENT MODALITIES IN CHRONIC MYELOID LEUKEMIA: A SINGLE INSTITUTION EXPERIENCE

J. Mohm, F. Kroschinsky, M. Bornhäuser, R. Ordemann, B. Mohr, C. Thiede, A. Neubauer, G. Ehninger. *Medizinische Klinik und Poliklinik I, Universitätsklinikum Carl Gustav Carus Dresden, Germany*

With growing knowledge about the pathophysiologic mechanisms and therapeutic options in chronic myeloid leukemia (CML), individually tailored, risk-adapted treatment regimens get more and more important. Besides the results of multicenter studies, the local, single institution experience is an important basis for therapeutic decisions.

We thus evaluated the data of all patients with CML, which were treated in our institution since 1995 (n = 53). Among the 26 females and 27 males were 50 with typical, Philadelphia-chromosome positive CML, 3 were Philadelphia (and Bcr-Abl) negative. The age at diagnosis ranged from 7 to 76 with a mean of 40.6 years. 25 patients were treated conventionally with interferon-α, hydroxyurea or busulfan. 28 patients underwent stem cell transplantation of different kinds: 5 autologous, 8 sibling donor and 15 matched unrelated donor transplants.

We demonstrate the follow-up of all patients with regard to their known risk factors (e.g. age, stage of disease, response to the first treatment, chromosomal aberrations) and cytogenetic and moleculargenetic investigations at different disease stages.

From this analysis, it seems that the individual risk profile has a strong prognostic relevance and should influence the choice of therapy. Only an individualized, risk-adapted, multi-modal therapy concept will therefore be most beneficial for patients with CML.

P-1276 RECONSTITUTION WITH BCR-ABL NEGATIVE RECIPIENT HAEMATOPOIESIS AFTER ALLOGENEIC BMT FOR CML

J. Serrano, J. Román, J.A. Castillejo, J.A. Navarro, A. Pascual, I. García-Rubio, M. Falcón, C. Martín, J. Sánchez, C. Herrera, A. Torres. *Haematology Department. Reina Sofia Hospital. Córdoba, Spain*

Mixed Chimerism (MC), presence of both recipient and donor hematopoietic cells, is frequently identified in CML patients who receive a T-Depleted BMT. Usually these residual host cells show positive Bcr-Abl transcripts and the likelihood of subsequent clinical relapse is high.

We report a 46-year-old man diagnosed with Ph' chromosome(+) CML who underwent an HLA-identical T-Depleted Allogeneic BMT from a sibling donor one year after diagnosis. DNA polymorphism analysis amplified by PCR show complete donor chimerism immediately post-BMT, followed by MC by day +120 post-BMT. Highly purified cell population analysis disclosed that Granulocyte, Natural-Killer cells and B-Lymphocytes were mixed donor-host type, but T-Lymphocytes were host derived. RT-PCR analysis revealed negativity of Bcr-Abl transcripts at day +120 or subsequent samples two years post-BMT.

This patient is unique insofar as non-malignant cells of host origin were able to re-establish hematopoiesis 4 month after transplant and predominated for more than 2 years without molecular or haematology relapse. So, MC on manipulated BMT for CML is not a predictor of impending relapse, and more important, is not always associated with minimal residual disease (MRD). Lack of MRD in our patient is independent of graft versus leukaemia effect, because T cells are host origin and he did not developed GVHD, thus did not benefit from their immunologic advantage. In this case, non-malignant host myeloid progenitor cells survived the conditioning regimen and would have had a growth advantage over neoplasm cells. This finding could support the potential use of autologous BMT for CML in early-phase of disease when there are Ph'negative-stem cells, already.

P-1277 BONE MARROW TRANSPLANTATION AS TREATMENT FOR CHRONIC MYELOID LEUKEMIA (CML) IN CHILDREN — A REPORT FROM THE STUDY CML-PAED

M. Suttorp, T. Klingebiel, F. Zintl, S. Burdach, C. Bender-Götze, B. Kremens, A. Reiter. *BMT-Unit, Univ.-Children's Hospital, Kiel, Germany*

CML in childhood is rare and BMT offers the only option for cure. According to the multicenter protocol "CML-paed", patients (pts) should be transplanted within six months after diagnosis if a matched sibling donor is present and within one year if an unrelated donor can be identified. Initial treatment to achieve hematological remission includes hydroxyurea followed by interferon-alpha (IFN). As of Jan 1998, 55 pts (31 boys; median age: 14 yrs, range: 1–24 yrs) have been included in the protocol (median follow-up: 20 months, range: 1–33 mos). 33/55 pts have been transplanted with bone marrow (n = 27), peripheral stem cells (PBSC, n = 5) or cord blood (n = 1); 1 pt received autologous PBSC. 13 pts were grafted from HLA-fully matched siblings, while 5 received grafts from phenotypically identical (n = 1), or one antigen mismatched (n = 3), or three-antigen mismatched (n = 1) parental donors. 14 pts were transplanted from unrelated donors and 4 of these received an one antigen mismatched graft. T-cell depletion was performed in 5 cases. 4/33 pts have died from transplant-related complications (infection, GvHD) while 3/22 without transplantation died from progression of CML. Although IFN has become an established therapy for CML and will control myeloid hyperplasia in approximately 60% of the cases, it is not curative in the long term. Therefore the main efforts on the study will focus on the identification of an HLA fully or partially matched donor for all children.

P-1278 SIGNIFICANCE OF DETECTION OF BCR/ABL TRANSCRIPTS AFTER ALLOGENEIC AND AUTOLOGOUS TRANSPLANTATION IN CML — SINGLE CENTER EXPERIENCE

M. Kopera, J. Hołowiecki, M. Krawczyk-Kuliś, J. Wojnar. *University Department of Haematology and Bone Marrow Transplantation Silesian Medical Academy Katowice, Poland*

19 patients with CML in chronic phase received either autologous peripheral blood cell transplantation (APBCT) - 9 patients or unmanipulated allogeneic bone marrow transplantation (BMT) - 10 patients. The median age was 30 y in APBCT group (6 males and 3 females) and 32 y in BMT group (4 males and 6 females). All pts. in APBCT gr. received HU + INFα (6 MIU/m^2/d) until BCR/ABL negativity in nested RT PCR in 2 consecutive evaluations was achieved. G-CSF was used for mobilisation. BCR/ABL positivity was estimated in every single apheresis product and only BCR/ABL negative collections were transplanted. The mean number of NC equelled 9.2 × 10^8/kg (range 4–13.6) in APBCT gr. and 3.36 × 10^8/kg (R 2.45–5.9) in BMT gr. The number of CD34(+) cells equelled 7.5 × 10^6/kg (range 3–12.6) in APBCT gr. and 1.83 × 10^6/kg (range 0.9–3.25) in BMT gr. Busulphan (16 mg/kg) and cyclophosphamide (120 mg/kg) was used as conditioning regimen in both groups. The hematopoesis reconstitution was as follows: APBCT gr.- WBC > 1 G/l d. +26 (12–42); granulocytosis >1 G/l d. +32 (15–40); reticulocytosis >15‰ d. +37 (15–50); PLT > 50 G/l d. +74 (20–150) and BMT gr.-WBC > 1 G/l d. +20 (17–37); granulocytosis >1 G/l d. +28 (17–48); reticulocytosis >15‰ d. +23 (12–100), PLT > 50 G/l d. +38 (22–27). In both group BCR/ABL

fusion transcripts were monitored by nested RT PCR (BM and PB) every 3–6 months after transplantation.

Results: APBCT gr.-currently only one patient is persistently BCR/ABL (+), 4 pts - transiently BCR/ABL (+); in BMT gr. - 3 pts. are transiently BCR/ABL (+) and there is no correlation with GvHD grade.

Conclusions: 1/ BCR/ABL(−) cells could be obtained for APBCT in INF responding CML patients, 2/ BCR/ABL negative collections could be achieved in pts. who started INF treatment even 1–4 y from diagnosis, 3/ the current DFS of 13 months suggests that autologous PBCT offers a promising modality for CML pts. with no available donor, 4/ in both groups BCR/ABL positivity was trensiently present in some cases and a longer observation time is necessary for evaluation of its clinical relevance.

P-1279 ALLOGENEIC PERIPHERAL BLOOD PROGENITOR CELLS FOR TREATMENT OF RELAPSE AFTER BONE MARROW TRANSPLANTATION

D. Stamatović[1], B. Bela, S. Obradović[1], Lj. Tukić[1], S. Berger[2], Z. Trajković[2], L. Ristić[1], B. Todorić, O. Tarabar[1], M. Mateševič[1]. [1]*Clinic of Hematology;* [2]*Institute of Pathology, Military Medical Academy, Belgrade, Yugoslavia*

Leukemic relapse is the most frequent cause of treatment failure after allogeneic bone marrow transplantation (BMT). The optimal treatment strategy for pts relapsing after BMT is still open question. The infusion of donor's lymphocytes (DLI) as a novel way of adoptive immunotherapy proves to be highly effective in a substantial number of pts (up to 70% pts with CML and 30% of AML pts). Pancytopenia complicate the procedure in more than one third of pts.

Two pts - one with chrCML-Ph+ and one with AML-M5, in the relapse, 34 and 7 months after the first BMT, were treated with allo-PBSCT from the same donor. The goal of this treatment was the induction of GvL effect. CML pt in relapse was initially treated with DLI, which induced bone marrow aplasia, and after the conditioning with ATG he received allo-PBSC transplant AML pt received allo-PBSC in the aplasia after the induction chemotherapy. Mobilization of donor's progenitor blood cells were performed with rhG-CSF (10 μg/kg BM/d s.c.), and the number of harvested MNC was 4.1×10^8/kg BM (CD34+ 15.4×10^6/kg BM) for CML pt; and MNC was 4.2×10^8/kg BM (CD34+ 10.8×10^6/kg BM) for AML Pt.

On the 14th (CML pt) and 11th (AML pt) post infusion day, hematological recovery were found. The patients did not develop GvHD and currently are in complete remission, including cytogenetic and molecular complete remission, 8 months (CML pt) and 4 months (AML pt).

Allogeneic PBSC transplantation my be an useful treatment modality for relapse of leukemia after BMT, with the decreasing risk of aplasia during the DLI therapy, but further investigations are needed.

P-1280 ALLOGENIC PERIPHERAL BLOOD STEM CELLS TRANSPLANTATION IS A SAFE AND EFFECTIVE TREATMENT FOR HEMATOLOGIC MALIGNANCIES

P. Sobrevilla-Calvo, C. Alvarado, J. Labardini, J. Texcocano, A. Acosta, M. Sailes, E. Miranda, M.C. Zambrano, A. Marché, J.E. Martinez-Magaña. *Instituto Nacional de Cancerologia, Mexico, D.F., Mexico*

Allogenic bone marrow transplantation is the only curative treatment in certain hematologic malignancies like chronic myelogenous leukemia (CML), but its acute and chronic toxicity is still very important. Also relapse occurs in a substantial number of patients. To improve these results we modified the method in 2 ways: Decreasing the dose of busulfan (Bu) to decrease toxicities like veno-oclusive disease and mucositis and using allogeneic peripheral blood stem cell (AloPBSC) to decrease the period of myelosupresion and to enhance the graft versus leukemia effect. Patients and methods: From 1996 to 1997, we trasplanted 15 pts, median age 25 ys (14–43), 8 female, 7 male, diagnosis: CML 10 pts, acute myeloblastic leukemia 4 pts and 1 pt with lymphoblastic leukemia. Nine pts received Bu 14 mg/kg and cyclophosphamide (Cy) 120 mgs/kg and 6 pts, Bu 16 mg/kg with CY at the same dose. Graft vs host disease (GVHD) prophylaxis: methylpredisolone, cyclosporin and methotretaxe. HLA matched siblings donors, with a median age of 28 (1148) years, received filgrastim 300 mcg/12 hrs × 10, and PBSC were collected with a Fenwall CS3000 daily × 3.

Results: Eight pts received 1 apheresis product (the other 2 were cryopreserved), 5 pts; 2 apheresis products and 2 pts; 3. The median no. of monuclear cells/kg $\times 10^8$ transfused was 4.62 (1.39–12.87) and the median no, of CD34+ cells/kg $\times 10^6$ was 5 (0.2 a38.29). Median no. of days to achieve an absolute neutrophil count of >500: 14 (9–25); days to >20 $\times 10^3$ platelets: 16.5 (11–92). We observed acute GVH in 6 pts: 1 pt died, 4 pts G1-2 and 1 pt G3 and Chronic GVH in 6 pts: 3 limited, 3 extensive. There has been 4 deaths, 1 sudden, 1 Acute GVH, 2 encephalopathy. Actuarial survival is 72% at 692 days, with a median follow up of 248 days. We conclude that APBSC transplantation allows a very fast neutrophil and platelet engraftment, with an acceptable toxicity.

P-1281 OUTCOME OF PATIENTS AT HIGH-RISK OF DEVELOPING GVHD, AFTER ALLOGENEIC TRANSPLANTATION OF SELECTED CD34+ BLOOD CELLS

C. Chabannon, T. Aurran-Schleinitz, F. Viret, C. Faucher, A.M. Stoppa, P. Ladaique, N. Vey, R. Bouabdallah, G. Novakovitch, J.A. Gastaut, D. Maraninchi, D. Blaise. *Institut Paoli-Calmettes, Marseille, France*

The toxicity associated with allogeneic bone marrow or blood transplantation restricts the applications of this technique, as much as the lack of suitable donors. Toxicity is largely due to the occurrence of Graft-Versus Host Disease (GVHD), which is mediated by donor T-cells present in the graft. T-cell depletion of marrow graft reduces the occurrence and severity of GVHD, however at the expense of an unacceptable rate of graft failures and relapses. Substitution of allogeneic blood cells to allogeneic bone marrow cells, as well as the observation that donor lymphocyte infusion can cure or prevent post-transplantation relapses, offer new perspectives to evaluate again T-cell depletion. In this prospective, single-institution study, we have evaluated the use of a CD34+ cell selection device, the Baxter Healthcare Isolex® 300i, to deplete T cells from allogeneic aphereses collected from matched siblings, and to reduce the frequency and severity of GVHD in recipients at high risk of developing GVHD, either due to age, or to the status of the disease at the time of transplantation. 14 patients with a median age of 42 years were included after informed consent. Donors were mobilised with 10 μg/kg/d of rhG-CSF, after informed consent, and aphereses were started on day 5, after the 4th injection. 2 patients received unselected blood cells, because the donor poorly mobilized, and the number of collected progenitors was deemed too low. Following conditioning with TBI and cyclophosphamide (in addition, 2 patients received HD melphalan, and 10 patients received one dose of ALG),12 patients were transplanted with a median number of 4.7×10^6 CD34+ cells/kg, and 166×10^3 CD3+ cells/kg (6 patients received less than 105 CD3+ cells/kg). In addition to T-cell depletion, most recipients received immunosuppression with cyclosporine±methotrexate. 1 patient died early of sepsis. 8 out of 11 patients engrafted (2 patients had primary graft failure, one patient had secondary graft failure at the time of CMV disease, and treatment with ganciclovir). 4 patients developed grade 2–4 acute GVHD, and one, patient developed extensive cGVHD. 3 patients remain alive at days 60, 215 and 300 days respectively (actual survival rate at 100 days: 36%). No relapse has been observed. Although we only report a limited experience, we conclude that CD34+ cell selection of allogeneic blood cells did not drastically change the outcome of a cohort of patients at high risk of developing GVHD, while it appears as an effective tool to deplete T cells from aphereses. Also, the infusion of large numbers of progenitors (i.e. when compared with bone marrow transplantation) did not prevent the occurrence of graft failures in this group of recipients.

P-1282 COMPARISON BETWEEN ALLOGENEIC TRANSPLANTATION WITH CRYOPRESERVED PBSC VERSUS UNMANIPULATED BONE MARROW: RESULTS IN A SINGLE CENTRE

D. Hernández, J. Sevilla, M. Canales, P. Mateos, M.T. Jiménez, M.T. Alvárez, M.P. Martín, J.Ga. Bustos, M.J. Aguado, A. Martínez, A. Sastre, P. De Miguel, A. Lafuente, I. Del Pozo, R. Arrieta, E. Ojeda, F. Hernández-Navarro. *Servicio de Hematología, Hospital "La Paz", Madrid, Spain*

Two groups of allogeneic transplants with different sources of progenitor hematopoietic cells (BM or PBSC) performed in our institution and matched for diagnosis were retrospectively compared to evaluate the intratransplant evolution. Quality of harvest, times for engraftment (granulocytes and platelets recovery), support requirements and complications (including venooclusive disease and GVHD) were analyzed. Thirty-six patients with acute leukemia (lymphoblastic -ALL- or non-lymphoblastic -NLAL-) or chronic myeloid leukemia (CML) underwent allogeneic hematopoietic transplantation either with PBSC (18 patients: 8 NLAL, 4 ALL and 6 CML) or BM (18 patients: 8 NLAL, 8 ALL and 2 CML). The median age for PBSC group was 28 years and 10 years for BM group ($p = 0.01$). Median number of CD34 $\times$ 10^6/kg cells infused were 4.3 (1.6–10) for PBSC group and 3 (1–9) for BM group (p = NS). Engraftment was, faster for the PBSC group (median time for granulocytes >0.5 $\times$ 10^9/L of ten days and for >20 $\times$ 10^9/L platelets twelve days vs. fourteen and nineteen days for BM group; $p = 0.001$). No differences for transfusional requirements, days of hospitalization, days of fever (>38.5ªC), days of treatment with antibiotics, infectious complications, incidence of pneumonias, mucositis and VOD were founded between the two groups. Incidence of acute GVHD was 66% (12 cases) in PBSC group and 77% (14 cases) in BM group, but grades $\geq$3 were more frequent in the PBSC group (5 patients -27%- vs only one patient in BM group -5%). Six patients developed chronic GVHD: two of PBSC group (of thirteen patients that survived more than 100 days) - 15% - and four of BM group - 23% - (of seventeen patients at risk). Despite a faster engraftment in the PBSC group, no relevant differences were recorded in our series for other parameters; excluding GVHD, probably related to different ages of both groups.

P-1283 CHANGE OF MORPHOLOGICAL COMPLICATIONS AFTER BMT AND PBSCT

T. Friedrich[1], G. Wahn[1], M. Kubel[2], W. Pönisch[2], D. Wolff[2], R. Krahl[2], W. Helbig[2], G. Vogelsang[3]. [1]*Institute of Pathology;* [2]*Medical Clinic II, Department Hematology/Oncology, Leipzig University;* [3]*Johns Hopkins Medical Center, Baltimore, USA*

Bone marrow transplantation (BMT) and Peripheral Stem Cell Transplantation (PBSCT) are accompanied with a spectrum of typical complications. We analyzed the shift of these main problems after introduction of main new therapeutic schedules within a period of 30 years. We reviewed clinical and paraclinical data and main morphological findings from 485 autopsy cases after BMT and PBSCT in association to basic disease, conditioning regimen, kind of transplantation, and others. In the first phase (1967–1986) after conditioning with single dose whole body irradiation without lung shielding a predominance of severe toxic lung injuries, early and severe therapy-resistant acute GvhD with profound immune deficiency, and hemorrhagic diathesis was the typical setting. After the introduction of fractionated whole body irradiation with lung shielding, a better CMV-prophylaxis and a more sufficient supportive hemotherapy (1987–1991) these complications were mainly solved. The onset of acute GvhD was prolonged, late acute and chronic GvhD became a problem. PBSCT instead of BMT (since 1992) was accompanied with a quicker hematological reconstitution. Recurrence of basic disease remained a constant problem over the years. We found an unequivocal shift in prevalence, severity, and time of onset of main morphological complications.

P-1284 QUALITY OF LIFE OF THAI PATIENTS POST BONE MARROW AND PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

T. Intragumtornchai, S. Jootar, A. Ungkanon, S. Chancharunee, D. Swasdikul, V. Udomprasertkul. *Bone Marrow Transplant Unit, Department of Medicine, Chulalongkorn University, Ramathibodi Hospital, Bangkok, Thailand*

Objectives: Data on the post-transplant quality of life (QOL) in Asian patients is sparse. This study was conducted to determine the QOL of Thai patients undergoing BMT/PBSCT and compared data with those reported from the western countries. The variables which had impact on the patients' overall QOL were determined.

Population: Patients aged $\geq$15 years undergoing BMT/PBSCT at the two university hospitals who were in disease-remission were entered into the study.

Measurements: The QOL questionnaires, consisted of the demographic data and the QOL-BMT tools, were mailed to the patients. The QOL-BMT tool included 37 items representing the four domains of QOL, i.e., the physical status and abilities, the psychological well-being, the social interactions and the treatment-related symptoms. The overall QOL was also self-rated. The items are scaled on a 5 point Likert-type scale. Participants filled out the questionnaire based on their experience within the last two weeks and returned by mail. A randomly selected sample of patients were asked to repeat this survey two weeks later for the test-retest analysis.

Results: There were a total of 62 patients (90% of the eligible patients). The median age was 35.5 years (range, 16–56). The median time from transplant was 9.1 months (range, 1–62.8). Eighty-nine percent of the patients had malignant diseases. Thirty patients had HLA-identical allogeneic transplant and 31 were treated with autologous stem cells. Thirty-five patients had peripheral blood as the stem cell source. High-dose chemoradiotherapy preparative regimens were given in 58% of the patients. Reliability of the questionnaire measured as the test-retest (Pearson' correlation) and the internal consistency (Cronbach's α coefficient) were 0.79 and 0.87 respectively. The mean score of the overall QOL of the patients was 3.76 on a scale of 1–5 with "5" being their best. The mean scores of the physical, psychological, social well-being and the treatment-related domains were 3.94, 3.99, 4.12 and 4.20, respectively. By using stepwise multiple regression, four independent variables namely, despair, appearance, functional ability and the financial burden were found to have statistical significance on the QOL. These variables accounted for 53% of the variance in the overall QOL scores. None of the variables on the socioeconomic status and the treatment options had a significant impact on the QOL.

Conclusion: The overall QOL of Thai patients undergoing BMT/PBSCT was comparable to most studies from western countries. The four most important variables determined the QOL were despair, appearance, functional ability and the financial burden. The association of financial burden and QOL had never been reported.

P-1285 AUTOLOGOUS BONE MARROW (ABMT) OR PERIPHERAL BLOOD STEM CELL (ABSCT) TRANSPLANTATION AFTER INTENSIVE CHEMOTHERAPY IN MYELODYSPLASTIC SYNDROMES (MDS)

E. Wattel, X. Leleu, E. Solary, D. Caillot, F. Dreyfus, A. Brion, B. Mahé, J.P. Jouet, L. Hoang-Ngoc, F. Maloisel, A. Guerci, H. Rochant, N. Gratecos, A. Sadoun, A. Stamatoullas, P. Casassus, M. Janvier, A.M. Stoppa, B. Desablens, N. Fegueux, D. Guyotat, P. Brice, P. Lepelley, P. Fenaux. *Groupe Français des Myélodysplasies (GFM) and GOELAMS, France*

Intensive chemotherapy (IC), in MDS, gives lower complete remission (CR) rates and shorter CR duration than in de novo AML in spite of consolidation chemotherapy (CT). Only retrospective studies of ABMT have been reported in MDS who reached CR with IC. We designed a trial of IC in pts aged $\leq$65 years with "high risk" MDS. Pts received Mitoxantrone 12 mg/m^2/d d_{2-5} + AraC 1 g/m^2/12 h d_{1-5}, with or without (randomized) quinine 30 mg/kg/day. Pts <55 years with no HLA identical sibling and achieving CR were scheduled to receive ABMT preceded by a consolidation CT course. As bone marrow harvest was insufficient in some pts, peripheral blood stem cell (PBSC) harvest was subsequently proposed after mobilization by consolidation CT followed by G-CSF. The conditioning regimen was: Cy 50 mg/Kg/d and Bu 4 mg/Kg/d during 4 d. From Oct 1992 to May 1996, 162 pts were included. 66 pts (41%) achieved CR. Median age of the 96 pts aged $\leq$55 was 48.5 years (range: 18–55). Cytogenetic analysis (64 pts) was abnormal in 41 pts: del 5 q: 4 pts; -7: 8 pts; other single abn: 14; complex abn: 15 pts. 45 pts (47%) achieved CR. 5 of them were allografted in CR. 23 of the remaining 37 pts (62%) received ABMT (17 pts) or ABSCT (6 pts). Reasons for not performing ABMT or ABSCT were: early relapse (5 pts), poor clinical condition (5 pts), poor marrow stem cell harvest (2 pts), patient refusal (2 pts). ABMT and ABSCT were performed 1 to 7 months (median: 3) after CR achievement. 2 pts were found to be in relapse at the time of autograft. Hematological reconstitution occurred in all pts. In the 17 pts who received ABMT, median duration of leukopenia, neutropenia and thrombocytopenia were 17.5 (range 11 to 30), 18 (range 11 to 36) and 46.5 days (range 11 to 99), respectively. In the 6 pts who received ABSCT, median duration of leukopenia, neutropenia and thrombocytopenia were 14.5 (range 11 to 18), 15 (range 11 to 23) and 60 days (range 11 to 80), respectively (differences NS). Median follow up was 17.5 months after autograft. 2 pts died from the procedure, 11 relapsed after 2 to 26 months and 12 (53%) were still in CR after 8 to 55 months. In autografted pts, median

Kaplan-Meier (KM) DFS was 26 months from the autograft. KM estimate of survival from the autograft was 68±9% at 12 months, 52±11% at 24 months and 46±12% at 36 months. Median KM DFS from the onset of treatment of pts who achieved CR and were not autografted (excluding allografted cases) was 10. In conclusion 1) This prospective study shows that ABMT or ABSCT can be performed in about 60% of MDS who achieve CR with IC. However, it is too early to determine if ABMT and ABSCT will increase CR duration in some pts, by comparison with consolidation CT, although results of the present study are encouraging. 2) PBSC collection may yield higher numbers of stem cells than marrow collection in some cases, and could improve the percentage of MDS pts autografted in CR. However, it does not seem to shorten the duration of aplasia.

P-1286 EVOLUTION AND SURVIVAL AFTER AUTOLOGOUS HEMATOPOIETIC TRANSPLANTATION WITH STEM CELLS IN PATIENTS DIAGNOSED OF CHRONIC MYELOID LEUKEMIA

M.J. Aguado, F. Hernández-Navarro, J.L. Bello[1], E. Ojeda, J. G[a]-Bustos, T. Perez-Pino, R. Arrieta, V. Jimenez, M.P. Martin, B. Sanchez, A. Martinez, M. Canales, D. Hernandez-Maraver, T. Jimenez, J. Sevilla, T. Alvarez, M. Morado. *Hospital La Paz Madrid and Hospital Universitario Santiago de Compostela, Spain*

Actuarial survival and evolution was analysed in 14 patients with Ph positive Chronic Myeloid Leukemia treated with autologous hematopoietic transplantation with peripheral, blood stem cells. Eleven patients were in chronic phase, two in accelerated phase and one in complete remission after lymphoblastic crisis. The median age was 41 (range 27–58) nine were female and five male.

Mobilization was carried out using Hydroxyurea (UH) at 1 gr/m^2/day and was stopped when less than 1 × 10^9/L leukocytes on peripheral blood was reached. The day after finalization of UH, G-CSF was administered s.c. at 10 mcg/kg body weight and was continued until the last day of harvesting. FISH studies were carried out in 11 patients using the m-bcr/abl translocation DNA probe (ONCOR), and Nested-PCR in three patients. All patients were successfully harvested using this mobilization procedure and they were autotransplanted using as conditioning Busulfan in eleven pts and Busulfan-Cyclophosphamide in two pts.

To date, with an observation period time, which varies between five and twenty four months, ten pts are alive and four were died all of whom were in accelerated phase prior to transplant (3 to 17 mounths after the procedure). The pts autografting in a chronic phase are alive with a mean survival of 49,13 months and of 30,93 months for the patients autografting in accelerated phase. Kaplan-Meier test proved a survival plot of 100% for patients in a chronic phase and 30% for patients in accelerated phase with a statistical difference ($p < 0,02$) between this groups 24 weeks after transplant.

The cytogenetic analysis revealed negative results or a very low number after transplant. Between 7 and 19 months all patients revert to Ph positive hematopoiesis and all of them need treatment with UH, alpha-Interferon or both drugs to disease control.

Autologous transplantation is not a curative procedure but it looks to improve the survival and to affect positively the clinical course of CML, however a more prolonged follow-up of these patients will be required to assess its efficacy.

P-1287 LATE AUTOLOGOUS STEM CELLS TRANSPLANTATION IN THE FIRST REMISSION OF ACUTE MYELOGENOUS LEUKAEMIA — YES OR NO?

S. Popovic, V. Uzurov, S. Stoicevic, A. Savic, I. Savic, D. Pejin. *Clinic of Haematology, Novi Sad, Yugoslavia*

Results of autologous stem cells transplantation (ASCT) in the first remission (CR1) of acute myelogenous leukaemia (AML) is time depended. According to EBMT Group data (1980–91 year) chance for cure, risk of relapse and mortality after ASCT in the first three months and after six months of CR1 are 23%, 61%, 16% and 48%, 46%, 6%, respectively. This differences were explained by in vivo purging of autotransplants. We made mathematical analysis based on this data.

Cumulative effect ASCT after intensive consolidation chemotherapy. Modern chemotherapy in CR1 give 40% chance for cure with 12% mortality. At least 10% pts relapsed within six months of CR1 before ASCT, so ASCT is applied ill 78% of pts. Than is cumulative risk of relapse (RR), therapy related death (TRD), and chance for cure (CC): RR = 0.10 + 0.78 × 0.44 = 44.3%, TRD = 0.12 + 0.78 × 0.06 = 16.7% and CC = 100% − (44.3% + 16.7%) = 39.0%. Cumulative effect of ASCT and intensive consolidation chemotherapy are similar.

In vivo purging? If consolidation chemotherapy in CR1 eradicated leukaemic cells in 25% pts (real outcome in period 1980–91) than 75% of pts has minimal residual disease. In the first group ASCT have two outcomes: death = 0.25 × 0.06 = 1.5% or "repeat cure" = 25%–1.5% = 23.5% and in the second group have all three outcomes: all relapses are in this group (46%), death = 0.75 × 0.06 = 4.5%, cure = 75% − 46% − 4.5% = 24.5%. Pure antileukaemic effect of late ASCT in pts with residual leukaemia (24.5%) is similar to antileukaemiac effect of ASCT in the first three months of CR1 (23%). Total cure after late ASCT is 23.5% + 24.5% = 48% and total mortality is = 1.5% + 4.5% = 6.0%. Mostly, in vivo purging of autotransplants are consequence of application of ASCT in pts cured by prior chemotherapy.

Late ASCT in CR1 yes or no? No. We perform selective ASCT in the first remission of AML.

P-1288 DONOR LYMPHOCYTE INFUSIONS (DLI) FOR RELAPSE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT). HEMATOLOGIC, CYTOGENETIC AND MOLECULAR RESULTS

A. Thiébaut, M. Michallet, L. Gebuhrer, Y. Lasne, C. Charrin, I. Mollet, A. Belhabri, E. Archimbaud†. *Hématologie, LYON, France*

We studied 14 patients (pts) who underwent DLI for relapse after allogeneic BMT: 4 CML (3 1st and 1 2d chronic phase), 6 AML, 2 ALL, 1 MM, 1 CLL. All pts relapsed after BMT (12 medullary (H) and 2 extra-medullary) with a median delay of 23 months [4–93].

Date of transplant	Date of relapse	Date of DLI	CD3/kg	Cytogenetic	VNTR	MBCR/ABL
03/03/94	no CR			06/94 20%Ph1+		
LMC				12/94 100%Ph1+	m	
		21/02/95	1.29×10^8	02/95	m	
(1)				04/95 100%Ph1-	m	
				05/95	c	
				06/95 100%Ph1-	c	
		23/11/95 = 2dBMT		11/95 100%Ph1-	c	93%
				10/96		0%
				12/96		0%
				01/97		0%
				04/97 100%Ph1-		
				06/97 100%Ph1-	c	
				10/97		c
07/07/94	11/95			11/94	c	0%
LMC				08/94 100%Ph1- XY		
				11/95 100%Ph1+ XY		
(2)		06/02/96	2.22×10^8	01/96 63%Ph1+ XY	m	
				02/96	m	85%
				05/96 63%Ph1+ XY	m	
				07/96		60%
				08/96 38%Ph1+ XY	m	23%
				11/96 97%Ph1+ XY	m	23%
				04/97 100%Ph1- XY	m	
				01/97	m	6%
				03/97	m	0%
				09/97	m	
26/11/93	11/96			12/95 100%Ph1- XY	m	
LMC				11/96 99%Ph1+ XY	m	179%
(3)		05/12/96	1×10^7	12/96		93%
		12/12/96	5×10^7	01/97 96%Ph1+	m	
		20/02/97	1×10^8	02/97 100%Ph1+	m	188%
				03/97		29%
				04/97	c	
				06/97	c	
				08/97 100%Ph1-	c	
				10/97	c	
10/05/90	05/96: 2dBMT			06/96 100%Ph1- XY	c	
LMC				01/97	m	
(4)				02/97 100%Ph1+ XX		273%
	03/97	19/03/97	5×10^7	03/97	m	126%
		05/05/97	1×10^8	05/97	m	155%
				06/97 100%Ph1+ XX		
				07/97 100%Ph1- XY	c	
				08/97	c	0%
				09/97	c	?

Date of transplant	Date of relapse	Date of DLI	CD3/kg	VNTR
13/07/89	03/96	26/04/96	1×10^7	ND
LAM	(5)	H	03/05/96	5×10^7
05/10/95	03/96	09/05/96	1×10^7	09/95 cc (R)
LAM	H	17/05/96	7×10^7	05/96 mc
(6)				06/96 mc
				08/96 mc
21/06/96	10/96			07/96 cc (D)
LAL	mening. and H			09/96 mc
(7)		12/12/96	5×10^7	12/96 cc
				03/97 cc
				05/97 cc
				08/97 cc
03/08/95	12/96			11/96 cc
LAL	renal			01/97 cc
(8)		13/02/97	5×10^7	02/97 cc
				04/97 cc
				05/97 cc
26/09/96	04/97			10/97 cc
LAM	H	18/06/97	5×10^7	06/97 cc
(9)		17/07/97	1×10^8	7/97 mc
15/09/94	05/97			05/97 mc
LAM	H	25/06/97	5×10^7	06/97 mc
(10)		17/07/97	1×10^8	07/97 cc
				08/97 cc
27/11/95	07/97			08/97 mc
LAM	H	16/09/97	1×10^7	09/97 cc
(11)				10/97 cc
09/01/97	07/97	02/09/97	1×10^7	
LAM	H	09/09/97	5×10^7	09/97 mc
(12)		15/10/97	1×10^8	10/97 mc
27/09/90	11/96	27/02/97	5×10^7	
MM	H			03/97 cc
(13)		20/05/97	1×10^8	05/97 cc
				07/97 cc
				09/97 cc
16/02/89	11/96			01/97 mc
LLC	H	06/03/97	1×10^7	06/03/97 mc
(14)		13/03/97	5×10^7	13/03/97 mc
		03/05/97	2.2×10^8	
				10/97 cc

We performed longitudinal cytogenetic and molecular studies by PCR to document chimerism (VNTR) in all pts and minimal residual disease (% of MBCR/ABL) in CML pts. Eleven pts had a mixed chimerism (mc) before DLI, 2 extra-medullary relapses (1MM, 1ALL) presented a complete chimerism (cc) and 1 AML presented the recipient profile. Delay between hematological relapse and DLI number varied (cf table). All CML pts achieved cytogenetic complete responses, correlated to complete chimerism in 3 pts and disapearance of MBCR/ABL transcript in 3 pts. They are all alive from 18 to 44 months. For acute leukemias (6 AML, 2 ALL), all pts received chemotherapy regimen before DLI with obtention of complete remission (CR) in 2 AML and 1 ALL with extra-medullary relapse, prolonged with DLI. In the others pts, after DLI we observed no hematological nor chimerism in 2 AML, a reappearance of chimerism mixed with only hematological CR in 1 AML and in the 3 other AML, hematological response were correlated with cc in 2 cases. In the other ALL, a partial hematological remission with cc were obtained. Two pts are alive at 5 and 22 months, 3 pts died of leukemia, 2 pts died of GVHD hepatic in CR and 1 pt died of GVHD hepatic and leukemia. We observed a very good GVL effect in the 2 last pts (1MM, 1CLL) with disappearance of monoclonal component in MM and of tumoral syndrom and lymphocytosis in CLL with reappearance of a complete chimerism. These 2 pts are alive and well at 35 and 105 months.

P-1289 OUTCOME OF REPEATED ALLOGENEIC STEM CELL TRANSPLANTATIONS IN CHILDREN, TRANSPLANTED BETWEEN 1968 AND 1997

J.J.H. Bleesing, P.M. Hoogerbrugge, M.H. Van Weel, J.M. Vossen. *Dept. of Pediatric BMT, Leiden University Medical Center, Leiden, The Netherlands*

Between 1968 and 1997, 370 patients (pts) have received an allogeneic stem cell transplantation (aSCT) at our institution. In total, 29/370 (7.8%) pts have

received a second aSCT. In addition, 5/29 pts also underwent a third aSCT. Indications for a second aSCT included; graft failure in 25/29 pts (86%), and relapse of the original leukemia in 4/29 pts. The indication for a third aSCT was always graft failure. Median time between first and second aSCT was 2.7 months (range: 0.9–68 months), and between second and third aSCT; 1.7 months (range: 0.9–3.3 months). Donor profiles for the second aSCT were; HLA-matched sibling (donor: 10/29, all of which were performed before 1987, if the indication was graft failure; HLA-(mis)matched related donor: 9/29; HLA-matched unrelated donor (MUD): 10/29. For the second aSCT, the same donor was used in 69% (20/29), and for the third in 3/5 cases. T-cell depletion (TCD) of the graft was performed in 16/29 (55%), 12/29 (41%), and 2/5 (40%) for the first, second, and third aSCT, respectively. Overall, 24% (7/29) have survived 35 to 280 months after the first aSCT (as of July, 1997). No patient has survived a third aSCT. In the survival group, 4/7 received the second aSCT from the same HLA-matched sibling donor, 1/7 from the same HLA-mismatched related donor, and 2/7 from another unrelated donor. Reciprocally, 22/29 pts have not survived repeated aSCT. Median time between death and second aSCT was 1.9 months (range: 0.1–15.6 months). The main cause of death was transplant-related (86%). In the above mentioned time period, the profile of repeated aSCT's has changed from non-TCD, HLA-matched sibling transplantations, to TCD, MUD transplantations. In parallel, outcome has changed from 50%, for the period 1968–1977, to 81% mortality, for the period 1988–1997. Graft failure has remained the main indication for retransplantation, especially in the case of certain diseases, such as SAA, FA, and primary immunodeficiency diseases. In conclusion, repeated aSCT carries a high risk of mortality. This should put emphasis on careful selection of the donor, conditioning regimen, and graft manipulation. This should also be taken into account before an aSCT is repeated with an unrelated (volunteer) donor.

P-1290 INTENSIFIED CONDITIONING REGIMEN IN CHILDREN TRANSPLANTED FOR AML IN FIRST AND ALL IN SECOND COMPLETE REMISSION

P.M. Hoogerbrugge[1,2], H.P.M. Versluis[1], E.R. Van Wering[2], J.Fv.W. Weerden[2], A. Van der Does-v.d. Berg[2], M. Van Weel[1], M. Van Rossum[1], L. Van 't Veer, J.H. Bleesing[1], J.M. Vossen[1]. *[1]Dept. of Pediatric BMT, Leiden University Medical Center, Leiden; [2]Dutch Childhood Leukemia Study Group, Den Haag, The Netherlands*

A relapse of the original leukemia is still the major cause of death in children following HLA-identical allogeneic BMT. To reduce the incidence of relapse, we included, from 1989 onward, high-dose AraC (4 × 1 gr./m^2) in the conditioning regimen of children treated for AML in first remission (CR1) and we included VP16 (2 × 350 mg/m^2) in children transplanted for ALL in CR2. In addition to AraC or VP16, cyclophosphamide (120 mg/kg) and TBI (Cy-TBI) were given. The results were compared with patients treated before 1989, who had received only Cy-TBI.

40 patients with **AML** were included, 18 were conditioned with Cy-TBI, 22 with AraC-Cy-TBI. One patient (Cy-TBI group) died of BMT-related complications. The groups were comparable with respect to prior treatment, duration of CR1 before BMT and incidence of 'good risk chromosomal translocations'. The 5-years survival after BMT was similar in both groups (72±11% in the Cy-TBI group, vs. 69±10% in the AraC-Cy-TBI group). These data indicate that the addition of AraC did not result in better survival of patients transplanted for AML in CR1.

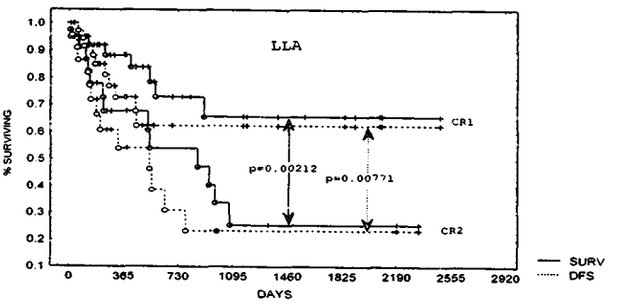
Of the 41 patients with **ALL** in CR2, 14 received Cy-TBI, 27 VP16-Cy-TBI. 4 patients died of complications (3/14 in Cy-TBI group; 1/27 in VP16-Cy-TBI group). The groups were comparable with respect to duration of CR1 and site of relapse. The initial therapy in the Cy-TBI group, which received BMT before 1989, was slightly less intensive than in the VP16-Cy-TBI group. Despite this, the survival tended to be lower (48±14%) in the Cy-TBI group than in the VP16-Cy-TBI group (62±10%, NS). These data, therefore, suggest that the inclusion of VP16 in the conditioning of children with ALL in CR2 may improve survival.

P-1291 PROMISING RESULTS OF ABMT IN ADULT ALL PATIENTS

J. Hołowiecki, J. Wojnar, M. Krawczyk-Kuliś, S. Giebel, Ł. Kachel, T. Krużel. *University Department of Haematology, Katowice, Poland*

Among 282 bone marrow transplantations performed between 1991 and 1998 autologous BMT (ABMT) was carried out in 63 ALL patients aged 11–50 years, median 22 years (40 high risk CR1 patients and 23 in CR ≥ 2). Bone marrow was stored for 72 hours in 4°C. In all patients CAV was used as conditioning regimen. The following prognostic factors for outcome were analysed: age, sex, WBC, splenomegaly and immune phenotype at diagnosis, time to obtain CR1, time interval from CR to ABMT, CNS involvement and stage at ABMT (CR1 vs. CR ≥ 2). The Cox proportional hazards model was used in a multivariate analysis of the data and groups were compared using Cox F-test.

Results:



Two patients (3,2%) died during first 30 days after ABMT. Ten patients in CR1 and 13 patients in CR2 relapsed. In CR1 7-years DFS was better than in CR2 (p = 0.0077). The factors associated with relapse were: CNS involvement, male gender, preABMT dose/time reduced treatment schedule.

P-1292 PERIPHERIL BLOOD STEM CELL TRANSPLANTATION FOR TREATMENT OF PATIENTS WITH REFRACTORY BLOOD DISEASES

Da Wanming, Wang Cunbang, Liu Yuan, Bai Hai, Wei Yaming, Ji Menju. *Department of Hematology, Lanzhou General Hospital, Lanzhou, 730050, Gansu, China*

Peripheral blood stem cell transplantation (PBSCT) have been increasingly performed in place of bone marrow transplantation. The benefits of PBSCT include more rapid hematopoietic recovery, lesser complications, lower costs, and avoidance of a general anaesthetic of donor. We used allogenic PBSCT from HLA-identical siblings for treatment of three patients with refractory blood diseases. Among them, one is chronic myeloid leukemia in blast phase, two are malignant lymphoma who never archived complete remission after several intensive chemotherapy and local irradiation. Harvest of PBSC started after mobilization with GM-CSF or G-CSF for 5 days. Performing 3–4 procedures, we collected at least 2.6 × 10^8/kg BM nucteated cells, 7.4 × 10^4/kg GM-CFU, and 2.6 × 10^6/kg CD34$^+$ cells. The conditioning regimen included high dose combination chemotherapy (VCR, Vp16, Ara-C, Mitoxantrone and cyclophosphemide), and total body irradiation (TBI). After allogenic PBSCT, the Hematopoiesis recovered in 3 weeks, and acute graft-vesus-host disease was observed in one patient. Complete remission are obtained in all three patients post-transplantation, and continually up to know. The results suggest that PBSCT is a potential choice to cure the refractory malignant blood diseases.

P-1293 WHOLE BLOOD RICH ON (PBPC) — SUPPORTIVE HAEMOTHERAPY AFTER INTENSIVE CHEMOTHERAPY IN BREAST CANCER

S. Filip, J. Vaňásek, V. Medková, M. Bláha[1], P. Meřička[2]. *Dept. of Oncology and Radiotherapy; [1]Dept. of Clinical Hematology; [2]Dept. of Tissue Bank, Charles University Hospital Hradec Krlové, Czech Republic*

To support multicyclic, dose-intensive chemotherapy in breast cancer, we assesses the effects of reinfusing hematopoietic progenitors either as a leukaphereis product or as mobilized unprocessed whole blood. In this clinical study sixteen consecutive female breast patients were given 6 cycles of chemotherapy regimen EC-epirubicin 150 mg/m^2 and cyclophosphamide 1250 mg/m^2 on day 1. In the first cycle, 24 hours after chemotherapy, mobilization of the peripheral blood progenitor cells (PBPC) was started with growth factor G-CSF at a dose of 5 μg/kg/day for 13 days. In all other cycles G-CSF had been given at the same dose from day 7. On days 11, 12 and 13 the leukapheresis were performed and their products cryopreserved. On day 14 whole blood was collected. The median peak incidence of CFU-GM in peripheral blood was approximately 50-times the baseline levels. The leukapheresed PBPC were divided into portions and reinfused after the fourth, fifth and sixth therapeutic courses. In leukapheresis harvests performed on day 13 after initiation of chemotherapy, the mean number of CD34+ cells was 4.93 × 10^6/kg, range 0.36–10 × 10^6/kg, and the amount of CFU-GM was 2.18 × 10^6/kg, range 0.07–4.2 × 10^5/kg. The yields of PBPC in 450 ml whole blood on day 14 reached 0.51 × 10^5/kg, range 0.05–1.5 × 10^5/kg CFU-GM and 1.3 × 10^6/kg, range 0.18–2.58 × 10^6/kg CD34+ cells. PBPC yields in 450 ml of unprocessed whole blood were in some cases not sufficient for good hematopoietic recovery after EC cycles. Grade 4 leukopenias and thrombocytopenias were two times higher in cycles with whole blood support than in cycles with cryopreserved PBPC support.

P-1294 AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PATIENTS WITH HAEMATOLOGICAL MALIGNANT DISEASES — ONE CENTER EXPERIENCE

H. Minigo, A. Planinc-Peraica, I. Kardum-Skelin, S. Ostojic, Z. Siftar, O. Jaksic, M. Strauss-Patko, C. Maglov, B. Jaksic. *University Hospital "Merkur" and Croatian Institute for Transfusion Medicine, Zagreb, Croatia*

Fifty-six patients with malignant haematological diseases were transplanted with autologous bone marrow and/or peripheral blood stem cells from November 1993. to February 1998. Forty-four had malignant lymphomas (20 with HD and 24 with NHL). Three patients had multiple myeloma and 9 acute leukaemia (6 AML and 3 ALL). Median age was 32 years (14–57). Twenty patients were transplanted with autologous bone marrow, 20 with bone marrow and peripheral blood stem cells, and 16 with peripheral blood stem cells only. Myeloablative chemotherapy was BEAM for lymphomas and ALL, Bu-Cy for AML and high-dose melphalan for multiple myeloma. Only 5 patients were transplanted in CR, and all others had active disease. Patients with malignant lymphoma were poor risk, heavily pre-treated. All but one patient with NHL were supported after transplantation with G-CSF 5 μg/kg BW during the period of leukopenia of less then 1.0 × 10^9/l. Median duration of cytokine support was 10 days. All patients were also supported with platelet and 39/56 with RBC concentrates. Median number of febrile days in pancytopenic period was 4 (0–15). Transplant related mortality for patients with lymphoma was 6.8% (3/44), for AML 11.1% (1/9) and for multiple myeloma 33.3% (1/3). CR was obtained in 25 and PR in 14 out 42 patients with active lymphoma. Projected survival from ABMT or PBSCT for patients with lymphoma was 68% at 4 years. Projected DFS was 72% at 4 years. Patients with HD had significantly better DFS then NHL patients ($p = 0.045$). All patients with multiple myeloma and ALL died after 6 months to one year while 4 patients with AML are still alive. We conclude that autologous stem cell transplantation after myeloablative chemotherapy is safe, highly efficient for remission (re)induction in heavily pre-treated patients with malignant lymphomas, with durable remissions and prolonged survival. Patients with multiple myeloma and with ALL had poor prognosis in spite of myeloablative chemotherapy with autologuos stem cells transplantation.

P-1295 NON-CRYOPRESERVED PERIPHERAL BLOOD STEM CELLS AUTOTRANSPLANTS FOR HEMATOLOGICAL MALIGNANCIES CAN BE PERFORMED ENTIRELY ON AN OUTPATIENT BASIS

G.J. Ruiz-Argüelles, A. Ruiz-Argüelles, B. Pérez-Romano, A. Marín-López, J.L. Delgado-Lamas, M.A. García-Martínez. *Centro de Hematología y Medicina Interna de Puebla, Laboratorios Clínicos de Puebla, Centro Médico de Occidente-Guadalajara and Hospital ISSTEP-Puebla, México*

Entirely on an outpatient basis, we have prospectively performed peripheral blood stem cell (PBSC) autotransplants in ten patients with haematological malignancies: One M3 acute myelogenous leukaemia (AML) in first remission (CR), 2 transformed non-Hodgkin's lymphomas, three M2 AML, two in first and one in second CR, two chronic myelogenous leukaemias in haematological CR, one multiple myeloma associated with breast carcinoma, and one refractory Hodgkin's disease. Patients were conditioned with high-dose melphalan and received a median of 4.2 × 10^8/Kg non-cryopreserved, non-purged mononuclear cells, containing a median of 3.9 × 10^6/Kg CD34+ cells. The median time to achieve >500 granulocytes/ul was 21 d, with a range of 13 to 40, whereas the median time to achieve >20 000 platelets/ul was 38 d, with a range of 20 to 48. Three patients were transfused with platelets and red blood cells were transfused in two. All patients survived 60 days after the graft and seven are alive at a median of 240 d (range 30–1740) after. One patient had to be admitted to the hospital on day +10 because of fever. A median of 6 500.00 USD per patient was calculated as the total cost of each procedure. Since outpatient autologous transplants with non-frozen PBSC are feasible, restrictions to PBSC autotransplant programs may be overcome and costs may be diminished.

P-1296 LIFE AFTER STEMCELL TRANSPLANT: A FOLLOW-UP

M.G.J. Hendriks, H.C. Schouten. *University Hospital Maastricht, The Netherlands*

Introduction: High dose therapy followed by stemcell transplantation is increasingly used because of its curative potential in a subset of patients. However, only limited data are available on the impact of this modality on quality of life. In 1995 we performed a study on quality of life in transplant patients using the EORTC QLQ-C30 and semi-structured interviews (n = 52). One and a half year later we used the EORTC QLQ-C30 in a follow up, to see how the quality of life changes through time. Furthermore we were interested to know how the partner and the patient's physician estimate the patients quality of life, functioning and symptoms.

Methods: We asked all respondents (adult patients) from the former study to participate in a second analysis for quality of life using the EORTC QLQ-C30 questionnaire. The questionnaire was answered by 63% (n = 33) of the patients (21% non-response, 16% not included because of a relapse or death), 29 partners and 31 physicians. Forty-five percent had malignant lymphoma, 25% breast cancer and 30% acute leukemia. Allobmt was applied in 21% and autobmt in 79% of the cases. Median age at transplant 40 years.

Results: The structured interviews in 1995 revealed significant problems with fatigue and in relation to sexuality and return to work. The overall quality of life was 75. With 18 months longer follow-up it was 73 on a 100 pts scale. There are no significant differences between these 2 measure moments. However, there appeared to be a trend that the outcomes on functioning scales (except role functioning) were less high in the follow-up. The partner's view on the patient did not differ much with the results from the patient. Between the physician and the patient were significant differences at 3 functioning scales and 6 symptom scales or single items with the doctor judging Quality of Life better than the patient.

Conclusions: From this analysis we conclude that high dose therapy has a significant impact on several quality of life issues, which should be considered in the application of this modality in palliative situations. The EORTC QLQ-C30 may be helpful to analyze overall quality of life after transplantation, but may underestimate the effects on quality of life. The patient's partner has a good view on quality of life. The physician, however, overestimates the quality of life of the patient.

P-1297 ACCORDING WITH DIAGNOSIS, COULD BE ESTABLISHED RISK TOXICITY GROUPS IN AUTOLOGOUS PBSC TRANSPLANTATION?

J. Sevilla, D. Hernández, M. Canales, M.T. Jiménez, M. Morado, M.T. Alvarez, C. Hernández, M. Rodríguez, M.P. Martín, V. Jiménez, J.G. Bustos, M.J. Aguado, R. Arrieta, E. Ojeda, F. Hernández-Navarro. *Servicio de Hematología y Hemoterapia, Hospital "La Paz", Madrid, Spain*

Data about autologous PBSC transplantation in three groups of malignancies in 145 patients was prospectively recorded. Evaluation of quality of harvest, time for engraftment (granulocytes and platelets recovery), support requirements and complications was performed. The three groups were composed

as follows: breast cancer -61 patients-, lymphoma -46 patients-, and acute leukemias and myelomas -38 patients-. Conditioning, regimens were: STAMP-V for breast cancer, BEAC for lymphoma and BuCy2 or Busulfan + Melphalan acute leukemia & myeloma (AL&MM) group. Despite differences in diagnosis and chemotherapy, there is not statistically significant differences between three groups in harvest characteristics (CD34 $\times$ 10^6/kg and CMN $\times$ 10^8/kg). Median number of days to recover >0.5 $\times$ 10^9/L neutrophils (from day of infusion) was 13 for AL&MM group, 11.2 days for lymphoma group and 10.4 days for breast cancer group (p = 0.001), although neutropenia time (days with <0.5 $\times$ 10^9/L neutrophils) was not statistically significant: 10 days as median (p = 0,08). Platelet engraftment (>20 $\times$ 10^9/L platelets) was slower for AL&MM group-median 27 days- (p = 0.001) and in this group more platelet transfusions were needed. Complications during transplantation period were similar from the three groups except for gastrointestinal toxicity: patients that underwent autologous PBSC transplantation for breast cancer displayed more mucositis, more diarrhoea and vomits due to their conditioning regimen STAMP-V). There were no relevant differences between these three groups in incidence of intratransplant mortality, infections, VOD, and support requirements (red blood cells units, number of days with antibiotics). So, only differences in gastrointestinal toxicity and platelet support could be established, in our experience, for these threee groups of patients. They could be considered with the same toxicity risk for PBSC hematopoietic transplantation.

P-1298 AUTOGRAFTING FOLLOWED BY NONMYELOABLATIVE THERAPY/ALLOGRAFTING IS A FEASIBLE PROCEDURE IN ADVANCED HODGKIN'S DISEASE AND BREAST CANCER

A.M. Carella[1], E. Lerma[1], F. De Stefano[2], L. Celesti[1], F. Frassoni[1], A. Bacigalupo. *[1]Dept. of Hematology, Ospedale San Martino; [2]Forensic Medicine, Genoa, Italy*

Fludarabine (Flu) and Cyclophosphamide (Cy) are both potent immunosuppressive drugs and can also modulate GVHD. We started a pilot study with Flu-Cy protocol followed by HLA-matched sibling progenitor cells mobilized with G-CSF. Five patients were enrolled. Two patients had advanced resistant Hodgkin's disease (HD), two patients had metastatic breast cancer and one patient was in myeloid blastic crisis-CML. All patients were previously treated with Cy/G-CSF followed by autologous progenitor cells collection. After that, autografting was given to all patients in the attempt to reduce tumor bulky before allografting. At a median of 15 days after engraftment, we started the allografting procedure. The conditioning regimen consisted of Fludarabine (30 mg/m^2/day for 3 days) and cyclophosphamide (300 mg/m^2/day for 3 days) (Flu-CY protocol). Allogeneic progenitor cells were given to the patients 48 hours after. GVHD prophylaxis consisted of CSA/MTX. Flu-Cy treatment was extremely well tolerated in all patients. No aGVHD developed. Chimerism analysis was studied every 10 days on bone marrow and blood cells by STR polymorphirms via PCR and in 4/5 patients $\geq$95% of donor cells were found between 45 and 150 days post-transplant. All patients are alive between 63 and 210 days post allografting. One patient with HD who achieved PR after autografting, obtained CR after allografting. Two patients (HD, Breast cancer) mantained PR achieved after autografting, one patient mantains PH-positive second CP-CML achieved after autografting and the last patient is in progressive disease with new localizations in the liver and in bone.

P-1299 CHEMOTHERAPY AND AUTOLOGOUS PERIPHERAL BLOOD PROGENITOR CELL TRANSPLANTATION (aPBPCT) FOR HIGH RISK LYMPHOMA

S. Sica, P. Salutari, B. Innella, F. Sora', P. Chiusolo, L. Laurenti, N. Piccirillo, E. Ortu La Barbera, G. Leone. *Dept. Hematology-Universita' Cattolica S. Cuore, Roma, Italia*

The feasibility and the efficacy of a two-step protocol combining second line chemotherapy and aPBPCT was assessed in 70 pts with high risk lymphoma (NHL 49, HD21, median age 38 range 16–63, F/M 30/40). Chemotherapy included mitoxantrone 10 mg/m^2 day 1, carboplatinum. 100 mg/m^2 day 1–4, aracytin 2 g/m^2 day 5 and methylprednisolone 500 mg/m^2 day 1–5 (MiCMA) + G-CSF 5 μg/kg/day sc from day 10 until completion of leukapheretic (LKP) procedure. BM involvement was present in 25 out of 70 pts at diagnosis. At entry 30 pts (42.8%) were in untested relapse, 22 pts (31.4%) were in partial remission (PR), 9 pts (12.8%) were resistant and 9 (12.8%) showed disease progression (DP) to standard chemotherapy. A median of 3 cycles of MiCMA (range 1–6) was administered and response was as follows: complete remission (CR) in 20 pts (28.6%), PR in 29 pts (41.4%), DP in 19 pts (27.1%). Two pts are under evaluation. PBPC collection was obtained in 65 out of 70 pts (92.8%) with a median of 3 LKP (range 1–10). A median of 7 $\times$ 10^8 Kg MNC (range 0.01–17.9), 8.3 $\times$ 10^6/kg CD34+ (range 1.2–33.8) and 43 $\times$ 10^4/kg CFU-GM (range 3–258) was collected. PBPCT was performed in 46 out of 70 pts (65.7%). Their status at PBCT was: PR 27 pts (58.7%), CR 11 pts (23.9%), DP 6 pts (13%) and relapse 2 pts (4.4%). Conditioning regimen was BuCy2 in 35 pts, BEAM 8 pts and BuMel 3 pts. PBPCT related mortality was 0%. Hemopoietic recovery was obtained at a median of 12 d (9–25) to ANC > 0.5 $\times$ 10^9/l, 11 d (0–62) to Plts >50 $\times$ 10^9/l. After PBPCT 36 pts achieved CR (78.3%), 3 pts PR (6.5%) and DP in 7 pts (15.2%). At a median follow-up of 14.5 m (range 3–68) 22pts are alive in CR, 1 pt is alive in PR at a follow-up of 37 m, 16 pts relapsed at median of 8.5 m (range 2–58) and 8 of them died from the disease, 8 are alive in relapse, 7 patients with DP died at a median of 7 m (range 4–10) after PBPCT. Twentyfour pts did not receive PBCT for the following reason: DP 12 pts, refusal 4 pts, involved field radiotherapy 2 pts, medical decision 2 pts, 4 pts are waiting to be submitted to PBPCT. Ten pts died from DP at a median of 2.5 m (1–9) 6 pts are alive in CR at a median of 57 m (range 15–67), 2 pts were lost to follow-up and 2 pts are alive in PR and DP. Our protocol was both feasible (65.7% pts completed the protocol), efficacious (70% CR+PR to MiCMA), almost all pts were able to collect PBPC. Sensitivity to chemotherapy was the most important prognostic factor both for survival and freedom from progression. The impact of the international prognostic index for NHL is also included.

P-1300 FLEXI-VACOP/B FOLLOWED BY HIGH-DOSE CYTOXAN (CY) AND HIGH DOSE THERAPY + AUTOLOGOUS PERIPHERAL BLOOD PROGENITOR CELL (PBPC) RESCUE FOR AGGRESSIVE NHL WITH BONE MARROW INVOLVEMENT AT DIAGNOSIS

A.M. Congiu, P. Coser, P. Leoni, L. Salvagno, G. Santini. *For the Non-Hodgkin's Lymphoma Co-Operative Study Group. Department of Haematology, S. Martino Hospital, Genova, Italy*

Aggressive NHL with BM involvement at diagnosis has a poor prognosis. Survival probability at 3-years is about 20% using a conventional chemotherapy. In 1992, following the encouraging results achieved in patients with persistent BM involvement after conventional treatment, a new study was started using VACOP-B until maximum response followed by high-dose cytoxan and PBPC autografting as first-line therapy. 40 successive patients (groups F-G-H-K/WF) entered the study. Median age of pts. was 51 yrs. (range 20–60); 25 were males and 15 females; median BM involvement was 35% (range 8%–90%). Patients received a median of 8 VACOP-B courses (range 4-12), followed by CY (7 gr/m^2/single dose) plus G or GM-CSF (5 mcg/Kg) in order to reduce tumor burden and to collect PBPC. Median number of aphereses was 3 (range 1–7); median of collected cells was 6.1 $\times$ 10^8/Kg; median of CD34+ was 11.3 $\times$ 10^6/Kg and median of CFU-GM was 35.3 $\times$ 10^4/Kg. All pts. are evaluable for response. Eleven patients did not conclude the procedure: 1 was ineligible; 2 refused procedure; 6 died in progression; 1 died of pulmonary fibrosis and 1 of cardiac failure after CY administration. Other pts. underwent PBPC autografting after Melphalan + TBI or BEAM regimen. According to intention to treat, 28/40 pts. (70%) obtained CR: 9/40 (22.5%), after VACOP-B treatment; 8/40 (20%), after high-dose CY; 11/40 (27.5%), after high-dose therapy • PBPC autografting. The statistical analysis shows a 5-year probability of survival of 35%, with a probability of DFS and PFS of 35% and 19%, respectively. This study suggests that this procedure is superior to conventional treatment and a randomized study is now ongoing to establish its impact in improving outcome for this category of patients.

P-1301 PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (PBSCT) FOR MULTIPLE MYELOMA AND LYMPHOMA. A SINGLE CENTER EXPERIENCE

C. Salvador-Osuna, P. Giraldo, G. Perez-Lungmus, J.J. Gimeno, P. Delgado, J.C. García-Zueco, P. Mayayo, D. Rubio-Félix, M. Giralt. *Haematology Department, Miguel Servet Hospital, Zaragoza, Spain*

Purpose: To analyze the results using PBSCT in patients with haematological malignancies other than leukaemias in a single center.

Patients & Methods: *From 08/95 to 01/98, 30 patients (17 NHL: high grade 7, intermediate grade 2, low grade 8, 4 Hodgkin's disease and 9 multiple myeloma) entered in a PBSCT program. Mean age 44.9 years (range 14–68). M/F 18/12. Disease status at transplant CR 16 (56.7%); PR 14 (43.3%) Mobilization: G-CSF alone 8 cases; G-CSF + Cyclophosphamide 4 cases and G-CSF + Polychemotherapy 18 cases. Apheresis procedures were performed according to the CD34 level. Two different systems (Cobe Spectra® and Fenwall 3000®) were used for apheresis procedures. Cellular products were cryopreserved using a programed cryopreservation system and stored at -196° until use. Conditioning regimens: Melphalan alone (MM), BEAC (NHL) and BEAM (HD).*

Results:

Evaluated parameters	MM	NHL	Total
Apheresis: mean (range)	5 (3–10)	3 (2–7)	4 (2–10)
MNC × 10^8/kg	10.3±2.7	8.1±3.4	8.2±3.2
CD34 × 10^6/kg	4.7±2.3	6.2±5.9	5.2±4.6
CFU-GM × 10^5/kg	2.0±1.6	2.2±1.4	2.0±1.4
ANC > 0.5 × 10^9/L	10.8±1.3	10.4±1.9	11.0±2.0
Platelets >20 × 10^9/L	13.1±3.1	13.1±3.1	14.4±6.4
Transfussion RBC/PLT	2.5/19	3.0/21	3.2/20
Days of hospitalization*	28.5±8.8	25.0±6.4	27.5±8.1

* including days on conditioning regime.

After a median follow up 9 months (range 2–30), 23 patients (76.6%) were alive (14 NHL; 7 MM; 2 HD), 4 of them had relapsed at 2, 7, 15 and 17 months after PBSCT.

Seven patients died: (4 viral infection, 1 relapse, 2 transplant related)

Comments: In our short experience PBSCT shows encouraging and similar results to previously reported.

P-1302 EVALUATION OF AMIFOSTINE FOR PREVENTION OF CYCLOPHOSPHAMIDE INDUCED HEMORRHAGIC CYSTITIS

A. Srivasatava[1], S.C. Nair[1], V.M. Srivastava[2], A.N. Balamurugan[3], M. Chandy[1], S. Gunasekharan[3]. *[1]Departments of Hematology; [2]Pathology; [3]Physiology, CMC Hospital, Vellore, India*

Hemorrhagic cystitis (HC) commonly occurs in patients receiving high dose cyclophosphamide (Cy) despite preventive measures. Amifostine (Af) is an aminothiol that provides cytoprotection against various chemotherapeutic agents in different organs but its possible role in preventing HC has not been explored.

We have evaluated the cytoprotective effect of Af on urothelium in the rat model. Forty eight albino rats, weighing 130–230 grams, were divided into 4 groups of 12 rats each. The three experimental groups received 150 mg/kg of Cy with no Af (Gr.I), 100 mg/kg of Af (Gr.II) and 200 mg/kg of Af (Gr.III) while the control group (Gr.IV) received no drugs. Both drugs were administered intraperitoneally, Af 15 minutes prior to Cy. Six animals from each group were sacrificed 24 hours later and the rest after 7 days. The bladders were examined for evidence of HC. Histopathological changes were graded as per clearly defined criteria.

All rats in Gr.I receiving Cy alone, developed severe HC by 7 days. In Gr.II, 6/10 evaluable rats had no HC while 4/10 had mild HC. In Gr.III, none of the 12 rats had any evidence of HC. There was no HC in any of the Gr.IV rats.

AF provides excellent protection against Cy induced HC in rats. This needs to be confirmed in humans. It can then be used for prevention of HC in a wide variety of clinical situations.

P-1303 UMBILICAL CORD BLOOD TRANSPLANT (UCBT) FROM UNRELATED MISMATCHED DONOR IN HIGH RISK (FIR) LEUKEMIA

W. Arcese, C. Guglielmi, A.P. Iori, M. Screnci, F. Boecklin, D. Carmini, G. Cimino, L. De Felice, L. Elia, L. Laurenti, A. Mengarelli, M.L. Moleti, P. Perrone, A. Romano, A.M. Testi, F. Mandelli. *Dipartimento di Biotecnologie Cellulari ed Ematologia, University "La Sapienza" Rome, Italy*

Fourteen eligible patients (pts) with HR leukemia underwent UCBT from unrelated HLA mismatched donor (twelve for 1 locus and two for 2 loci, after DRB1 high-resolution typing) at median intervals of 99 days from the start of the search, 52 days from the formal identification of the eligible UCB unit and 90 days from their last CR. Two were transplanted in CR1 achieved after failure of primary induction regimen (1 AML, 1 ALL), eight in CR2 (1 AML, 7 ALL), three ALL pts in second relapse occurring shortly after CR2 and one with CML in accelerated phase. Median age was 7.5 years (2–16) and median body weight was 27 Kg (12–67). Conditioning regimen (fractionated TBI, VP16, cyclophosphamide and anti-lymphocyte serum) and prophylaxis of graft-versus host disease (GVHD) (cyclosporin-A and 6-methylprednisolone) were identical for all pts. All pts recovered neutrophils (>0.5 × 10^9/L) at a median of 33 days (22–74) after UCBT, but four showed an autologous hematopoietic reconstitution (three spontaneous, one after autologous bone marrow rescue). Platelets >20 × 10^9/L were achieved in nine pts in a median of 66 days (17–135). Acute and chronic GVHD were observed in 8 and 2 cases, respectively, two pts dying with grade III–IV disease. Three ALL pts relapsed so far, and two of them had been transplanted in relapse. After a median follow-up of 1 year the actuarial disease-free survival at 1 and 2 yrs were 63% and 42%, respectively. Our experience suggests that UCBT from HLA mismatched unrelated donor is a valid option for the treatment of HR leukemia since it may be performed shortly after the start of search. With our selection criteria, conditioning regimen and GVHD prophylaxis, graft-failure and fatal graft versus host disease represented the main obstacles to a prolonged survival.

P-1304 THE JERUSALEM HUMAN UMBILICAL CORD BLOOD (HUCB) PROGRAM

G. Varadi, C. Brautbar, U. Elchalal, P. Cohen, A. Nagler. *Human Umbilical Cord Blood Bank, Tissue Typing Unit, Gynecology & Obstetrics Dept., Hadassah University Hospital, Jerusalem, Israel*

From July 1994 until November 1997 we have performed 9 HUCB transplantations. Six were for genetic diseases (SCID-2; Hurler's syndrome 1; Thalassaemia major-1; Fanconi's anemia-1; Adrenoleukodystrophy-1) and three for haematological diseases (AML M4 in 2nd CR, MDS RAEBT and biphenotypic AL in CR). Six HUCBT were performed from fully matched siblings, two from matched unrelated donors (New York Cord Blood Bank) and one underwent autologous HUCBT. Five patients were Arabic origin, 3 were Askenazi Jews and one an Oriental Jew. Five were female and 4 male, with a median age of 4.8 years (range 0.3–11.6 years). The median weight was 15.5 kg (range 5.5–40 kg). The 2 SCID patients received no conditioning. Five patients received myeloablative conditioning (2 with TBI) and two patients (FA 1; AL 1) received non-myeloablative conditioning containing fludarabine 30 mg/m^2 × 6, busulfan 4 mg/kg × 2 or cyclophosphamide 10 mg/kg × 2 and ATG 10 mg/kg × 2. For GVHD prophylaxis cyclosporine A was administrated. Two patients did not engraft (autologous rescue -1; autologous reconstitution -1). The other patients achieved ANC > 0.5 × 10^9/L on day +28 (9–37), WBC > 1 × 10^9/L on day +29 (8–38) and platelets >20 × 10^9/L on day +48 (19–57). Three patients developed acute GVHD (grade I–II - 2; grade IV - 1). One patient recieved cord blood that was contaminated with *Klebsiella pneumoniae*. He was treated with antibiotic from the day of HUCBT and did not develop clinical sepsis. One child developed CMV pneumonitis, was treated with gancyclovir and IVIG and recovered. No child developed severe mucositis, VOD or renal toxicity. Two patients died of TRC (GVHD grade IV-1; cardiomyopathy - 1). The fludarabine-based protocol which avoids irradiation should be evaluated in a larger group of HUCBT.

Cell modifiers: proapoptotic treatment, anti-signal transducing and anti-sense drugs, ribozymes, anti-fusion proteins

P-1305 ULTRASOUND IRRADIATION INDUCES APOPTOSIS IN HL-60 CELLS AND IN NORMAL AND PATHOLOGICAL BLOOD MONONUCLEAR CELLS

L.A. Rozenszajn[1], N. Gozdanker[1], H. Ashush[1], H. Shapira[2], U. Rosenschein[3], J. Radnay[1,2]. [1]*Bar-Ilan University, Ramat-Gan;* [2]*Meir Hospital, Kfar-Saba;* [3]*Ichilov Hospital, Tel-Aviv, Israel*

Apoptotic cell death occurs in response to diverse stimuli including TNFα, Fas ligand, anti-IgM, chemotherapeutic agents as well as UV and ionizing irradiation. To determine whether the effect of low frequency ultrasound irradiation on hemopoietic cells results in apoptotic cell death we studied sonication on human HL-60 leukemic cell line and peripheral blood mononuclear cells (MNC) and MNC from chronic lymphatic leukemia cells. Attention has been devoted to the investigation of the acoustic effect, produced by low frequency ultrasound, defined as generation and oscillation of gas bubbles in the experimental media. Here we present evidence that low intensity ultrasound irradiation on hemopoietic cells cause cellular lesions inducing apoptosis. Leukemic cells were found sensitive to ultrasound irradiation, where as normal cells were less sensitive, at different levels in the sonication. Fluorescent measuring of viable and apoptotic cells by ethidium bromide and acridine orange provide a fast and objective method for studying the effect of ultrasound irradiation on cell suspension. Changes in the surface of treated cells undergoing apoptosis includes the break up of phospholipid of their plasma membrane and expressed phosphatidylserine from the inner to the outer side of the membrane layer. In apoptotic cells, reduced DNA content was found by flow cytometry following staining of cellular DNA presented by a sub-G_0 peak (A_0). After ultrasound, morphological alterations of apoptotic cells involve: cell shrinkage, membrane blebbing, chromatin condensation, nuclear fragmentation and apoptotic body formation exhibiting hyperchromasia. No significant changes in cell cycle distribution were observed in sonicated live cells. Our results indicate that low frequency ultrasound irradiation may induce apoptotic cell death in normal and pathological hemopoietic cells. However, the understanding of the initiation step responsible for ultrasound-induced apoptosis signaling is not yet well defined.

P-1306 LOSS OF MITOCHONDRIAL TRANSMEMBRANE POTENTIAL AND ACTIVATION OF CASPASE-3 ARE NECESSARY BUT NOT SUFFICIENT TO INDUCE APOPTOSIS

Y. Kawabata, M. Hirokawa, J. Kuroki, A. Kitabayashi, T. Horiuchi, A.B. Miura. *Department of Internal Medicine III, Akita University School of Medicine, Akita, Japan*

Recently it has become apparent that apoptotic signals irrespective of the stimulation used are transduced to the common pathway, the reduction of mitochondrial transmembrane potential and activation of caspase-3 leading to DNA degradation. We have previously reported that cell-permeable ceramide inhibits the growth of B lymphoma Raji cells by inducing G0/G1 cell cycle arrest but not apoptosis, (Leukemia 10: 1950, 1996). We have taken advantage of this cell line to ask whether these two events are sufficient to induce DNA fragmentation during apoptosis. Following the treatment with cell permeable C2-ceramide or mitochondrial permeability transition inducer (t-butylhydroperoxide), not only apoptosis-inducible cell lines (HL-60, Jurkat and Daudi cells) but also apoptosis-resistant Raji cells showed the loss of mitochondrial membrane potential and activation of caspase-3. HL-60, Jurkat and Daudi cells showed apoptotic cell death, whereas Raji cells did not show detectable level of apoptosis. In the cell-free system, the cell lysates prepared from HL-60 cells treated with t-butylhydroperoxide induced apoptosis of Raji nuclei, whereas the cell lysates from Raji cells treated with t-butylhydroperoxide did not induce apoptosis of HL-60 nuclei. These results indicate that the reduction of mitochondrial transmembrane potential and the activation of caspase-3 are not sufficient to induce apoptosis.

P-1307 BCL2-EXPRESSION, SPONTANEOUS AND DRUG INDUCED APOPTOSIS IN CHILDHOOD LEUKEMIA: A PILOT STUDY

E.G. Haarman[1], G.J.L. Kaspers[1], R. Pieters[1], G.J. Broekema[1], C.H. Van Zantwijk[1], K. Hählen[2], A.T.P. Veerman[1]. [1]*University Hospital Vrije Universiteit, Amsterdam;* [2]*Sophia Children's Hospital, Rotterdam, The Netherlands*

The antileukemic activity of cytotoxic drugs is increasingly thought to be the result of induction of apoptosis. Several proto-oncogenes have been related to the regulation of this process. In this study bcl2 expression and its relation to spontaneous and drug induced apoptosis were investigated. We tested 46 lymphoblastic and 16 myeloid leukemia samples taken at initial diagnosis (iALL, iAML) and 31 lymphoblastic and 6 myeloid samples taken at relapse (rALL, rAML). The leukemic cells were incubated in culture medium, with and without dexamethasone (DXM, in ALL) or cytarabine (ara-C, in AML). Bcl2 expression and apoptosis were measured flowcytometrically, the latter using DNA histogram analysis. Bcl2 expression was 1.4 fold higher in rALL than in iALL ($p = 0.008$). Spontaneous apoptosis increased significantly from 0 to 48 hours (up to 71%), but in AML samples less then in ALL samples. Bcl-2 expression correlated with the extent of spontaneous apoptosis after 24 hours in iALL ($rho = -0.40$, $p = 0.05$). DXM and ara-C induced apoptosis time- and dose-dependently (up to 81%). In iAML samples the extent of ara-C induced apoptosis was correlated with the degree of spontaneous apoptosis ($rho = 0.77$, $p = 0.01$). This correlation was not found in the other groups. In rALL, but not in iALL, high bc12 expression was correlated with a low rate of drug induced apoptosis ($rho = -0.66$, $p = 0.005$). In conclusion, DXM and ara-C exert their cytotoxic effect at least partly by means of apoptosis. Bcl2 appears to inhibit drug induced apoptosis in rALL. This study will be continued including more recently described cell-death regulators. (Supported by the Dutch Cancer Society, VU 97-1564.)

P-1308 CHEMOTHERAPY-INDUCED APOPTOSIS RESULTS IN CPP32 ACTIVATION BUT DOES NOT REQUIRE FAS/FAS LIGAND INTERACTIONS

D.S. Richardson, P.D. Allen, S. Patel, S.M. Kelsey, A.C. Newland. *Dept. of Haematology, St. Bartholomew's & Royal London School of Medicine, London, UK*

It has been suggested that one means by which chemotherapeutic agents exert their effect on leukaemic cells, is via autocrine induction of fas-ligand which then binds to fas (CD95), activates the caspase pathway and results ultimately in apoptotic death. In order to test this hypothesis, we have treated leukaemic cell lines with various chemotherapeutic agents (idarubicin, etoposide, fludarabine and 2-CdA). The fasligating antibody, CH11 and all drugs were found to increase CPP32 activity in a colorimetric assay; interestingly, with less activity at 6 hours seen in parental T lymphoblastic leukaemia cells, CEM, than their mdr-expressing drug resistant counterpart, CEM/VLB. Cells were also treated with chemotherapeutic drugs +/−pre-treatment with fas (ZB4) and fas-ligand (NOK-1) blocking monoclonal antibodies. Cell cycle analysis and quantitation of apoptosis were performed by flow cytometry following propidium iodide staining. HL60 cells were found to be sensitive to the induction of apoptosis with all drugs tested but were highly resistant to treatment with CH11. Apoptosis was not inhibited in parental CEM cells nor in CEM/VLB by pre-treatment with either ZB4 or NOK1. In addition, CEM/VLB were slightly more sensitive to treatment with CH11 (100 ng/ml) than parental CEM cells (%age apoptosis = 30.35 and 23.675, $p = 0.024$) and at least as sensitive to recombinant fasligand (50 ng/ml) (%age apoptosis = 26.63 and 20.2, p = NS).

We conclude that although chemotherapeutic agents activate CPP32, it is unlikely that fas/fas-ligand interactions play a significant role in the induction of apoptosis by these drugs in the leukaemic cell lines tested.

P-1309 ARSENIC TRIOXYDE AND MELARSOPROL INDUCE APOPTOSIS IN MYELOMA CELLS IN VITRO AND INHIBIT PLASMA CELLS GROWTH IN VIVO

Ph. Rousselot, S. Labaume, J.P. Marolleau, J. Larghero, J.C. Brouet, J.P. Fermand. *Hôpital Saint-Louis, Paris, France*

We studied the effect on malignant and normal plasma cells of AS_2O_3 and melarsoprol, two arsenical compounds that have recently been shown to induce apoptosis of promyelocytic leukaemic and B lymphoid cells.

AS_2O_3 and melarsoprol (10^{-8} to 10^{-6} M) caused a dose- and time-dependent inhibition of survival and growth in plasma cell lines. A 36 hour incubation in presence of 10^{-6} M AS_2O_3 resulted in a reduction of thymidine incorporation about 100%, 85%, 50% and 40% for NCI, OPM2, U266 and LP1 cell lines respectively. Results were similar using the organic salt melarsoprol. Treatment of NCI induced a rapid apoptosis with 15% of annexin-positive, PI-negative apoptotic cells after 18 hours in the presence of As_2O_3 10^{-6} M as compared with about 40% in the presence of melarsoprol. Treatment with AS_2O_3 10^{-6} M for 24 hours promoted DNA fragmentation as assessed by the TUNEL method in 72, 25, 30 and 15% of NCI, OPM2, U266 and LP1 cells respectively.

As_2O_3 and melarsoprol also inhibited the viability and growth of plasma-cell enriched preparations (containing more than 80% of plasma cells) from bone marrow or blood of 11 myeloma patients. Exposure to 10^{-6} M AS_2O_3 for 48 hours induced a mean decline in cell viability to 47% (range 26 to 64%) as compared with 15% (range 0 to 36%) in control cultures. In a representative study of a bone marrow sample initially containing 45% of plasma cells (CD38 ^{high} +), 12% of these expressed annexin V after 2 days of culture with 10^{-6} M AS_2O_3, whereas 0.9% of myeloid cells (CD38 ^{low}, CD15 +) were positive for the apoptosis marker.

In primary myeloma cells as in cell lines, IL6 did not prevent arsenic-induced cell death or growth inhibition. No synergistic effect was observed with alpha-Interferon. In contrast to AS_2O_3, melarsoprol did not reduced PWM-induced plasma cell differentiation and did not affect the targeting of PML onto nuclear bodies in malignant plasma cell lines as well as in PWM-induced normal plasma cells.

Preliminary studies of AS_2O_3 and melarsoprol in SCID mice transplanted with human myeloma cells appear to confirm *in vivo* the *in vitro* effects of the two arsenical compounds. These results have prompted us to initiate a phase I–II trial in refractory myeloma patients.

P-1310 ANTIPROLIFERATION EFFECTS OF ANTISENSE OLIGOMER C-MYB/IGF-1R ENWRAPPED BY HEXADECYLPHOSPHOCHOLINE ON LEUKEMIA AND SOLID TUMOR CELL LINES IN IN-VITRO AND IN VIVO

Yu-Zhi Wang[1], Lan-Jun Wu, Xiu-Ying Liu, Qin-Sheng Xu[2], Dong Fu. *Institute of Radiation Medicine of AMMS, Beijing 100850, China*

The human leukemia HL-60 cell line was exposed to an antisense oligonucleotide (ASON) complementary to an 18-base-pair sequence of c-myb encoded mRNA, 47–64% growth inhibition was observed in cultured for 3–5 days comparing to untreated HL-60 cells. Growth inhibition was not observed *in random oligomer sequence treated cells. The c-myb mRNA expression* by RT-PCR also was examined that c-myb mRNA expression was inhibited by ASON, while c-myb mRNA expression was detected in untreated HL-60 cells. In the cell differentiated phenotype, about 50% NBT-positive in HL-60 exposed to c-myb ASON, only <5% in untreated cells. These results indicated that the nuclear protein encoded by the c-myb is required for maintenance of proliferation in certain leukemia cell line and may be involved in the differentiation of myeloid leukemia cells.

In order to enhance the ASON-uptake of cells, the liposome forming property of Hexadecylphosphocholine (HePC) was investigated. Using Laser Scanning Confocal Microscope to detect that HePC-liposome-encapsulated ASON (ASON labeled with fluorescein) was able to be internalized and increasing the activity of the ASON-c-myb by 20% in HL-60 in-vitro, while of the ASON-IGF-1R by 70% in human pancreatic cancer cell line SW1990 in nude mice in-vivo. HePC-liposom could combine with ASON and demonstrate synergistic therapeutic effects against some tumors. HePC is alkylphospholipid analogue which demonstrated widely antitumor activities we had shown period, but did not interference significantly with cell proliferation of normal BMC by testing CFU-gm and would be an excellent antitumor agents for selective killing tumor cells and with low toxicity comparing with conventional cytotoxic agents. We also suggests that HePC-ASON might be used for bone marrow purging agent.

P-1311 VALIDATION OF A MATHEMATICAL MODEL FOR IMPROVED ANTISENSE OLIGONUCLEOTIDE THERAPY IN HAEMATOLOGICAL MALIGNANCIES

D.A. Fennell[1], M. Corbo[1], B. Kuss[1], F. Dazzi[2], J.M. Goldman[2], F.E. Cotter[1]. *[1]LRF Molecular Haematology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH; [2]LRF Dept of Haematology, RPMS, Hammersmith, London, UK*

Objective: Antisense oligonucleotides (ASO) "switch off" the abnormal genes mediating a failure of apoptosis in haematological malignancies. Mathematical modelling of ASO may improve their drug development. This study examines the hypothesis that leukaemia antisense pharmacodynamics can be analysed quantitatively and experimental predictions examined in practice.

Design, Methods and Results: A mathematical framework for interpreting pharmacodynamics of antisense oligonucleotides based on steady state antisense kinetics have been designed. These provide predictive results. Three SCID-Hu models of malignant B-cell lymphoma, biphenotypic leukaemia and CML, all with high Bcl-2 expression and chemoresistance were set up. In vitro treatment of corresponding cell lines with 10 μM of Bcl-2 ASO with a phosphorothioate backbone (G3139, Genta Inc) showed downregulation of Bcl-2 protein in a dose dependent manner, and subsequent treatment with etoposide revealed a marked increase in apoptosis (morphology, sub G1 analysis, CPP32 activation, annexin V and MTT assay). In vivo, Bcl-2 ASO similarly showed downregulation of Bcl-2. Treatment of the mice by IV etoposide at doses between 1.25 μg and 250 μg (following Bcl-2 ASO) resulted in a log fold increase in apoptosis in all models. The kinetics of these experiments were compared to the mathematical model and shown to fit the hypothesis. Subsequently application of the model, predictively, to additional antisense molecules are under investigation.

Conclusion: G3139 Bcl-2 AS has already been used in a human phase I study for lymphoma which demonstrated efficacy. The mathematical model may assist in rational design and improvement of ASO therapy for malignancy for the future.

P-1312 HIGH EFFICIENT LONG TERM INHIBITION OF HIV-1 REPLICATION BY NEW RETROVIRAL ANTI-HIV RIBOZYME VECTORS

C. Klebba[1], S.A. Klein[1], T.S. Dobmeyer[1], M. Grez[2], J.W. Engels[3], O.G. Ottmann[1], D. Hoelzer[1]. *[1]Uniklinik Frankfurt, Med. Klinik III; [2]Georg-Speyer-Haus, Frankfurt; [3]Universitat Frankfurt, Institut für Organische Chemie, Germany*

Retrovirally expressed ribozymes have been demonstrated to inhibit HIV-replication efficiently. To achieve an improved long-term inhibition of HIV-replication we synthesised new ribozymes targeting alternative highly conserved sequences of the HIV genome. Additionally improved vectors for the expression of ribozymes as well as packaging and transduction technologies were optimised. Based on the analysis of the secondary structure of the HIV genome and the identification of highly conserved sequences, 20 different hairpin and hammerhead ribozymes against not yet targeted sequences of the HIV genome were synthesised and cloned into different advanced retroviral vector backbones. As the only reporter gene, the non-immunogenic intracellularly truncated low affinity nerve growth factor receptor (LNGF-RΔ) was added. HIV susceptible cell lines such as HUT78, U937, Molt 4 and Jurkat and primary $CD4^+$ T-cells were transduced using virus supernatants derived from Phoenix, FlyRD-18, GP+ envAM-12 and PG-13 packaging cell lines. Transduced cells expressing the LNGF-RΔ were quantified and sorted by FACS followed by infection with HIV. In vitro HIV replication was monitored by p24-ELISA in culture supernatants. Ribozymes targeting the 5'-LTF, the protease, and the integrase region of the HIV genome: were highly effective in inhibiting HIV replication. p24 concentrations in culture supernatants of HIV III infected cells were reduced 2 to 3 logs for up to 8 weeks. Transduction efficiency in CD4+ T-cells could be increased to more than 40% i.e. >80% of cycling cells using an improved stimulation and anti-apoptotic protocol with OKT3, IL-2 and IL-1 and FlyRD-18 virus supernatants.

P-1313 RIBOZYME-MEDIATED CLEAVAGE OF AML1/MTG8 FUSION-TRANSCRIPTS

J. Krauter, O. Heidenreich, A. Ganser, G. Heil. *Dept. of Hematology/Oncology, Hannover Medical School, Hannover and Dept. of Cell Biology, University of Tübingen, Germany*

The reciprocal translocation t (8;21) (q22;q22) in acute myeloblastic leukemia (AML) fuses the N-terminal part of the AML1-gene of chromosome 21 to the nearly complete MTGS-gene of chromosome 8 resulting in a chimeric AML1/MTG8 fusion mRNA. The AML1-gene encodes for a transcription factor essential for normal hematopoiesis. The function of MTG8 is still unknown. AML1/MTG8 fusion transcripts can be detected in all patients with t (8;21). However, the role of this chimeric gene product in leukemogenesis is still unclear. We constructed hammerhead ribozymes which bind to the MTG8-portion but cleave within the AML1-portion at a CUC-triplet 3 nucleotides 5′ of the fusion site of the chimeric transcript. These ribozymes effectively bind and cleave the AML1/MTG8 run-off transcript and inhibit in-vitro translation of the AML1/MTG8 protein. In contrast, the wild-type AML1b mRNA and protein synthesis is not affected. After ribozyme-binding and cleavage, the fusion site of AML1/MTG8 cannot be amplified by RT-PCR whereas the flanking regions of the AML1- and MTGS-portion remain unaffected further demonstrating the specifity of the ribozyme for AML1/MTGS. HL60 cells transfected with the ribozymes showed a stable expression of the ribozyme-RNA without a negative effect on clonal cell growth as compared to the untransfected cells. Moreover, the ribozyme-RNA endogenously expressed by HL60 cells effectively targets AML1/MTG8 transcripts. In conclusion, the stable expression of ribozymes which specifically target the synthesis of the AML1/MTG8 fusion product in hematopoietic cell lines might be a suitable approach to analyse the role of AML1/MTG8 in leukemogenesis.

P-1314 DIPHTHERIA TOXIN FUSED TO GM-CSF CAN BE USED FOR IN VIVO TARGETING OF PRIMARY AML CELLS IN IMMUNODEFICIENT MICE

A.C.M. Martens, H. Rozemuller, W. Terpstra, E.J.C. Rombouts, R.J. Kreitman, D.J. FitzGerald, B. Löwenberg, I.P. Touw, A. Hagenbeek. *Institute of Hematology, EUR, Rotterdam, Lab. of Mol. Biol. N.I.H., Bethesda, USA; Dept. of Hematology, University Hospital Utrecht, Utrecht, The Netherlands*

The utility of Diphtheria toxin fused to GM-CSF (DT-GM-CSF) for treatment of leukemia was studied in the AML-SCID mouse model. When human AML cells are injected into conditioned SCID mice leukemic cells continue proliferation and AML/SCID mice can thus be used to study the anti-leukemic efficacy of DT-human GM-CSF. Dose escalation studies revealed a maximal tolerated dose (MTD) in SCID mice for DT-hGM-CSF of 75 μg/kg/day given i.p. for 7 consecutive days. Seven AML's were studied. Conditioned SCID mice were injected on day 0 with primary AML cells and treated with 75 μg/kg/day DT-huGM-CSF from days 3–10. Around day 40 the percentage of AML cells in the SCID mouse BM was determined using a panel of monoclonal antibodies and flow cytometry (with a lower level of detection of human AML cells of 0.5%) and compared to untreated controls. In 4 AML's no leukemic cells were detectable in the BM of treated mice whereas in control mice between 2 and 90% human AML cells were found. In 1 AML a 40% reduction was found and in 2 AML's the number of leukemic cells in the BM of treated and control mice was similar. The latter cases did not respond to GM-CSF in vitro.

Because there is no cross-species reactivity for GM-CSF, DT-murineGM-CSF was used for evaluating the in vivo toxicity of DT-mGM-CSF to normal progenitor cells. Normal mice were treated for 7 days at the MTD level of 25 μg/kg/day and the effect on the various hemopoietic progenitor cell subsets was determined from days 8–21. In DT-mGM-CSF treated animals no reduction in the number of BM stem cells subsets (measured as cobble stone area forming cells, CAFC) was found throughout the observation period. A typical observation, however, was a steep drop in the number of the committed progenitor cell type CFU-GM on day 11 in the BM (3 days after treatment) with a concurring steep rize in the spleen that fully compensated for the BM decrease. Three days later the numbers in BM and spleen returned to normal levels. Because normal hemopoietic stem cells survive at dose levels that yield in the AML/SCID model up to 3 to 4 log leukemic cell kill, these studies show that a "therapeutic window" exists to exploit the GM-CSF-R on leukemic progenitor cells for selective targeting of DT-GM-CSF.

P-1315 RETINOIC ACID AND ARSENIC IN ACUTE PROMYELOCYTIC LEUKEMIA

H. De Thé, J. Zhu, F. Quignon, M. Koken, M. Gianni, M. Chelloi-Alix, V. Lallemand, Z. Chen. *CNRS 9051, Hospital St. Louis, Paris France, Rui-Jun Medical School, Shanghai, China*

Acute promyelocytic leukemia (APL) is specifically associated to a t (15, 17) translocation which fuses PML, and RARa. There is evidence to suggest that PML/RARa antagonises both differentiation and apoptosis. While RARa promotes differentiation, PML suppresses growth, possibly as a result of its specific binding to nuclear subdomains (PML nuclear bodies). In APL, the PMI,/RARa fusion delocalises PML onto distinct nuclear microspeckles which was proposed to induce proliferation.

Retinoic acid (RA) leads to remissions by inducing differentiation. The critical molecular events triggered by RA are not fully characterized. However, disruption of PML localisation is reversed upon RA exposure, as a consequence of RA-induced PML/RARa degradation. Arsenic trioxide (As), was identified as an APT, therapy which induces apoptosis, rather that differentiation. Remarkably, As, like RA, induces the degradation of the PML/RARa fusion protein. As appears to target the PMI, moiety of the fusion, as it accelerates NB-targeting of PML and induces its degradation. Thus, these two APL therapies each specifically target the PML or RARa moiety of the fusion protein and both induce its degradation, identifying two complementary oncogene-targeted therapies.

We have recently examined associations between RA and As either in APL cell-lines or *in vivo* and observed a clear synergy for both differentiation and apoptosis. We will also report experiences demonstrating that As induces covalent modifications of PML by PIC-1 ubiquitin-like peptide. As also greatly enhances PML-mediated growth suppression which could account for As-induced apoptosis.

P-1316 PILOT STUDY OF ARSENIC TRIOXIDE (ATO) IN ACUTE MYELOID LEUKEMIA

P.H. Wiernik, R.E. Gallagher, S. Kim, E. Paietta, J.P. Dutcher. *Albert Einstein Cancer Center, Montefiore Medical Center, Bronx, NY, USA*

6 patients (pts) with relapsed/refractory myeloid leukemia have been treated with ATO, 10 mg/day IV infusion. Diagnoses: blast crisis of chronic myeloid leukemia (CMLBC)-1, refractory anemia with excess blasts in transformation (RAEBT)-2 (1 too early to evaluate), AML-2, and APL in 1 pt. The duration of treatment is 1–60+ days to date. 1 AML pt is off study due to exacerbation of diabetic neuropathy. No significant toxicity has been noted to date in other pts. The APL pt achieved complete immunophenotypic and marrow response within 28 days (CD117 decreased from >90% to 0% by day 22. A week later >750% CD11b+ noted). All metaphases initially had t (15;17), and at day 22 only 2/20 were +. A relapsed AML pt had improved WBC differential count in parallel with decreasing CD117 positivity and increasing CD11b positivity, first seen on day 29. The CMLBC pt had reduction in splenomegaly and WBC and is continuing beyond 60 days, despite no immunophenotypic response to date, and development of a tetraploid clone while on study. A marked decrease in clonogenic growth in the APL pt, with transient decreases in an RAEBT pt and the CMLBC pt were observed in vitro. Arsenic trioxide has activity in APL and other myeloid leukemias, the magnitude of which will be determined in this study.

P-1317 ENDOGENOUS PRODUCED INTERLEUKIN-6 PROTECTS K562 CELLS FROM CISPLATIN MEDIATED CYTOTOXICITY

G.V.Z. Dedoussis, P. Menounos, M.-C. Kyrtsonis, A. Karameris, A. Maniatis. *Laboratory of Research, Nursing Military Academy, Department of Pathology, 401 General Army Hospital, Laboratory Haematology and Transfusion Medicine, University of Patras Medical School, Greece*

Cisplatin is an efective chemotherapeutic agent. However in most patients who initially responded to CDDP, the tumor became unresponsive to CDDP upon continuing treatment. IL-6 is a cytokine produced by various tumor cell lines and has been associated with drug resistance in some cases.

Objective: The aim of our study was to determine the contribution of IL-6 in the regulation of drug-induced cytotoxicity we investigated whether treatment of K562 cells with anti-IL-6 mAb enhances their sensitivity to anticancer chemotherapeutic agents.

Design and Methods: K562 cells were cultured for different time points (1–4 days) with various concentrations of cisplatin with or without the presence of anti-IL-6 neutralizing antibodies. After the end of the incubation time drug induced cytotoxicity was tested by the MTT colorimetric assay.

Results: After 4 days in culture we observed that K562 cells incubated with 10 ng/ml of cisplatin together with 10 μg/ml of anti-IL-6 mAb exhibited the same level of cytotoxicity as 10 μg/ml of cisplatin alone. Treatment of K562 cells with isotype-matched control antibody had no effect on their sensitivity to CDDP. In addition we observed synergy of the two agents since their combined use in low doses (10–100 fold less than when used alone) achieved similar cytotoxicity. We then examined the sensitivity of a K562/CDDP resistant subline produced in our laboratory, to the synergistic effect of CDDP in combination with anti-IL-6 mAb. The CDDP-resistant K562 subline was rendered sensitive after 3 days of incubation with 10 μg/ml anti-IL-6 mAb and 100 ng/ml of cisplatin.

Conclusions: This study demonstrates that neutralization of protective factors produced by the tumor cells reverses drug resistance of tumor cells. Therefore, the therapeutic use of CDDP in combination with anti-IL-6 mAb might be useful in the treatment of patients with CDDP-resistant cancer cells.

P-1318 *IN VITRO* CYTOTOXICITY OF PURINE ANALOGUES IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

M. Rafel, D. Colomer, F. Bosch, B. Bellosillo, F. Cobo, N. Villamor, E. Montserrat. *Department of Hematology, Hematopathology Unit, Institut D'Investigacions Biomèdiques August Pi Sunyer, Hospital Clinic, Barcelona, Spain*

To analyze the possible interactions between different purine analogues in inducing *in vitro* cytotoxicity in B-chronic lymphocytic leukemia (B-CLL) cells, peripheral blood lymphocytes from 23 B-CLL patients were tested with either deoxycoformycin (DCF), fludarabine (FAMP) or 2-chlorodeoxyadenosine (2-CDA) alone or in combination. Cell viability was analyzed after 48 hours of drug incubation by means of the MTT assay. The drug concentration required to reduced cell viability to 50% (IC_{50}) was 10 μg/ml for DCF, 5 μg/ml for FAMP and 1 μg/ml for 2-CDA. The mean of spontaneous (sv) viability was 77%. The mean viability for the purine analogues was as follows (p values as for its comparison with sv): DCF 71% ($p = 0.3$), FAMP 41% ($p < 0.001$) and 2-CDA 45% ($p < 0.001$). The *in vitro* cytotoxic effect of FAMP or 2-CDA was higher than those observed with the DCF ($p < 0.001$). The cellular mean viability observed with the combination of these drugs (FAMP + DCF, FAMP + 2-CDA and 2-CDA + DCF) was lower than the sv ($p < 0.001$ in all cases). The addition of 2-CDA to FAMP did not increase its cytotoxic effect (FAMP 41%, FAMP + 2 CDA 32%; $p = 0.19$.). *In vitro* cytotoxicity induced by FAMP and 2-CDA alone were significantly higher than DCF. No differences were observed between FAMP and 2-CDA alone ($p = 0.5$). The combination of FAMP + 2-CDA did not increase the cytotoxic effect of these drugs individually evaluated.

Chemotherapy resistance – molecular pharmacology

P-1319 EXPRESSION OF MDR 1 AND BCL-2 GENES IN LEUKEMIAS AND MULTIPLE MYELOMA

M. Cioch[1], J. Kocki[2], A. Dmoszyńska[1], I. Hus[1], M. Wojtaszko[1], M. Koktysz[1]. [1]*Department of Haematology, Department of Medical Genetics, University School of Medicine, Lublin, Poland*

Expression of P-glycoprotein 170, an ATP-dependent drug efflux pump, which reduces the intracellular drug accumulation is suggested as one major mechanism of multidrug resistance (MDR) in hematologic malignancies. Several studies have also demonstrated that resistance to chemotherapy is connected with high expression of bcl-2 gene inhibited apoptosis, which is a common mechanism of death in malignant cells treated with anti-cancer drugs.

In a series of 12 myelomas bone marrow and 10 leukemias blood samples we have studied mdr 1 and bcl-2 genes. Total cellular RNA was extracted by modified guanidinum thiocynate acid-phenol method, cDNA was synthetized and PCR products of genes fragments were amplified with specific bcl-2 and mdr 1 oligonucleotides.

They were 11 cases of multiple myeloma, 1 case of Waldenström's macroglobulinemia, 5 cases of blast crisis of chronic myelogenous leukemia (CML), 4 cases of acute myeloid leukemia (AML: M2 - 1, M4 - 2, M5 - 1) and 1 case of T-cell ALL. We found simultaneous expression of mdr 1 and bcl-2 genes in all cases of multiple myeloma. The situation in leukemias was different. We found high expression of bcl-2 in 9 cases (only not in T-cell ALL), but mdr 1 only in 2 patients (blast crisis of CML). These 2 cases of double bcl-2 and mdr 1 expression was characterized by strong drug resistance. Lack of correlation between expression of bcl-2 and mdr 1 genes may be characteristic for acute proliferation, especially ALL and AML, which generally have high primary sensitivity to chemotherapy. The pleiotropic drug resistance based on expression of both bcl-2 and mdr 1 genes may be implicated in the strong resistance of patients with myeloma chemotherapy.

P-1320 EVALUATION OF THE CHEMORESISTANCE MEDIATED BY THE MDR-1 GENE AND THE PRESENCE OF BCL-2 REARRANGEMENT IN PATIENTS WITH NON HODGKIN LINPHOMA (NHL)

M. Correnti[1], M. Di Stefano[1], R. Somoza[1], M.A. Torres[1], M.E. Cavazza[2], N. Salma[1], A. Neri[3], J.J. Desenne[1], G. Acquatella[1], A. Muller[1]. [1]*Inst. of Oncology and Hematology M.S.A.S.- Universidad Central de Venezuela;* [2]*Biomedicine Institute;* [3]*Policlinico Milano, Venezuela*

The expression of the mdr-1 gene in lymphoproliferative diseases could be an important element for the resistance to the chemotherapeutic agents such as: anthracyclines and alkylating agents. Equally, cells with high levels of bcl-2 protein are relatively resistant to chemotherapy.

Objective: To determine the presence of the Pgp-170 as a product of the mdr-1 gene by inmunocytochemistry and the presence of the t (14:18)-bcl2 in patients with NHL relating their expression to chemotherapy response.

Methods: 21 PB samples from NHL patients of the Oncology and Hematology Institute were analyzed. CHOP/MINE/ESAP regimens were given to all the patients included in the study. The lymphocytes were isolated using density gradient separation with Ficoll-Hypaque and were fixed on slides for their analysis. AVIDINE/BIOTHINE.-PEROXIDASE technique was used with the monoclonal antibody C219 (Oncoscience), for the determination of the mdr-1 phenotype. At the same time, the presence of the bcl-2 rearrangement was determined with the polymerase chain reaction (PCR) technique and subsequent molecular hybriditation of the product.

Results: The expression of the Pgp-170 was found in 52.4% (11/21) of the samples, 47.6% (10/21) were negative. The Pgp-170 expression was more intense in the membrane cell than in the perinuclear and cytoplasmatic area. 33.3% (7/21) of the samples were bcl2+. Among the mdr-1 positive samples, 63.6% (7/11) were bcl-2+, 36.4% (4/11) of these patients were negative for the bcl-2 oncogen. No significant differences were observed in the progression of the disease between the group of patients mdr-1+/bcl-2+ and the group mdr-1−/bcl-2+. Nevertheless, statistically significant difference was found, between the mdr-1 (+) and the mdr-1 (−) phenotype respect to the partial or no response to the treatment comparing the two groups ($p = 0.00387$). It was possible to evaluate the expression of the mdr-1 phenotype before and after the treatment in 9 patients. 5 of 9 samples (55%) showed an increase over 50% of the mdr-1+ cells after the treatment. Of these 5 cases, 3 had a partial response and 2 died as a consecuence of disease progression. These results suggest that the treatment of NHL with chemotherapeutic agents associated to the mdr-1 phenotype can induce an mdr-1 gene product overexpression, demostrated by a partial or no response to the treatment.

Project financiated by CONICIT/CNR PI-122

P-1321 ABSENCE OF PGP EXPRESSION AND HIGH INTRACELLULAR DAUNORUBICIN ACCUMULATION DISTINGUISH ANLL WITH GOOD PROGNOSIS

D. Damiani, M. Michieli, S. Grimaz, A. Ermacora, P. Masolini, M. Baccarani. *Division of Hematology, Department of Morfological and Clinical Research, Udine University Hospita, 33100 Udine, Italy*

Cytoflurimetric detection of the PGP and Intracellular Daunorubicin Accumulation (IDA) were evaluated in 118 consecutive patients with de novo Acute Non Lymphocytic Leukemia (ANLL) admitted for treatment to the Division of Hematology of the Udine University Hospital, between 1991 and 1996.

PGP was detected using the MRK16 antibody and patinets with a MRF-16 Mean Fluorescence Index (MFI) $\geq$6 were considered as PGP overexpressing. Intracellular DNR was assayed after a 2 hour incubation in 1 μg/ml of the drug. The cut off to distinguish an impaired drug accumulation was set at a DNR NMFI < 280. Among the 118 de novo ANLL, 73 patients aged $\leq$55 ys and the 45 > 55. In the first group (age $\leq$ 55) 29/73 (40%) showed and 44/73 (60%) did not show a PGP overexpression. An impaired IDA was seen in 41/73 (56%). Complete remission was obtained in 41/44 (93%) PGP-, and in 13/29 (45%) PGP+ cases ($p < 0.001$). Considering the IDA, on patients aged $\leq$55, CR was achieved by 28/30 (93%) cases showing DNR NMFI $\geq$ 280 and by 24/41 (59%) patients with a DNR NMFI < 280 ($p = 0.0027$). 26/45 (58%) patients aged more than 55 were PGP+, 19/45 (42%) were PGP-. A DNR NMFI < 280 was observed in 31/43 (72%) patients. In this groups of 10/19 PGP- (3%) and 8/26 PGP+ (31%) achieved complete remission ($p = 0.06$). 10/31 (22%) with DNR NMFI < 280 and 9/12 (73%) with DNR NMFI $\geq$ 280 entered complete remission ($p = 0.0028$). In conclusion in patients less than 55 the absence of PGP overexpression and high DNR uptake distinguish a group of ANLL with a good prognosis. In older ones only the impaired DNR uptake was significantly is associated with an higher proportion of complete remission.

P-1322 IMPACT OF CELL CYCLE ANALYSIS AND DRUG RESISTANCE FOR MONITORING AND FOLLOW UP OF HEMATOLOGIC MALIGNANCIES

D.M. Gengenbacher[1], K. Romanakis[3], R. Knipper[3], B. Schmetzer[2], M. Bender[2], A.A. Fauser[1,2]. *Department of Bone Marrow Transplantation and [1]Hematology/Oncology, European Institution for Research and [2]Development of Transplantation Strategies (EUFETS), Idar-Oberstein, Department of Human Biology and [3]Genetics, University of Kaiserslautern, Germany*

Hematologic malignancies are caused by the disruption of cell proliferation resulting in alterations of the cell cycle with increase of aneuploid tumor cells. Either "de novo" or stressed by repeated chemotherapy, these cells aquire resistance to cytostatic agents and subsequently the clinical management becomes difficult. We evaluated 34 patients (pts), i.e. 8 ALL, 18 AML, 5 MDS, 1 MM, 1 NHL and 1 CML in myeloid blast crisis undergoing allogeneic bone marrow transplantation (BMT, 26 pts) or chemotherapy (CT, 8 pts). Cell cycle analysis of bone marrow cells was performed by flow cytometry, and drug resistance (MDR) was analyzed in a 24 hour "in vitro assay", testing the cytostatic agents: Ara-C, Cyclophosphamide, Etoposide, MTX, Idarubicin, Doxorubicin and Mitoxantrone. The number of analysis performed per pts ranged from 2 to 8 at different time points with a follow up of 2 to 11 months. In 13 cases aneuploidy was detected by cell cycle analysis, correlating with the disease activity or the prediction of relapse. Six pts were aneuploid before BMT and diploid post BMT confirming engraftment. Ten pts revealed a high proliferative capacity indicated by increased S/G2 phases, due to prevalent disease or beginning relapse. Six of 15 pts who demonstrated positive MDR prior to BMT developed sensitivity to the cytostatic agents tested at day +30 post BMT. These pts remained in CR with a follow up time ranging from 4 to 9 months. In 5 cases we measured positive MDR at different time points during CT and all these pts failed to achieve a remission. Our test system including cell cycle analysis and "in vitro" drug resistance provides additional information with regard to the biology of the malignancy and supports the understanding to tumor related therapy.

P-1323 QUANTITATIVE DETERMINATION OF MULTIDRUG RESISTANCE EXPRESSION AND FUNCTION IN HAEMATOLOGICAL MALIGNANCIES

K. Jakab, G. Szakács, S. Nahajevszky, Zs. Holló, B. Sarkadi. *National Inst. of Haematology and Immunology, Budapest, Hungary*

Quantitative determination of the expression of multidrug resistance proteins might help the physicians to modify the standard chemotherapy protocols to achieve better clinical response. Here we describe the application of combined functional and immunological flow cytometry based approach for the detection of P-glycoprotein (MDR1) and the multidrug resistance-associated protein (MRP) in leukemic cells from haematological patients. By using the calcein accumulation assay recently we have demonstrated that the transport activity of MDR1 and MRP can be quantitatively characterized in a series of human cell lines. The measure of transport activity, the MDR activity factor MAF is calculated from the rate of calcein accumulation in the presence and absence of an inhibitor (e.g. verapamil), respectively, and is given as a dimensionless value. This quantitative functional assay has been combined with flow cytometry immunophenotyping, or Western blotting by using anti-MDR1 antibodies.

We have found close correlation between MAF values and MDR1 or MRP expression in various tumour cells. Preliminary data suggest that in some cases of the refractory AML higher MAF values correlate with other established poor prognostic factors (age, karyotype, CD34 count). We have carried out multivariance analysis of the data obtained in untreated, refractory or relapsed AML and ALL patients.

P-1324 ASSOCIATION BETWEEN REPRODUCIBLE FLOW CYTOMETRIC METHODOLOGY FOR MEASURING MULTIDRUG RESISTANCE AND REMISSION RATES IN AML

M. Pallis, J. Turzanski, G. Harrison[1], K. Wheatley[1], S. Langabeer[2], A.K. Burnett[3], N.H. Russell. *Nottingham City Hospital and University of Nottingham, Nottingham; [1]CTSU, Oxford; [2]UCL hospitals, London; [3]University of Wales College of Medicine, Cardiff, UK*

We have used a combination of flow cytometric assays to define MDR positive and negative blasts in cryopreserved samples from 47 adult AML patients entered into MRC trials. Our primary test is a recently reported standardised and reproducible assay for daunorubicin (dnr) accumulation. Confirmatory assays for MDR comprise a cyclosporin modulation assay for functional p-glycoprotein and also measurement of lung resistance protein and multidrug resistance associated protein (with LRP-56 and MRPr1 respectively). 27/47 (57%) samples had both low dnr accumulation and at least one positive confirmatory test (a modulated functional assay and/or protein overexpression) and were categorised as "confirmed MDR". 15/47 patients (32%) were MDR negative in all 5 assays. 5/47 (11%) patients had unconfirmed low dnr accumulation. None of the patients in this cohort had high dnr accumulation alongside overexpressed lrp or mrp or functional p-glycoprotein. The Mantel Haenszel test for trend was used to compare our findings with patient response to induction chemotherapy. Of 35/47 patients (74%) who achieved CR, 17 had confirmed MDR, 4 had unconfirmed MDR and 14 had high daunorubicin accumulation ($p = 0.03$). Of the 7 patients with resistant disease 6 had confirmed MDR, 1 had unconfirmed MDR and none had high daunorubicin accumulation ($p = 0.04$). The use of reproducible assays with clear cut off points to define MDR positivity and the increase in sensitivity brought about by combining the results of more than one assay will help to bring MDR analysis into a clinical setting.

P-1325 FUNCTIONAL ASSAY OF MULTIDRUG RESISTANCE IN ACUTE IN MYELOID LEUKEMIA USING JC-1, A CARBOCYANINE FLUORESCENT PROBE

V. Ugo, J.Y. Perrot, O. Legrand, A.M. Faussat-Gonzales, R. Zittoun, J.-P. Marie. *Service d'Hématologie, Hôpital Hôtel Dieu, Paris, France*

JC-1 is a fluorescent lipophilic carbocyanine dye forming aggregates above a critical concentration. On excitation at 490 nm, aggregates display a specific red emission band centered at 597 nm, whereas the JC-1 monomers display a green emission band centered at 540 nm. In AML cell lines, JC-1 accumulation is correlated with the level of P-gp expression. JC-1 is avidly accumulated in sensitive cells where it displays both a green cytoplasmic and a red mitochondrial fluorescence, and in contrast JC-1 is poorly accumulated in resistant cells, which show only a slight green fluorescence. The specific fluorescence properties of JC-1 allows accurate discrimination between low-level resistant cells and sensitive cells.

We measured JC-1 fluorescence by flow cytometry in 60 samples of blood or bone marrow from AML patients. Incorporation of JC-1 was measured with and without adding ciclosporin A (CsA), a P-gp modulator. Both red and green fluorescence emissions were analyzed, and the results were expressed as a mean ratio of fluorescence calculated as follows: mean fluorescence with CsA/ mean fluorescence without CsA. P-gp expression was determined using the UIC2 monoclonal antibody.

Incorporation of JC-1 was correlated with P-gp expression in CD34 positive blasts, but not in CD34 negative cases. Correlation with the status of the disease (de novo versus relapse or secondary AML) was also significant.

	green fluorescence		red fluorescence	
P-gp+/CD34+	6.18	p = 0.009	18.11	p = 0.057
others (P-gp- and/or CD34-)	1.94		5.28	
relapse or secondary AML	4.61	p = 0.004	14.16	p = 0.01
de novo AML	2.04		3.81	

Our results show that JC-1 is a sensitive probe for assessing P-gp function. The use of a P-gp modulator is mandatory for the interpretation of JC-1 assay. Discrepancies between P-gp expression and functional assays for P-gp have been already described, and we confirm that correlation with CD34 expression is needed for the evaluation of a functional assay. Further investigations are warranted for comparing the relative informations from JC-1 and rhodamine 123 assays.

P-1326 MEASUREMENT OF mRNA AND PROTEIN LEVELS OF MRP1 AND LRP IN ELDERLY *de novo* ACUTE MYELOID LEUKEMIA

P.J.M. Vossebeld, E.A.C. Wiemer, P. Sonneveld. *University Hospital Rotterdam, The Netherlands*

Multidrug resistance to chemotherapy is one of the major problems in the treatment of acute myeloid leukemia in elderly patients. Classical multidrug resistance is caused by the expression of P-glycoprotein (P-gp). However additional mechanisms of multidrug resistance have been reported to exist. Expression of the multidrug resistance-related protein (MRP1), which is analogous to P-gp, an ABC-transporter, was shown to be associated with multidrug resistance. Furthermore the overexpression of lung resistance-related protein (LRP) in many multidrug resistant cell lines, and a variety of tumors of different histogenetic origin, strongly suggests a role in multidrug resistance for the vault complex; the subcellular organelle of which LRP is the main structural component.

We developed a reverse transcriptase-polymerase chain reaction (RT-PCR) for the quantitation of the mRNA levels for MRP1 and LRP. As an internal standard a synthetic RNA was generated in which the same primer sequences are present, but which gives rise to a PCR-product with a different size. Quantitation was based on the competition between cellular mRNA and the internal standard RNA. We also generated a specific polyclonal antiserum against LRP that could be used in immunoblotting.

We measured the mRNA and protein levels for both LRP and MRP1 in different multidrug resistant cell lines and their corresponding drug-sensitive parent cell lines. The mRNA and protein levels of MRP1 and LRP were determined next to the P-gp expression in bone marrow samples of *de novo* acute myeloid leukemia from elderly patients. A series of 60 uniformly treated patients was investigated who received standard Daunorubicin/Cytarabine induction treatment to which PSC833 was added. Our goal was to determine if the adverse prognostic role of P-gp on response was bypassed by *in vivo* treatment with PSC833. Secondly, the LRP/MRP1 clinical significance could be determined in the same cohort of patients. The results so far demonstrate that PSC833/Daunorubicin/Cytarabine can establish complete remissions in P-gp-positive leukemia. From patients, that were strongly positive for P-gp, 9 from 13 attained a complete remission, while from patients, that were negative for P-gp, 8 from 11 attained a complete remission. Significant expression of LRP and MRP was observed in a subgroup of patients, and seems to be of limited prognostic value.

This study was supported by Novartis Pharma AG, Switzerland.

P-1327 LUNG RESISTANCE PROTEIN (LRP) GENE EXPRESSION IN ACUTE MYELOID LEUKEMIA (AML) SAMPLES: A CRITICAL EVALUATION BY THREE TECHNIQUES

O. Legrand, G. Simonin, R. Zittoun, J.-P. Marie. *Hematology Department, Hôpital Hôtel-Dieu, Paris, France*

The role of LRP in clinical MDR in AML is controversial: 2 studies using immunocytochemistry (ICC) showed correlation between LRP + AML and treatment failure, but 2 other studies using flow cytometry (FC) did not. The use of different techniques could explain this discrepancy. We therefore compared multiple assays (including RT/PCR, ICC and FC) in 47 fresh and thawed adult AML in order to validate and quantitate measures for the LRP phenotype detection. The reproductibility of LRP expression by RT/PCR was good ($r = 0.94$, $p < 0.0001$). When ICC was used, we have collected two types of data: first, the intensity of antibody (LRP56) staining and secondly, the percentage of LRP positive cells. The correlation between both types of data was good ($r = 0.87$, $p < 0.0001$). The reproductibility of LRP staining measured by ICC was also good ($r = 0.88$, $p = 0.003$, when measured by the intensity of antibody staining and $r = 0.88$, $p = 0.003$, when measured by the percentage of positive cells). When FC was used, protein values were expressed by two methods: first as the ratio of arithmetic mean fluorescence LRP56/IgG2b control; secondly, LRP protein staining was compared with the one of a control cells by the Kolmorogov-Smirnov test. These two methods were correlated ($r = 0.83$, $p < 0.0001$). When LRP staining was measured by FC, there was also a good correlation between fresh and thawed AML samples ($r = 0.83$, $p = 0.002$). Between the three methods used, there was only a weak correlation between FC and ICC ($r = 0.37$, $p = 0.04$), no correlation between RT/PCR and ICC ($r = 0.22$, $p = 0.15$) and no correlation between RT/PCR and FC ($r = 0.30$, $p = 0.08$). LRP gene expression, measured by FC, was a prognostic factor for achievement of complete remission ($p = 0.03$), but not when measured by RT/PCR or ICC. LRP gene expression, measured by RT/PCR, ICC or FC, was not a prognostic factor for DFS and overall survival durations. In conclusion, the weak correlation between FC and ICC may explain the discrepancies observed between several studies. Therefore, as for MDR1/Pgp, at least two different techniques should be used for validation, preferably single cell detection methods. The absence of correlation between RT/PCR and methods for LRP protein detection may be explained by a post transcriptional regulation, given that major vault protein are multi-units proteins structure. Therefore, FC and ICC may be prefered to RT/PCR for LRP detection. In addition, the reproductibility of these two methods (ICC and FC) for LRP detection is good. In our study, LRP is not a prognostic factor for DFS and overall survival durations.

P-1328 INTRACELLULAR LOCALIZATION OF THE VAULT COMPLEX IN NON-P-GP MULTIDRUG RESISTANT CELL LINES

E.A.C. Wiemer, P.J.M. Vossebeld, P. Sonneveld. *University Hospital Rotterdam, The Netherlands*

To date two proteins have been identified that are able to confer multidrug resistance when they are expressed in drug-sensitive cells. Both P-glycoprotein and the multidrug resistance-associated protein (MRP1) function as efflux pumps in the plasma membrane reducing the intracellular drug accumulation. Additional mechanisms seem to exist decreasing the intracellular drug concentrations by sequestering drugs into (exocytotic) vesicles. To investigate whether the cytoplasmic vault complex functions in this kind of detoxification processes we examined the intracellular localization of the vault complex in parental and drug-resistant cell lines.

We characterised the cancer cell lines GLC4 (small cell lung cancer) and SW1573 (non-small cell lung cancer) and their drug resistant derivatives GLC4/ADR and SW1573/2R120 for the expression of P-gp, MRP1 and the

major vault protein (MVP/LRP) in immunoblotting experiments, RT-PCR and by FACS analysis. P-gp expression could not be detected, however MRP1 was clearly induced in the drug-resistant cell lines as was MVP. In the parental cells no MRP1 was detected but considerable amounts of MVP were present. The intracellular localization of the major vault protein - and as a consequence vaults - and MRP1 was examined by immunofluorescence using specific monoclonal and polyclonal antibodies. MRP1 was found to be abundantly present in the plasma membrane in GLC4-ADR cells. In contrast MRP1 was found to be intracellular in SW1573/2R120. Vaults were dispersed throughout the cytoplasm in parent cell lines. In the drug resistant cell lines the MVP staining pattern was heterogeneous; most cells showing an intense, but diffuse, staining of the cytoplasm but some cells contained additional large, brightly fluorescent, vesicular structures in their periphery. A fusion protein consisting of the green fluorescent protein (GFP) linked to the C-terminal end of MVP was transiently expressed in SW1573/2R120 cells. Again the fluorescent pattern was heterogeneous with cells expressing low levels of the fusion protein displaying a diffuse cytoplasmic staining whereas strongly expressing cells contained - in addition to the diffuse cytoplasmic staining - fluorescent vesicular structures similar to the pattern visible in IF experiments. The nature of these structures is not yet known, most likely they represent vault clusters or vaults associated with other subcellular structures. It was also investigated if the GFP-labelled vaults permanently or temporarily colocalize with the fluorescent (exocytotic) vesicles that originate when SW1573/2R120 cells are treated with daunorubucin. Initial experiments indicated that the daunorubucin containing vesicles do not colocalize with vault i.e with the vault clusters in the cell's periphery.

P-1329 IN-VITRO DEFINITION OF CRITICAL DRUG EXPOSURE LEVELS IN INDUCTION OF DNA EXCISION REPAIR: APOPTOTIC THRESHOLD CONCENTRATION (ATC)

C.L. Lannon, L.M. Ball, G.R. Langley, L. Leahey, D. Van Velzen. *Dalhousie University, Halifax, Nova Scotia, Canada*

Objective: To establish the feasibility of determining a maximal in-vitro drug exposure level which is still associated with effective DNA excision repair in assessing drug sensitivity in human leukaemic cells.

Designs and Methods: Mononuclear cells from bone marrow from normal healthy donors (obtained at orthopaedic surgery procedure) were subject to 1 hour of in-vitro exposure to stepwise increased levels of DaunoXome®, followed by a 2 hour culture period to allow for induction of DNA excision repair. After Single cell Gel Electrophoresis, 30 μm frozen sections of SCGE gels were analysed by high resolution Confocal laser Scanning Microscopy (Zeiss Axiomat LSM 410, 40x oil objective, N.A.1.3) with quantitation of results by computer assisted image analysis (Quantimet 570 C). Cytospin preparations of suspensions of selected cell cultures were analysed using Frag-EL (CalBiochem, USA) in-situ labelling specific for DNA fragments released by apoptosis specific endonuclease.

Results: Size distribution of DNA excision repair derived fragments remains stable and is dose independent. Fragment quantity increases with dose level. In all cases high dose levels inhibit DNA excision repair evidenced by a complete absence of any comet tails. Analysis of cell preparations after 2 hour culture at these levels confirmed high levels of commitment to apoptotic cell involution.

Conclusions: In addition to the ability to establish dose response relationship for DNA directed chemotherapeutic agents, short term incubation followed by SCGE/high resolution Comet tail analysis may be used to establish the critical exposure level for induction of apoptosis rather than DNA excision repair. Studies of the relationship of the Apoptotic Threshold Concentration (ATC) to treatment outcome and clinical drug sensitivity would be of interest.

P-1330 INCREASED IN VITRO INDUCTION OF DNA EXCISION REPAIR BY 10-FOLD REDUCED LIPOSOMAL DAUNORUBICIN (DAUNOXOME) AS COMPARED TO CONVENTIONAL DAUNORUBICIN

C.L. Lannon, L.M. Ball, G.R. Langley, L. Leahey, D. Van Velzen. *Dalhousie University, Halifax, Nova Scotia, Canada*

Objective: To compare dose-response relationships between conventional and liposomal daunorubicin with respect to induction of DNA excision repair activity.

Methods, Design: Mononuclear cells were isolated from samples of normal human bone marrow cells obtained from healthy donors at orthopaedic surgery. These were exposured for 1 hour to conventional daunorubicin (at levels 0.5, 1.0, 1.5 and 2.0 μg/ml) and a liposomal formulation (DaunoXome®, Nexstar, USA, at levels of 0.05, 0.1, 0.15, and 0.2 μg/ml). After 2 hours post exposure culture, to allow for development of maximal nuclear content of DNA excision repair derived DNA fragments, cells were embedded in agar for Single Cell Gel Electrophoresis (SCGE). 30 μm frozen sections of SCGE gels were analysed by high resolution Confocal laser Scanning Microscopy (Zeiss Axiomat LSM 410, 40 x oil objective, N.A. 1.3) with quantitation of results by computer assisted image analysis (Quantimet 570 C). All exposures and analyses were carried out in duplicate.

Results: DNA fragment size distribution and maximal particle size (tail length/moment) did not differ between the two formulations indicating identical patterns of DNA damage. For both agents a clearly defined dose response curve was obtained with inhibition of DNA excision repair at highest dose levels. Maximal, comparable tail moments were acheived at a concentration of DaunoXome® of 0.1 μg/ml and conventional daunorubicin of 1.0μg/ml.

Conclusions: DaunoXome® induces comparable in vitro DNA damage at one tenth the dose of conventional daunorubicin. These findings indicate the usefulness of combined SCGE/high resolution CLSM in the in-vitro comparative study of chemotherapeutic agent efficacy and reflects the clinical experience of dosing schedules with DaunoXome®.

P-1331 EFFICACY OF ACETYLDINALINE IN RESISTANT BNML SUBLINES TO DAUNOMYCIN, ARA-C OR CYCLOPHOSPHAMIDE. PRECLINICAL STUDIES IN A RELEVANT RAT MODEL

H.M. El-Beltagi[1], A.C.M. Martens[2], A. Hagenbeek[2]. [1]*Clinical Pharmacology Dept. Mansoura Faculty of Medicine, Mansoura, Egypt;* [2]*Hematology Dept., University Hospital, Utrecht, The Netherlands*

One of the major drawbacks in successful leukemia treatment is the presence of subclones of leukemic cells that are resistant to the cytostatic agents employed to eradicate the disease.

The cytostatic drug acetyldinaline [ACD, CI-994, 4-acetylamine-N-(2-aminophenyl) benzamide] shows an extreme antileukemic effect in the Brown Norway rat model for acute myelocytic leukemia (BNML) with only minor toxicity for normal pluripotent hemopoietic stem cells.

The efficacy of acetyldinaline aganist resistant leukemia cells for currently used antileukemic drugs; Daunomycin, Ara-C or Cyclophosphamide; was evaluated and compared with those observed in the sensitive parent BNML cells.

Acetyldinaline treatment in Daunomycin resistant subline with 11.85 mg/Kg/day (low dose) for 5 days resulting in increase in median survival time compared with control group with 14 days which is expressed as 227%. With repeated daily administration of Acetyldinaline in a dose of 11.85 mg/Kg/day in Ara-C or Cyclophosphamide resistant sublines for 5 consecutive days resulting in an increase in life span (ILS) corresponding with a 5 log cell kill (LCK) or 4 LCK, respectively. In conclusion, this striking antileukemic effect of acetyldinaline against resistant BNML sublines denotes that there is no cross resistance between acetyldinaline and Daunomycin, Ara-C or Cyclophosphamide. This impressive antileukemic effect of Acetyldinaline warrants the introduction of this compound in clinical evaluation especially in the patient suffering from refractory acute myleocytic leukemia (AML) to these currently used antileukemic drugs.

P-1332 CLINICAL TREATMENT WITH CGP 41251 INCREASES SENSITIVITY OF CLL CELLS TO CONVENTIONAL THERAPY

K. Ganeshaguru, S.M. Hart, D.T. Jones, R.G. Wickremasinghe, A.E. Virchis, H.G. Prentice, A. Man[1], K. Csermek[1], A.B. Mehta. *Department of Haematology, Royal Free Hospital & School of Medicine, London, UK; [1]Novartis Pharma AG, Basel, Switzerland*

Protein Kinase C (PKC) is currently a potential target for drug development to achieve specific cytotoxic effects in tumour cells. CGP 41251, an analogue of the natural product staurosporine, is a selective inhibitor of PKC isoforms α, β, γ and η. In vitro and in vivo studies in animals have shown that CGP 41251 can reverse P-glycoprotein (P-gp) mediated resistance. CGP 41251 is currently undergoing clinical trial in chronic lymphocytic leukaemia (CLL) where a transient response is observed during treatment. Cells from patients with CLL before and at the end of 14d treatment with CGP 41251 were investigated in vitro for effect on the IC50 of drugs conventionally used for the treatment of CLL, ie., 2′-Cholodeoxyadenosine (CdA), Chlorambucil (Chl) and Fludarabine (Flu) and Daunorubicin (DNR). IC50 was studied using a 72 h MTT assay. The IC50 for CdA in 5/9 patients was reduced by a mean 66% to 870 ng/ml (3.0 uM), Chl in 9/10 by 54% to 16,731 ng/ml (55 uM), Flu in 8/11 by 93% to 2577 ng/ml (9 uM) and DNR in 7/10 by 80% to 318 ng/ml (0.56 uM). In cells from 4/11 patients, there was a reduction in IC50 in all four drugs investigated. In vitro treatment of CLL cells with a combination of either of the conventional drug and CGP 41251 could predict increased sensitivity of the cells after clinical treatment in 4 of the 5 that showed reduction in IC50 for CdA, 7/9 for Chl, 6/8 for Flu and 5/7 for DNR. Decrease in IC50 for all four drugs does not appear to be related to expression and function of P-gp. Clinical studies of combination chemotherapy incorporating CGP 41251 is now indicated.

P-1333 MULTISPECTRAL NATURAL FLUORESCENCE IMAGING TO STUDY LEUKEMIC CELL - DRUG INTERACTION

M. Monici[1], P. Mazzinghi[1], G. Agati[1], F. Fusi[2], A. Degl'Innocenti o Nocentini[3], B. Bartolozzi[3], P.A. Bernabei[3]. *[1]Istituto di Elettronica Quantistica - CNR, Florence; [2]Dip. di Fisiopatologia Clinica, University of Florence; [3]Div. di Ematologia, Policlinico di Careggi, University of Florence, Italy*

In this paper, the application of a new multispectral natural fluorescence imaging technique to the study of leukemic cell-drug interaction is described.

Cells, excited with light of suitable wavelenghts, spontaneously emit UV-visible fluorescence. This natural fluorescence (NF), in the visible, is produced by nicotinic coenzymes and flavins, molecules engaged in cell metabolism. Our previous results demonstrate that NF pattern allow for cell identification and provide information on their functional state. Moreover, since the most utilized differentiating and antiblastic agents exhibit fluorescence properties, with the multispectral NF imaging technique it is also possible to study the intracellular localization of drugs.

Leukemic cells from stabilized human leukemic lines HL60, U937 and FLG29.1 were treated with differentiating and antiblastic agents and analyzed by microspectrofluorometry and fluorescence imaging. The equipment consists of an epifluorescence microscope with oil-immersion CF-UV fluor objective 100× (N.A. 1.30); a high pressure mercury lamp with interference filters or an Argon laser, used as excitation sources; a dichroic mirror and a multispectral digital CCD camera (768 × 512) or, alternatively, a multichannel spectral analyzer to collect and analyze fluorescence emission. The experiments are carried out on single living cell in physiological conditions, the interaction with drug being the unique perturbation: the analytical technique does not require any manipulation or treatment of the samples and does not produce cell damage.

The results show that cell–drug interaction produces deep changes in cell NF pattern in all the populations examined. The contribute of the different fluorophores (cellular compounds and drug) to the emission and their localization can be revealed. Information about the functional state of cells can be deduced. In conclusion the multispectral natural fluorescence imaging technique let us able to study cell-drug interaction in order to evaluate uptake, retention, localization of the drug and its effect on cell metabolism.

Supportive care and quality of life

P-1334 A NEW METHOD IN DETECTION DOXORUBICIN INDUCED CARDIOTOXICITY: FLOW-DEPENDENT DILATATION MEASUREMENT

F. Szabó[1], L. Nagy[1], J. Palatka[1], G.L. Kovács[1], E. Röth[2], L. Németh[1], J.L. Iványi[1], J. Tarján[1]. *[1]Univ. Teach. Hosp., Szombathely; [2]Med. Univ. Pécs, Hungary*

Since 1995 we introduced a new method to detect early signs of doxorubicin (DOX) induced cardiotoxicity: flow-mediated dilatation (FMD) measurement with ultrasound. Free-radicals, secondary to DOX administration, are rapidly neutralised in vessels wall by nitric oxide. Endothelial dysfunction caused by overproduction of free-radicals is well detected by FMD measurement. 22 patients were entered is this study. 16 males and 6 females with an average age of 40 years (range 17–65). Diagnosis consisted of non-Hodgkin's lymphoma (18 pts) and Hodgkin's disease (4 pts). Standard measurements before DOX were: physical examination, electrocardiography, blood pressure, radionuclid ventriculography, echocardiography and FMD. The mean actual dose of DOX was 70 mg (40–100 mg). We detected the level of creatin kinase in all pts. and of troponin-T in 15 pts. at 0, 6, 12, 24 and 48 hours after DOX administration. FMD was measured at 0, 6, 12, 24 and 48 hours with simultaneously detection, in 9 patients, of levels of reduced glutation (GSH), oxidate glutation (GSSG), malondialdehide (MDA) and myeloperoxidase (MPO) from blood samples. The longest follow-up was 29 months, the cumulative dose of DOX was in average 230 mg/m^2. None of patients presented significant changing in ECG, blood pressure, CK and troponin-T level after DOX administration. By contrast, FMD decreased significantly from 9.4±3.4% to 5.8 ± 4% ($p < 0.02$). *Nitrate-mediated dilatation wasn't significant* (20.4±6.1% *vs* 18.8±5%). The lowest value of FMD was detected at 6 hours after administration of DOX. We could not detect any significant changing in the level of GSH, GSSG, MDA and MPO before and after administration of DOX, but DOX induced decreasing of FMD correlated well with decreasing in GSH/GSSG ratio. When patients who presented >5% decrease in FMD after DOX were pretreated with 1000 mg vitamin C, decreasing of FMD and GSH/GSSG could be prevented. Until now only two patients developed symptoms of cardiac failure *with significant decreasing in ejection fraction. Both presented previously a* significant decreasing of FMD after DOX. In conclusion, our method is easy to use and able to detect those patients who's antioxidant capacity is lower and are of risk to develop cardiac failure induced by doxorubicin administration.

P-1335 CYTOSINE ARABINOSIDE (ARA-C) SYNDROME AND ARA-C INDUCED PERICARDITIS

T. Yamada, H. Tsurumi, M. Sawada, T. Hara, H. Moriwaki. *First Department of Internal Medicine, Gifu University School of Medicine, Gifu, Japan*

Ara-C syndrome characterized by fever, myalgia, bone pain, and occasionally by chest pain, maculopapular rash, and conjunctivitis is one of special toxicities by Ara-C. Ara-C induced pericarditis is one of Ara-C syndrome. 8 patients (pts) who developed Ara-C syndrome including 2 pts with Ara-C induced pericarditis from May 1995 to March 1997 were analyzed. The underlying disorders were 4 acute myelogenous leukemia (AML) and 4 acute lymphoblastic leukemia (ALL). Incidence of Ara-C syndrome was 5.0% in AML and 14.8% in ALL in our hospital. The age ranged from 33 to 66 with a median age of 56. Among 8 pts, fever occured in 7; maculopapular rash in 7; conjunctivitis in 2; liver dysfunction in 2; and chest pain in 2. 2 pts with chest pain developed pericarditis and pleuritis. Pericardiocentesis in 2 pts with pericarditis showed exudate effusion with many neutrophils without bacterial decontamination. 3 pts were treated with low dose Ara-C including oral cytarabine ocphosfate and 5 pts with intermediate or high dose Ara-C. The Ara-C syndrome developed when they were exposed to 280–10800 mg of Ara-C. 6 pts recovered by discontinuance of Ara-C and/or administration of prednisolone. In 2 pts with pericarditis, pericardiac drainage as well as steroid pulse therapy were very effective. Prednisolone successfully prevented recurrence of Ara-C syndrome when Ara-C was again used for 3 pts with this syndrome. Ara-C syndrome should be considered when symptoms as above appear under administration of Ara-C.

P-1336 THE EFFECTS OF APPLYING WAIT-AND-SEE POLICY ON THE WELL-BEING OF PATIENTS WITH LOW-GRADE

NON-HODGKIN'S LYMPHOMA OR CHRONIC LYMPHOCYTIC LEUKEMIA: A PILOT-STUDY

E. Kruijssen, A. Vingerhoets, J.J. Keuning, G. Vreugdenhil. *Department of Psychology, Tilburg University and Department of Internal Medicine, St. Joseph Hospital Veldhoven, The Netherlands*

Patients with Low-grade non-Hodgkin's Lymphoma or Chronic Lymphocytic Leukemia often hardly experience symptoms in case of limited stage of these disorders. Only in exceptional cases will treatment lead to a complete remission. In case of limited disease, early treatment does not prolong overall survival as compared to applying the so-called Wait-and-See (WaS) policy, provided regular medical check-ups are carried out. Treatment is withheld until progression is observed, or symptoms occur. However, until now, the possible psychological effects of this strategy have not been investigated. The aim of this pilot-study is to gain insight into the consequences of the WaS policy on the quality of life (QoL) of these patients. Information was collected through semi-structured interviews and the QLQ-C30 quality of life questionnaire in 21 patients. The investigated patients consisted of two groups: those who were never treated (N = 12), while the other group (N = 9) had gone through one or more cycles of treatment after a WaS period of over one year.

The results indicate that, if the patients fully understand why the WaS is applied, have confidence in the physician's expertise, and experience no physical symptoms, WaS generally does not cause distress. If these criteria are met, most patients (57%) mention positive effects or cannot imagine any negative effects (71%) of such an approach. Seventeen patients (81%) judge their QoL as positive during WaS. The main reasons for concerns are the repeated confrontation with their disease and the awareness that progression may once occur. However, the decision of the doctor to continue WaS, while the patient experiences bodily changes may cause considerable tension. Careful communication with the patient is then essential to prevent severe distress in the patients. In certain cases the distress may be so severe that treatment for a limited period may be considered after weighing the benefits and disadvantages in order to reduce the psychological burden.

P-1337 QUALITY OF LIFE ASSESSMENT IN MULTIPLE MYELOMA

S.E. Crofts, A.G. Smith. *Haematology Department, Royal South Hants Hospital, Southampton, Hants, Great Britain*

Myeloma is still largely incurable despite the advent of intensive treatments and transplants. Despite this lack of cure vital assessments of Quality of life (QOL) have been largely ignored in studies of multiple myeloma (MM). This study aimed to directly assess QOL in an unselected group of consecutive MM patients. A total of 41 patients, 20 male, 21 female aged 45–84 (mean = 63.6) were studied. The EORTC QLQ-C30 questionnaire was used to assess QOL pre-treatment, 3 monthly for a year and then 6 monthly until death. The EORTC QLQ C-30 has 5 function scales, 3 symptom scales, and 7 single item scales to assess specifically health related QOL in cancer patients. This sequential study is ongoing, so to maximise analysis pre treatment and 9 months will be compared. Of the 41 patients at baseline 29 went on to complete questionnaires at 9 months, (3 died, 1 non responder, 8 too early to assess). The 3 deceased and the non responder were all male, with ages not dissimilar to the group mean.

For the function scales and QOL the higher the scores the better the performance. Symptom and single items are reversed, with the higher the score the more symptoms and problems they have.

Data on 41 baseline patients show:-

Physical	mean = 50.73 SD = 27.3	QOL mean = 53.32 SD = 24.7	
Fatigue	mean = 40.4 SD = 22.9	Pain mean = 42.9 SD = 31.5	

Data on 29 patients at 9 months show:-

Physical	mean = 71.31 SD = 22.3	QOL mean = 67.83 SD = 20.7	
Fatigue	mean = 33.04 SD = 20.5	Pain mean = 16.35 SD = 15.1	

Changes over time show:-

Physical	+20.58	QOL	+14.51
Fatigue	-7.36	Pain	-26.55

Differences >10 are regarded as clinically significant. Treatment improves physical functioning, reduces pain and fatigue and consequently improves overall QOL. Studies need to compare different treatment regimes using QOL as an endpoint.

P-1338 RANDOMISED PLACEBO-CONTROLLED TRIAL OF TOPICAL ORAL G-CSF (FILGRASTIM) IN HIGH-GRADE LYMPHOMA PATIENTS WITH SEVERE ORAL MUCOSITIS

M. Karthaus, C. Rosenthal, G. Huebner, H. Paul, C. Elser, T. Scharmann, B. Hertenstein, J. Krauter, R.G. Geissler, J. Novotny, G. Heil, A. Ganser. *Dept. Haematology and Oncology, Hannover Medical School, Hannover, Germany*

Oral mucositis is one of the dose-limiting side effects of intensive chemotherapy. It is caused directly by a cytotoxic effect of chemotherapeutic agents and indirectly by sustained neutropenia. Severe oral mucositis is an important predisposing factor for life-threatening septic complications during aplasia. Besides, it reduces quality of life. At present, no effective causal prophylaxis or treatment against oral mucositis is established. We performed a prospective randomised placebo-controlled trial using topical oral r-metHuG-CSF (filgrastim) in high-grade lymphoma patients treated according to the B-NHL protocol, which contains high-dose methotrexate and causes severe oral mucositis (WHO Grade I–IV) in >50% of patients. Between August 1996 and July 1997, a total of 32 chemotherapy cycles were documented in 8 patients (4 male, 4 female). Mucosal erythema and ulceration were recorded. All patients assessed their oral pain, and impact on swallowing daily using a subjective scale from no to maximal discomfort (1 to 10). In addition, oral mucositis was assessed according to the WHO-score. Filgrastim was administered in 16 cycles as a viscous mouthrinse (carboxymethylcellulose 2%, oleum citrii) 4 × 120 µg/d from day 10 to 16. Sixteen cycles were given in the control patients, of these 14 with placebo, and another two cycles with no treatment. Severe mucositis (WHO grade III/IV) was documented in 21out of 32 cycles (65.5%). A difference of borderline significance was observed for the maximum severity of oral mucositis between G-CSF vs. placebo ($p = 0.058$), with a 50% reduction of oral mucositis WHO grade IV in the G-CSF group (4 vs. 8 courses). The number of days in hospital were reduced significantly in the G-CSF group ($p = 0.02$). In conclusion, topical oral G-CSF mouthrinses may be beneficial to reduce oral mucositis.

P-1339 HAEMATOLOGICAL AND QUALITY OF LIFE (QOL) OUTCOMES IN ANAEMIC CANCER PATIENTS TREATED, WITH EPOETIN ALFA

J. Glaspy[1], G.D. Demetri[2]. [1]*University of California, Los Angeles;* [2]*Dana-Farber Cancer Inst., Boston, USA*

To further validate the clinical and QOL outcomes of epoetin alfa in anaemic patients (pts) with haematological malignancies undergoing chemotherapy (CT) in community-based oncology practice, two phase IV, open-label, 4-month, non-randomised, multicentre studies were conducted. Studies 1 and 2 included 466 and 515 pts respectively with haematological malignancies. Epoetin alfa dosage was initiated at 10,000 IU (SC) tiw and increased to 20,000 IU tiw if response at week 8 was inadequate (study 1) or Hb rise was <1 g/dL at week 4 (study 2). The study populations had the following mean baseline characteristics: age, 63.9 (study 1) and 65.5 (study 2) years; Hb, 9.0 and 9.2 g/dL; endogeneous serum erythropoietin level, 191 and 132 mU/mL; overall QOL as determined on a 100 mm linear analogue scale, 45.1 and 45.7 mm. The most common malignancies were Non-Hodgkin's lymphoma (46%, study 1; 45%, study 2) and multiple myeloma (26% and 31%). More than 90% of pts in each study received non-platinum-containing CT. Statistically significant ($p < 0.001$) mean changes in Hb from baseline to study end were demonstrated (1.8 g/dL, study 1; 2.0 g/dL, study 2). From one month pre-study to month 4, the percentage of pts transfused decreased from 26% to 13% in study 1 and from 39% to 8% in study 2; mean units transfused per pt decreased from 0.73 to 0.44 in study 1 and from 1.51 to 0.22 in study 2 (changes in both transfusion parameters were significant after month 2 ($p < 0.05$) in study 1; after month 1 ($p \leq 0.001$) in study 2). In both studies, mean scores improved for energy (by 41% and 34%, respectively), activity (39% and 33%) and overall QOL (28% and 25%). QOL positively correlated with increases in Hb, independently of associations between QOL and tumour responses to CT. QOL was not improved in pts who experienced a decrease in Hb level. The most common adverse events across both studies were disease progression (11.5%), pyrexia (10.7%) and fever (10.5%). In conclusion, these studies confirm that, in pts with haematological malignancies undergoing myelotoxic CT, epoetin alfa is well-tolerated and effective in improving QOL outcomes, increasing Hb, and reducing transfusion utilisation. Both Hb level increase and tumour response are important independent contributors to QOL improvement.

P-1340 SERUM ERYTHROPOIETIN LEVELS AND RECOMBINANT HUMAN ERYTHROPOIETIN USE FOR ANEMIA IN CHILDREN WITH CANCER

N. Çetingül, H. Öniz, D. Yılmaz, M. Kantar, K. Kavaklı, Y. Aydınok, A. Polat, G. Nişli, S. Öztop. *Ege University Faculty of Medicine. Department of Pediatric Hematology-Oncology İzmir, Turkey*

Cancer patients with symptoms of anemia frequently require red cell transfusions. Our aim was to determine erythropoietin (EPO) levels and the effect of weekly-administered recombinant human EPO (rHuEPO) (150 IU/kg/dose) on hemoglobin (Hb) levels, transfusion requirements red quality of life in anemic children with primary hematologic and solid malignancies. Seventeen children (9 male, 8 female; aged 1.5–17 years) with hematologic and solid malignancies were studied. Serum EPO and Hb levels were measured before initiation of rHuEPO therapy and repeated several times in the course. The rHuEPO therapy in our study was started when Hb levels decreased to 8 gr/dl or less.

EPO levels were not correlated with Hb concentrations. While the mean Hb levels decreased gradually during pre-study chemotherapy, the response to rHuEPO was defined as an increase of Hb following 1 month of therapy in each of hematologic and solid malignancies. During 12 weeks of rHuEPO treatment. Hb levels significantly increased in both solid (4.24 ± 2.8 g/dl) and hematologic (2.24 ± 1.98 g/dl) malignancies ($p = 0.04$ and 0.017, respectively).

Red cell transfusions did not decrease in the first two months of therapy. However a few patients were transfused, but transfusions were less per patient in the third months of rHuEPO therapy. The mean number of units transfused per patient per month during prestudy period and three months of rHuEPO therapy were found not to be significant (0.97 vs 0.86; $p > 0.05$).

We conlude that (1) rHuEPO therapy is safe and effective to increase red cell mass in anemic children with cancer, (2) If the patients with Hb levels below 10 g/dl were considered for rHuEPO therapy the response rate would have been optimised.

P-1341 RECOMBINANT HUMAN ERYTHROPOIETIN FOR THE TREATMENT OF ANEMIA IN IDIOPATHIC MYELOFIBROSIS

A. Falcone, P. Musto, C. Bodenizza, M. Carotenuto. *Division of Hematology, IRCCS "Casa Sollievo della Sofferenza" Hospital, S. Giovanni Rotondo, Italy*

Recombinant human erythropoietin (rHuEPO) has been used with moderate succes in the treatment of secondary anemia in pathologies involving the stem cell, such as myelodisplastic syndromes.

RHuEPO has not been used extensively in Idiopathic myelofibrosis (IMF).

We present eight patients (pts) with IMF treated with rHuEPO, at a dosage of 100–150 U/Kg sc, 3 times weekly, for a period at least 4–12-weeks. Six pts were males and two females, average age was 64 years (range 43–70 years), time from diagnosis and treatment with rHuEPO was 29 months (range 3–60 months). Seven pts had a transfusional requirement of 1–6 packed red cells monthly, one pt showed stable Hb levels of 8.0 g/dl. Splenomegaly more that 10 cm below arch of ribes was present in 6 pts. Cytoreductive chemotherapy with hydroxyurea (HU) was employed in 6 pts, before and during rHuEPO administration. Two pts achieved a response to rHuEPO treatment: one pt reduced transfusional requirement from 3 packed red cell to 1 monthly, with an increase in Hb level of 1 g/dl, another pt became transfusion independent; both were treated simultaneously with HU. One of the two responsive pt had inappropriately low serum EPO levels (14 mU/ml). Appropriate levels of sEPO, for the degree of their anemia, were observed in other responsive pt and in four unresponsive pts (respectively: 140, 1700, 1700, 390, 480 mU/ml). Very low levels of sEPO were detected in others two unresponsive pts (0.5, 34 mU/ml). Both responding pts showed an increase in reticulocyte count and an increase of soluble serum transferrin receptor. We observed: no spleen enlargement, no changes in platelets or WBC counts and no blastic phase evolution (follow-up 11 months).

In conclusion, in our experience, rHuEPO treatment showed no value in treating the anemia of IMF pts, and the levels of serum erythropoietin do not seem to be predective of the type of response.

P-1342 IMPAIRED RESPONSE TO rh HEPATITIS B VACCINE IN CHILDREN RECIVING ANTICANCER CHEMOTHERAPY

N. Umutlu, F. Atlihan, P. Gülez, C. Vergin. *Dr. Behçet uz Children's Hospital, İzmir, Türkiye*

The aim of this study is to evaluate the efficiacy of rh Hepatitis B vaccination for the prevention of Hepatitis B infection in different malignancies and chemotherapy regimens.

Serologic responses to rh Hepatitis B vaccine were investigated in 50 pediatric cancer patients ages between 2 and 15 years. Twenty of the patients were with hematologic malignancies, 30 with various solid tumors. Thirtyone patients were receiving intensive and 19 mild or modarete chemotherapy. Vaccination begun within one month following the diagnosis. Three doses of rh Hepatitis B vaccine (40 mcg) were given at 0, 1, 2 months and a booster dose was planned at the first year. Periodic serologic follow up by quantitative antibody titers were performed one month after each vaccination and also in the 5^{th} and 12^{th} month after vaccination. A titer equal to or greater than 10 IU/L were considered seropositive.

The seroconversion rates after the first, second and third doses were 4%, 28.57%, 34.09% respectively. In the control group seroconversion rates were 30%, 75%, 90% respectively. Among the solid tumors 10 patients (40%) and in among hematologic malignancies 5 patients (26.31%) produced positive seroconversion after three doses. The response is better in patients with solid tumors than in patients with hematologic malignancies. In addition the response is better in patients receiving mild or moderate chemotherapy (70.58%) than the patients who were receiving intensive chemothrapy (11.11%).

As a whole response is not effective, therefore combined administration of rh Hepatitis B vaccine and Hepatitis B hyperimmunoglobulin is needed.

P-1343 ONCE DAILY GENTAMICIN INDUCED OTOTOXICITY IN FEBRILE NEUTROPENIC PATIENTS

F. El Bakri, A. Pallett, A.G. Smith, A.S. Duncombe. *Departments of Medical Microbiology and Haematology Southampton University Hospitals Trust. Southampton, UK*

Aminoglycosides have traditionally had an adjunctive, synergistic role with β-lactam antibiotics, in the treatment of febrile neutropenic patients. Once daily dosing regimens, have been extensively studied and shown to be as effective, and no more toxic than multiple daily regimens. However very few

of these trials involved neutropenic patients. This is particularly important as these patients are at a higher risk of developing aminoglycoside toxicity due to their severe underlying disease, aggressive chemotherapy treatment, and their need for repeated, long courses of antibiotics. The incidence of inner ear damage is difficult to establish because of the small numbers studied and the lack of a standardized way of ascertaining cochlear or vestibular damage.

All cases of ototoxicity related to once daily gentamicin use among patients with haematological malignancies in our center, were studied (5 cases). They all received a dose of 7 mg/kg/day and serum levels were monitored 6–14 hours after the dose.

Age range was 41–64 years. All patients were neutropenic following intensive chemotherapy for acute myeloid leukaemia (AML) or Myeloma. They all had normal renal functions and satisfactory serum gentamicin levels. The duration of treatment with gentamic was 10–26 days. Ototoxicity manifested by vestibular and cochlear damage in 3/5 patients and vestibular or cochlear damage in the others. All patients with hearing deficits had abnormal audiograms.

Key features of once daily gentamicin induced ototoxicity were that it may consist of both cochlear and vestibular damage, may occur with the first course of gentamicin, and after stopping. Hearing deficit appeared irreversible although balance disturbance improved in some. Ototoxicity may occur even when renal function is normal and despite satisfactory serum levels. Prolonged administration, higher total dose and genetic predisposition appeared to be the major risk factors.

We recommend that the use of once daily gentamicin in lengthy neutropenia should be restricted to 7–10 days. Consideration should be given to a lower starting dose of 5 mg/kg/day, and clinicians should be vigilant for signs of ototoxicity.

P-1344 IMMUNOGENICITY OF TRIVALENT SPLIT INFLUENZA VACCINE IN PATIENTS AFTER SPLENECTOMY

L.B. Brydak[1], R. Rokicka-Milewska[2], P. Łaguna[2], M. Machała[1]. [1]*WHO Nat. Influenza Center, Nat. Inst. of Hygiene, Warsaw;* [2]*Dept. of Pediatrics Hematology and Oncology, Medical Academy, Warsaw, Poland*

The aim of this study was to assess the immunogenicity of influenza vaccine in patients after splenectomy. The study group consisted of 25 splenectomized patients aged 10–40 vaccinated in autumn 1997 with influenza vaccine containing 15 μg hemagglutinin of each of the following strains: A/Bayern/7/95 (HIN1), A/Wuhan/359/95 (H3N2) and B/Beijing/184/93. Neuraminidase activity of this vaccine amounted to 17.62×10^{-3} U/ml. Humoral response to hemagglutinin and neuraminidase of influenza vaccine was determined in sera collected before vaccination and after 1 month. The same procedures were carried out in a group of 25 healthy persons aged 5–40 who were also vaccinated against influenza with the same vaccine. After immunization, geometric mean titers (GMTs) of antihemagglutinin antibodies increased in both study groups when compared with the results before immunization. Mean fold increase (MFI) values amounted to 4.8 for hemagglutinin HI, 7.3 for hemagglutinin H3 and 5.9 for hemagglutinin HB in splenectomized patients, while in healthy persons MFI indexes were 8.5, 14.4 and 6.3 respectively. In patients after splenectomy, the protection rate, i.e. the percentage of subjects with antihemagglutinin antibody titers $\geq$1:40 was 4% for H1, 20% for H3 and 32% for HB before vaccination. After vaccination, these values increased and amounted to 32%, 80% and 72% respectively. In healthy persons, the protection rates were higher and before immunization they amounted to 20% for H1, 24% for H3 and 52% for HB, while after immunization they were 68%, 96% and 100% respectively. Response rate, i.e. the percentage of subjects with at least a fourfold increase of antihemagglutinin antibody titer after vaccination, amounted to 28% for H1, 68% for H3 and 44% for HB in patients after splenectomy and 52%, 80% and 60% in healthy persons respectively. In the case of neuraminidase, MFI indexes in splenectomized patients amounted to 3.5 for neuraminidase N1, 3.1 for neuraminidase N2 and 2.2 for neuraminidase NB, while in healthy subjects they were 5.2 for N1, 1.2 for N2 and 5.3 for NB. In conclusion, the present study indicate that influenza vaccine is immunogenic in patients after splenectomy.

P-1345 DOMICILIARY MANAGEMENT OF HEMORRHAGE FOR PATIENTS WITH ADVANCED-PHASE HEMATOLOGIC NEOPLASMS

C. Cartoni, M. Cedrone, C. De Gregoris, M. Giovannini, P. Niscola, C. Romani, L. Moleti, C. Girmenia, F. Mandelli. *Department of Cellular Biotechnologies and Hematology, University "La Sapienza" Rome, Italy*

Severe thrombocytopenia is a common feature of patients affected by hematologic neoplasm in advanced phase of disease. PLTS count lower than 20.000/mm3 is associated with an enhanced risk of hemorrhagic complications requiring medical intervention. In order to assess the incidence of bleeding in this category of patients and the feasibility of domiciliary management of this complication, our Home Care Unit started to approach the hemorrhagic episodes at home of the patients. Between September 1993 and June 1997, 256 pts with hematologic malignancies (73 acute leukemias, 20 lymphomas, 12 CML, 5 CLL, 8 myelofibrosis), in terminal phase had been assisted by Home Care Unit. In 118 pts. (46%) PLTS count was <20.000/mm3 with an average duration of thrombocitopenia of 28 days (range 1–358). All these patients received prophylactic therapy by oral antifibrinolytic drugs and showed pethechiae.

No prophylactic transfusions of platelets were given. In 74/118 patients (62%) bleeding was the reason of medical access at home, accounting for 140 total episodes (average 1.64, range 1–8). Major bleeding (defined as any bleeding more than petechiae or mucosal or conjunctival hemorrhage) was observed in 74/140 episodes (bleeding of urinary tract 16, gastrointestinal 35, respiratory 2, SNC 5, severe hematomas 16). Treatment consisted of platelets transfusions in 85 cases (60%) and i.v. antifibrinolytic agents in 44 (31%). This strategy allowed the resolution of the hemorrhagic complication in 127 episodes (91%) at home of the patient; in 12 cases (8.5%) bleeding was the cause of death (5 cerebral, 7 severe gastrointestinal hemorrhage).

These results indicate that: i) severe thrombocytopenia is a frequent clinical problem (46% of patients) to approach in palliative care; ii) not all patients with PLTS < 20.000/mm3 experienced hemorrhagic complication, with 38% of them not requiring medical intervention; iii) the domiciliary approach is feasible with 92% of episodes successfully resolved at home and avoiding an unnecessary hospitalization for severely ill patients; iiii) to achieve these results a close collaboration is needed between the Home Care Unit on call 24 hours daily and Transfusion Unit.

P-1346 CHARACTERISTICS OF DEATHS IN A DEPARTMENT OF ONCO-HAEMATOLOGY FROM A GENERAL HOSPITAL. A STUDY OF 81 CASES

F. Bauduer, C. Capdupuy, M. Renoux. *Centre Hospitalier de la Côte Basque, Bayonne, France*

A fatal outcome is often seen in patients (pts) treated in onco-haematology. Of note, characteristics of these deaths are rarely developed in the literature. Between 11/95 and 02/97, eighty-one pts deceased in our unit (about 50 deaths/year) which is mainly devoted to malignant blood diseases management and serves a population of about 290000 inhabitants. We report herein the main data concerning these cases which were prospectively assessed. Fifty were males (61.7%). The mean age was 67.8 (range: 19–96). Underlying diseases were: multiple myeloma (9), de novo (10) or secondary (12) acute myeloid leukaemia, non-Hodgkin (13) or Hodgkin (1) lymphoma, chronic lymphocytic leukaemia (6), acute lymphoblastic leukaemia (4), myelodysplastic syndromes (3), solid tumors (11), severe aplastic anaemia (1), anaemias of various causes (2), primary amyloidosis (1), immune thrombocytopenic purpura (1), HVC+ haemophilia (1), malignant hypereosinophilia (1), warfarin-induced cerebral haemorrhage (1), other (4). The previous disease duration was evaluable in 76 pts and ranged from 5 days to 276 months (mean: 31.9 months). The last hospital-stay duration before death varied between 0 (death at arrival or during taking to hospital) and 40 days (mean: 9.3 days). Two pts died in the emergency unit before entering our department (1 suicide). Only 15 pts were admitted for the first time. In 70% of the cases, death was the consequence of a refractory or of a end-stage disease and appeared as a predictable event. For these pts, home care was virtually impossible either because they lived alone or because their family members had to work or because of high degree of palliative support. For them, all the ≪do not resuscitate orders≫ were instituted at least 48 hours before death. No pt died in the intensive care unit. The most frequent clinical complaints were: severe and potentially treatable pain (27%), infection- or disease-related fever (40%), dyspnea (44%), haemorrhages (20%), CNS disturbances (25%). Chemotherapy (46%), antiinfectious agents (47%), transfusions (42%), major analgesics (27%) and steroids (20%) represented our main medications during the last stay. About half of the pts died in the absence of any family member. A better knowledge of dying pts profiles in haematology is needed for improving epidemiologic data. Optimization of quality of life in its final stages requires efficient palliative care including considerable psychological, sociological, technical and financial burden.

P-1347 CEFTAZIDIME AND AMIKACIN OR CEFTRIAXONE AND AMIKACIN IN NEUTROPENIC PATIENTS WITH HEMATOLOGICAL NEOPLASIAS

K. Antonis Zacharof, C. Petrogiannopoulos, C. Flevaris, H. Zacharof. *Hellenic Red Cross Hospital, Athens, Greece*

Purpose: To compare the efficacy of ceftazidime and ceftriaxone in association to amikacin for treatment of febrile episodes in patients affected by hematologic neoplasia and severe neutropenia.

Material and Methods: Fifty three patients with absolute neutrophil count (ANC) lower than 500/mm3 at the onset of febrile episode were randomly assigned to receive one of the following two regimens: regimen A (amikacin plus ceftazidime) or regimen B (amikacin plus ceftriaxone); ceftriaxone was administered once daily at a dose of 30 mg/kg (maximal dose 2 gr/day); ceftazidime was administered at a dose of 100 mg/kg/day in three divided doses (maximal dose 6 gr/day); amikacin was administered at a dose of 21) mg/kg/day in three divided doses (maximal dose 1.5 gr/day). All patients were treated in our Department. Sixty six patients were enrolled, 33 in each group; the response was evaluable in 53 (27 group A and 26 group B). Thirty one patients had ANC lower than 100/mm3 at the onset and 26 had ANC lower than 100/m3 at the onset or during the first week of antibiotic therapy. Thirty eight patients were affected by acute leukemia (26 AML, 12 ALL). No statistical differences concerning age, sex, hospital stay, patients with ANC lower than 100/mm3, etiology, were present between the two groups.

Results: Group A was effective in 19/27 (70%) patients; group B in 16/26 (62%). Among patients successfully treated, median time to defervescence was 2.9 days in Group A vs. 3.1 in Group B; study drugs were continued for 8.1 days and 7.8 days accordingly in the two groups. Median time to failure was 6.2 days in Group A and 5.4 days in Group B.

Conclusions: In our group of patients, when associated with amikacin, 6 gr/day of ceftazidime in three divided doses and 2 gr/day of ceftriaxone once daily are equally effective for the therapy of febrile episodes in severely neutropenic patients affected by hematological neoplasias.

P-1349 CYTOGENETIC CLONALITY ANALYSIS OF MEGAKARYOCYTES IN MYELODYSPLASTIC SYNDROME USING DUAL COLOR FLUORESCENCE IN SITU HYBRIDIZATION AND CONFOCAL LASER SCANNING MICROSCOPY

K. Van Lom[1], A.B. Houtsmuller[2], W.L.J. Van Putten[3], R. Slater[2], B. Löwenberg[1,2]. *[1]University Hospital Rotterdam; [2]Erasmus University Rotterdam; [3]Daniel den Hoed Cancer Center, Rotterdam, The Netherlands*

Myelodysplastic syndrome (MDS) comprises a group of clonal stem cell disorders characterized by anemia, leukopenia and/or thrombopenia. Numerical chromosome changes are often found in MDS and can be used as karyotypic markers to establish blood cell lineage involvement. In MDS, the blasts, granulocytic cells and erythroblasts are usually part of the cytogenetically abnormal clone. Little is known about the involvement of megakaryocytes. The variable ploidy of these cells hampers the investigation of numerical chromosomal abnormalities.

We applied dual color fluorescence in situ hybridization (FISH) to routinely made bone marrow (BM) smears of cytogenetically normal patients and of 7 MDS patients with monosomy 7 or trisomy 8. Probes specific for the centromeric regions of chromosome 7 and 8 were applied and detected with Fluorescein iso-thiocyanate (FITC) and Texas-Red respectively. We introduced confocal laser scanning microscopy to scan several optical planes of the megakaryocytes thus allowing us to count the FITC (#7) and Texas red (#8) FISH spots in different focus layers.

In normal cells the theoretical ratio between the numbers of chromosomes 7 and 8 is 1. In case of monosomy 7 and trisomy 8 these ratios are 0.5 and 1.5 respectively. Fifty-six megakaryocytes were analyzed in 6 normal BM smears providing a normal ratio for chromosomes 7 and 8 of 1.0±0.2 (mean±2SD). This ratio was applied to evaluate clonal involvement of individual megakaryocytes in patients with MDS. In two patients with monosomy 7 the majority of the megakaryocytes was monosomic. In one of the five patients with trisomy 8 all analyzed megakaryocytes were trisomic. In the other 4 patients the gain of chromosome 8 was apparent in a majority of the megakaryocytes.

These results provide conclusive evidence to indicate that in MDS megakaryocytes are involved in the malignant clone.

MDS: biology and diagnosis

P-1348 CYTOGENETIC STUDY OF 140 CASES WITH MYELODISPLASTIC SYNDROMES

M. Pantić, A. Novak, V. Đordević, D. Marisavljević, N. Suvajdžić, N. Radošević, G. Janković, M. Čolović. *Cytogenetics Laboratory, Institute of Hematology, Clinical Center of Serbia, Belgrade, Yugoslavia*

Myelodisplastic syndromes (MDS) are a heterogeneous group of hemopoietic stem cell disorders, whose diagnostic and prognostic properties could be evaluated by cytogenetic analysis. Bone marrow samples of 140 consecutive patients (pts) with primary MDS were cytogenetically studied during seven years (1990–1997). MDS pts were subclassified as: 54 pts with refractory anemia (RA), 14 pts with RA and ringed sideroblasts (RARS), 35 pts with RA and excess of blasts (RAEB), 13 pts with RAEB in transformation (RAEB-t), 22 pts with chronic myelomonocytic leukemia (CMML) and 2 pts as unclassified MDS. Cytogenetic analysis was performed after direct preparation and/or unstimulated 24–48 ^{h} short-term culture. The overall incidence of chromosomal abnormalities was 49%. The RAEB and RAEB-t pts showed chromosomal abnormalities more often than RA and CMML pts (71% and 62% vs. 35% and 32%). Also, a complex chromosomal aberrations were present in even 75% of RAEB-t comparing to 26% of RA or 14% of CMML pts. The most frequent anomaly, del(5q)/−5, was discovered in 29% (all but CMML) and del(7q)/−7 in 16% of cytogenetically abnormal pts, mostly associated with complex karyotypes. Trisomy of 8 and Y loss were found, as a single anomaly, in 9% and 6% pts, respectively. A significant number of patients (9 pts) had constitutional chromosome changes as pericentric inversion of chromosome nine (2 pts) or C-band heteromorphism of chromosome one (2 pts), nine (1 pts) and sixteen (4pts). One third of our patients (30%) underwent a cytogenetic follow-up, but only 31% of them showed a karyotypic evolution and even 21% patients lost chromosomal abnormalities as a consequence of treatment or spontaneous remission. In conclusion, one half of analyzed patients with MDS (49%) have karyotypic variations which could be further correlated with clinical, morphological and prognostic characteristics.

P-1350 DETECTION OF 'MASKED' MONOSOMY 7 IN MYELODYSPLASTIC SYNDROMES BY FLUORESCENCE *IN SITU* HYBRIDISATION

K. Jakovleva[1,4], I. Ögärd[3], I. Arvidsson[2], L. Shan[3], B. Jacobsson[2], B. Swolin[3], R. Hast[1]. *[1]Division of Hematology, Department of Medicine; [2]Department of Clinical Pathology, Karolinska Institute at Danderyd Hospital, Stockholm; [3]Department of Clinical Chemistry, Gothenburg, Sweden; [4]Department of Internal Medicine, University of Tartu, Tartu, Estonia*

Myelodysplastic syndromes (MDS) are associated with clonal chromosomal abnormalities in more than half of the cases and this has prognostic implications, particularly in cases of monosomy 7. With conventional cytogenetics it is sometimes difficult to obtain sufficient number of good-quality metaphases. Important prognostic information may therefore be lost. In this study we have used fluorescence in situ hybridisation (FISH) to look for the presence of masked monosomy 7, i.e. monosomy 7 which is not detected by metaphase cytogenetics, in a 114 MDS patients: 36 RA, 25 RARS, 32 RAEB, 3 CMML, 5 RAEB-t and 13 unclassified cases of MDS. The median age was 74 years (range 35–93), male:female 1.2:1. Two different methods were used to study monosomy; in 58 cases we used cryopreserved bone marrow smears (Danderyd) and in 56 cells from fixative solution prepared for conventional cytogenetics (Gothenburg) The material was analyzed by FISH using commercial DNA probes, specifying the pericentromeric region of chromosome 7. Studies of normal controls gave the cut-off level (mean of 10 normal controls +2SD) for bone marrow smears of >8% and for dropped slides of >6% of bone marrow cells with one fluorescent spot. By metaphase cytogenetics monosomy 7 was found in 9 of 114 cases, either a sole abnormality or as a part of complex abnormal karyotype. FISH confirmed the presence of monosomy 7 in the 9 patients where metaphase cytogenetics showed the aberration, and found additional 14 cases with masked monosomy 7. In the former group between 11.7 and 90% (median 72%) of the bone marrow cells showed one fluorescent spot compared to 6.1 and 23.4% (median 7%) among those with masked monosomy 7. Thus, the number of MDS patients with documented monosomy 7 increased from 9/114 (8%) by metapahase cytogenetics to 23/114 (20%) by FISH. Given the prognostic and therapeutic significance of monosomy 7, our finding of number MDS cases with masked monosomy 7 may be clinically important.

P-1351 REARRANGEMENT OF THE SHORT ARMS OF CHROMOSOME 8 IN MYELODYSPLASTIC SYNDROMES (MDS): A CYTOGENETICS AND FISH STUDY

P. Bernasconi[1], L. Malcovati[1], M. Boni[1], P.M. Cavigliano[1], M.G. Roberti[2], A. Bardi[2], A. Cuneo[2], G. Castoldi[2], C. Bernasconi[1]. [1]*Istituto di Ematologia, IRCCS Policlinico San Matteo, Pavia;* [2]*Dipartimento di Scienze Biomediche, Sezione di Ematologia, Università di Ferrara, Italy*

A structural abnormality of the short arm of chromosome 8 was detected in 6/428 MDS cases studied in a ten years period. One patient was classified as Refractory Anemia, 2 as Refractory Anemia with Excess of Blasts (RAEB), 2 as RAEB in transformation (RAEB-t) and one as Acute Myeloid Leukemia (AML) evolved from a MDS. At diagnosis no patient presented with marked eosinophilia or with lymphoadenopathy or had had a T or B non-Hodgkin lymphoma as it occurs in patients carrying the 8; 13 translocation. Bone marrow examination detected a marked tri-lineage dysplasia in 7 cases and only dysmegakaryocytopoiesis in the last one. Eosinophils were increased in no patient. In 4 cases the rearrangement of chromosome 8 was the only chromosome abnormality, while in 2 it was accompanied by other defects. In all cases, however, it was the primary chromosome change. In 5 cases cytogenetics interpreted the structural aberration as a terminal deletion involving band 8p21 in 3 cases and band 8p11 in the other 2. In the remaining case we detected the derivative of a chromosome 8 due to a 6; 8 translocation involving band 8p11. The other product of the rearrangement was lost. FISH with a painting probe for the whole chromosome 8, giving a red signal, was carried out in all cases and confirmed cytogenetic data. However, in order to better define our results, we are performing a FISH analysis employing a probe for the short arm telomere of chromosome 8.

Two cases (one RA and one RAEB) have a stable disease and have received supportive care only. One case, a RAEB, evolved in RAEB-t and 2, both RAEB-t, transformed in AML. These last 2 cases and the one diagnosed as AML underwent chemotherapy, without any response. In one young patient, however, a complete remission was achieved only after an allogeneic bone marrow transplantation.

Our results show that the structural abnormality of the short arms of chromosome 8 in MDS has a low incidence; the pathogenetic role of the genes mapped in that chromosomal region will be discussed when telomeric FISH data will be available.

P-1352 THE 17p- SYNDROME: A DISTINCT MYELODYSPLASTIC SYNDROME (MDS) ENTITY

L. Jary[1], H. Mossafa[2], N. Atkhen[3], C. Fourcade[1], P. Genet[4], M. Pulik[4], G. Flandrin[5]. [1]*Biologie clinique;* [4]*Hématologie, Argenteuil;* [2]*Cytogénétique Humaine, CERBA, St-Ouen-l'Aumône; Laboratoires d'Hématologie;* [3]*hôpital Boucicaut;* [5]*hôpital Necker, Paris, France*

The 17p- syndrome is a subset of MDS characterised by dysgranulopoiesis with pseudo-Pelger anomaly and a deletion of the short arm of chromosome 17. We describe 5 patients (47–87 years) 3 of them pretreated (1 by Misulban for CML, 1 by hydroxyurea for polycythemia, 1 had previous radiotherapy). All had anemia (77–100 g/L) and thrombopenia (27–148 $\times$ 10^9/L); 3 had neutropenia (0.09–0.8 $\times$ 10^9/L), 2 had leucocytosis (11.7–44.9 10^9/L). Blood and marrow smears showed dysgranulopoiesis, with round or bilobulated nuclei, cytoplasmic vacuoles and a myeloperoxydase deficiency involving neutrophils and their precursors in 5/5, and eosinophils in 3/5. Refractory Anemia with Excess of Blasts (RAEB) was diagnosed in 2 cases, RAEB in acute transformation in 1 case, and trilineage myelodysplasia in erythroblastic acute transformation in 2 cases. Bone marrow cytogenetic examination showed in 5/5 rearrangement of the short arm of chromosome 17 associated with an additional complexe karyotype and Ph+ chromosome in 1 patient. In 3 cases, FISH studies demonstrated the presence of chromosome 17p rearrangement. All patients had a refractory disease with a short survival (7–180 d).

Our findings suggest the possible correlation in MDS between cytogenetic and morphology as reported by Lai (1995). This new entity has a very poor prognosis. Rearrangements of 17p may involve the gene of p53, and alter the process of cell duplication leading to nuclear abnormalities. The relation between p53 in-activation and this subset of MDS needs further investigations.

P-1353 DE NOVO MYELODYSPLASTIC SYNDROMES: IS MLL GENE REARRANGED?

V.I. Pappa, T. Economopoulos, E. Papageorgiou, G. Vessalas, D. Lilington[1], J. Dervenoulas, P. Tsiotra, A. Kostourou, S. Melou, G. Bolas, B.D. Young[1]. *S. Raptis Second Department of Internal Medicine, Athens University, Evangelismos Hospital, Greece;* [1]*ICRF Department of Medical Oncology, St. Bartholomew's Hospital, London, UK*

Introduction: The MLL gene at 11q23 encodes a transcription factor of crucial importance in leukemogenesis since its disruption trough translocation with a variety of partner genes, or through internal duplication, or deletion, has been associated with acute myelogenous, acute lymphoblastic and mixed leukemias of adults, as well as with 90% of infant acute leukemias.

The involvement of MLL gene in de novo myelodysplastic syndromes has not been extensively studied. The aim of this study was to detect if rearrangement of MLL, of the type common found in acute leukemias, is also found in de novo myelodysplastic syndromes, a group of disorders affecting the early haemopoetic progenitor.

Patients and Methods: 34 patients not previously treated with de novo MDS (5RA, 3 RAS, 15 RAEB, 3 RAEB-t, 8 CMML), 2 acute myelogenous leukemias (AML) arising from preexisting myelodysplasia, and one AML (M6) were studies with bone marrow and peripheral blood cytology and classified according to FAB criteria. The involvement of MLL in these cases was studied with conventional cytogenetics and with Southern Blot. The probe used was the PCR product from MLL cDNA cloned in Bluescript plasmid using primers from exons 5 to 9 where most of the breakpoints of MLL in acute leukemias occur. Two restriction enzymes have been used: Bam HI and Eco RI for confirmation of the results. The probe was labeled with a^{32}P d CTP (Multiprime DNA labelling protocol, Amersham).

Results: The karyotype did not reveal any abnormalities in 27 cases (12 RAEB, 5 RA, 2 RAS, 1 AML (M6), 2 leukemic transformation of MDS, 5 CMML). Clonal karyotypic abnormality was found in 7 cases: 1 CMML with a 40% del 16(q22q24), 1 CMML with a 20% 47XX +8, 100/648 idem +8, 1 RAEB-t with a70% 47XY +8,1 RAEB-t with 30%del 9(q12u22), 1 RAEB with 10%47XX, 80% 48 idem +8, 1 RAEB with 2%47XX+8, and 1 RAEB with complex karyotype. In 3 cases karyotype was not performed. The analysis of MLL gene rearrangement with the above described probe detected in all cases non rearranged bands with both the Eco RI and Bam HI enzymes.

Conclusion: The most common karyotypic abnormalities in this series of MDS were trisomy 8 and tetrasomy 8. Abnormalities at 11q23 and rearrangement of MLL have not been found in any case. These findings indicate that MLL gene rearrangement at exons 5–9 is not common in de novo MDS. Further analysis of larger number of cases, as well as rearrangements of other parts of the gene or abnormalities of its expression in de novo myelodysplastic syndromes are needed to clarify the role of this gene in haemopoiesis.

P-1354 ADULT FAMILIAL MYELODYSPLASTIC SYNDROMES (*f*-MDS): TOWARDS THE GENETIC BASIS OF LEUKAEMIA

A. Mijovic, G.J. Mufti. *For the Adult Familial MDS Registry, King's College Hospital, London, UK*

Predisposition to MDS and acute leukaemia, usually manifesting in childhood, is a feature of several well-documented hereditary disorders; By contrast, there have been only anecdotal reports of adult-onset familial MDS.

In May 1997 we initiated the Adult Familial MDS Registry in order to collect data on kindreds with two or more first-degree relatives (fdr) with MDS or acute myeloid leukaemia (AML). In response to the initial enquiry, 19 families have been reported. As of February 1998, details were available on 12 families: 9 of British, 2 of Yugoslav, and 1 of South African descent. All families were of Caucasian race. Thirty-one cases of MDS (20 women and 11 men) have been observed among 94 fdr. More than one generation was affected in 4 kindreds, suggestive of a dominant mode of inheritance; in others, only siblings were affected and the inheritance pattern was difficult to ascertain. Dizygotic twins were the only affected members in one family. The median age at diagnosis of MDS was 38 (range 10–76) years. The probability of developing MDS for all fdr was 38±6% by the age of 50 years The risk was very low before the age of 18 years, but increased steadily thereafter. In 57 second-degree relatives with available information, only 1 myeloid malignancy (atypical CML) has been observed.

One MDS patient developed a second tumour. Nine other cancers have been observed among 94 fdr, occuring at the median age of 65 years, and resulting in an actuarial risk of cancer of 41±11% by age 70. In 2 kindreds, clustering of Ca Breast was also observed.

Eight of 22 MDS patients (36%) had an abnormal karyotype: unbalanced translocations or losses of chromosomes 7 and 5 were found in all. Notably, t(7;15) was found in two unrelated patients.

The variability of age at onset and inheritance patterns suggest different mechanisms of susceptibility to MDS, which in some families may be a part of a more general predisposition to cancer. Detailed molecular analysis of *f*-MDS is expected to provide insight into its genetic basis.

P-1355 INCREASE IN THE EXPRESSION LEVELS OF THE WILMS' TUMOR GENE (WT1) PREDICTS LEUKEMIC TRANSFORMATION IN MYELODYSPLASTIC SYNDROME (MDS)

H. Tamaki, H. Ogawa, K. Ohyashiki, Y. Oka, T. Soma, M. Tomonaga, K. Toyama, T. Kishimoto, H. Sugiyama. *Departments of Medicine III and of Clinical Laboratory Science, Osaka University Medical School, First Department of Internal Medicine, Tokyo Medical College, Department of Hematology, Atomic Bomb Disease Institute, Nagasaki University School of Medicine, Japan*

MDS comprises a heterogeneous group of hematopoietic stem cell disorders with a possible leukemic transformation. WT1 is highly expressed in almost all leukemia cells and thus is a tumor marker for leukemic blast cells (1). In this study, we assayed WT1 expression levels in MDS and found a strong correlation between WT1 expression levels and the disease progression to overt leukemia (OL). WT1 expression levels of thirty-seven BM and forty-three PB samples were evaluated for 67 patients with *de novo* MDS (36 RA; 15 RAEB; 9 RAEBt; 13 OL) by means of a quantitative RT-PCR method. WT1 expression levels in RA, RAEB, RAEBt and OL were 5.0×10^{-5}, 3.8×10^{-3}, 9.1×10^{-3} and 1.1×10^{-1}, respectively (WT1 expression level of K562 leukemic cell line was defined as 1.0), and thus the WT1 expression levels increased in proportion with the disease progression. The WT1 expression levels were significantly higher in RAEB or RAEBt patients with the disease progression to OL within 6 months than that in those without the disease progression (1.5×10^{-2} vs 8.5×10^{-4}, $p < 0.05$). We concluded that WT1 expression levels reflect the disease progression to leukemia and thus WT1 assay useful to predict leukemic transformation in MDS.

(1) Inoue, K., et al: Blood 84: 3071–3079, 1994

P-1356 PROGNOSTIC SIGNIFICANCE OF APOPTOTIC INDEX IN PATIENTS WITH MYELODYSPLASTIC SYNDROME AND COMPARISSON TO OTHER PROGNOSTIC SCORES

A.D. Bogdanović[1], D.P. Trpinac[2], G.M. Janković[1], V.M. Čemerikić[3], V. Bumbaširević[2], M.D. Čolović[1]. *[1]Inst of Hematology; [3]Dept. of Pathology Clinical Center of Serbia; [2]Inst. of Histology, School of Medicine, Belgrade, Yugoslavia*

Objective: To evaluate the prognostic significance of apoptotic index (AI) and to compare AI with Bornemouth (BS) and Spanish score (SS) in 30 patients MDS.

Methods: The bone marrow samples of 30 pts. with MDS were embedded for semithin morpho-logical analysis (Leukemia 1997) and number of cells in apoptosis were counted and expressed as percentage (AI). According to FAB there were RA and RARS (8 + 3 pts), RAEB + RAEB-t (14 + 2) and 3 pts with CMML. Analysis have been calculated on 50% survival basis ($SRV_{50\%}$).

Results: According to FAB, pts with low risk (RA + RARS) have significant better survival than RAEB/t (OS_{5y} 80% vs $SRV_{50\%}$ 20 m., log rank $p < 0.01$). According to Bornemouth score pts with low risk (4), intermediate (20) and high risk (6) have $SRV_{50\%}$ 50 m, 26 m and 6 m respectively. According to Spanish score pts with low risk (9), intermediate (15) and high risk (6) have $SRV_{50\%}$ >60 ms, 24 m and 6 m respectively. We have found that patients with low BS and SS scores have lower AI (2.37/2.86) than pts with high risk (3.60/3.27) which is on the level of significance ($p = 0.051$). We have introduced two apoptotic prognostic scores APS1 (low AI < 3 and high AI > 3) and APS2 (low AI < 2.5, intermediate AI 2.5–3.99 and high AI > 4). In analysis of APS1 we have found that there is no statistically significant difference in survival but pts with low APS1 survive longer (50 m) than pts with high APS1 (26 m) [Cox F-test 0.12]. In analysis of APS2 we have found again that low APS2 survive better then others (>68 m) while APS2 intermediate and high have worse survival (24 and 17 m). The survival according to APS2 was similar to survival according to BS and SS. We also found that pts. with low and high APS1 and APS2 have significantly different BS (t test $p < 0.05$) but not SS.

Conclusion: Our analysis is influenced by the small number of analyzed patients but we think that apoptotic prognostic score with cutoff of at least 3% of apoptotic cells (AI) can be used as a simple and reliable prognostic factor in patients with myelodysplastic syndrome.

P-1357 NO INCREASE OF APOPTOSIS CAN BE OBSERVED IN BONE MARROW HISTOLOGY BY TUNEL AND ELECTRON MICROSCOPY IN MYELODYSPLASIA PATIENTS

S.J.L. Brada, J. Koudstaal, S. Poppema, N. Blom, J.Th.M. De Wolf, D. Hendriks, H. Louwes, J.W. Smit, E. Vellenga. *Division of Hematology and Pathology, University Hospital, Groningen, The Netherlands*

In myelodysplasia (MDS) anemia is the presenting symptom in spite of a erythroid hyperplasia in the bone marrow. The cause of the ineffective erythropoiesis is not well defined, but it is suggested that apoptosis is a prominent feature in this process. Subsequently we performed electron microscopy and TUNEL staining of paraffin embedded bone marrow specimens to assess the amount of apoptotic cells in the erythroid lineage in 10 MDS patients (3 RA, 2 RARS, 4 RAEB, 1 RAEB-T) and 5 controls. Ki-67 was used as a proliferation marker in the same cases. The erythroid lineage was characterized by a positive staining with Glycophorin-A (GpA). The apoptotic index (AI), i.e. the total number of apoptotic cells/1000 nucleated cells was 2.15%±0.95 in MDS and 5.44%±1.69 in normal controls ($p < 0.05$). In RA/RARS the AI was 2.56%±1.15, in RAEB/RAEB-T 1.74%±0.53. Apoptosis in the GpA compartment did not differ between MDS and normal controls. In 8 cases (4 RA, 2 RARS, 2 RAEB) and 2 normal controls AI was measured by electron microscopy. AI for RA was 2.25%±1.50, for RARS 1.5%±0.70, for RAEB 2.0%±0, for normal controls 2%. Ki-67 positivity in the GpA compartment for MDS was 84%±12.24 vs 79.9%±20.16 for normal controls. In 4 cases of MDS Ki-67 activity was compared with erythron transferrin uptake (ETU) and soluble transferrin receptor (sTfR) as a measure for in vivo erythroid proliferation. No significant correlation could be established between Ki-67 positivity and ETU or sTfR. The results of this study demonstrate that the number of apoptotic cells found in paraffin embedded bone marrow specimens of MDS patients is significantly less than in normal controls. The hyperplasia of normoblasts seen in bone marrow specimens together with the low AI suggests a defect in the erythroid maturation in MDS which comes to expression at the final erythroid maturation.

P-1358 PROLIFERATION AND APOPTOSIS CHARACTERISTICS IN VITRO IN MYELODYSPLASIA, AML AND NORMAL BONE MARROW

L.F.R. Span, R.A.P. Raymakers, E.S.M. Rutten, Th. De Witte. *University Hospital Nijmegen, Nijmegen, The Netherlands*

Myelodysplastic syndromes are clonal stem cell disorders most commonly characterised by hypercellular bone marrow (BM) with peripheral cytopenia caused by premature programmed cell death.

Methods: BM mononuclear cells of 6 normal controls, 5 MDS and 6 AML patients, both with trisomie 8, were cultured in agar. After 4, 7 and 10 days of incubation *"proliferation"* was determined by counting the number (N) of clusters (10–40 cells) and colonies (>40 cells). The agar cultures were fixed on slides and processed with a modified In Situ End Labelling technique to determine the amount of apoptosis. *Apoptosis (A%)* is defined as the percentage of clusters or colonies showing apoptosis of more than 50% of their cells.

Results: *Normal BM* proliferated to a median of 261 clusters on day 4, showing a decline to day 7 (N: 100) and day 10 (N:73) because of apoptosis (approx. 50%) and continuous proliferation to colonies (N day 7: 217, N day 10: 229). These colonies showed a median A% of 20–25%. Cluster growth was higher for *MDS* on day 4 (N:342) and day 7 (N:337) but clearly declined to day 10 (N: 140) because of increasing A% (75%). The high cluster formation on day 4 caused a high number of colonies on day 7 (N: 280) which decreased rapidly to day 10 (N: 126) also because of increasing apoptosis found in these colonies (resp. 29% and 42%). *AML* was characterised by a low proliferation of clusters and colonies with a relatively high amount of apoptosis in its clusters (N, A% day 4: 62, 79%, day 10: 65, 62%) and a low amount of apoptosis in its colonies (N, A% day 7: 19, 24% and day 10: 57, 20%).

Conclusions: Normal BM colonies survived in vitro from day 7 to 10 with a decreasing amount of apoptosis. MDS marrows showed a decrease in their colony formation from day 7 to day 10 because of increased apoptosis in their clusters and colonies. AML had a low colony growth combined with low apoptosis causing an increase in their numbers of colonies. *CFU-GM of MDS patients in vitro show the same characteristics as in vivo - a high proliferation capacity combined with a high propensity of apoptosis.* This could be a nice in vitro model to unravel the mechanisms of apoptosis involved in myelodysplasia.

P-1359 CD34$^+$/CD36$^-$ CELLS FROM MYELODYSPLASIA PATIENTS HAVE A LIMITED CAPACITY TO PROLIFERATE BUT CAN DIFFERENTIATE IN RESPONSE TO ERYTRHOPOIETIN AND MAST CELL GROWTH FACTOR

S.J.L. Brada, J.Th.M. De Wolf, D. Hendriks, J.W. Smit, E. Vellenga. *Division of Hematology, University Hospital, Groningen, The Netherlands*

Myelodysplasia (MDS) is characterized by a normal or increased number of normoblasts in the bone marrow and an impaired in vitro colony formation. In this study we analyzed whether this might be due to a dissociation between proliferation and differentiation. CD34$^+$/CD36$^-$ sorted bone marrow cells of 18 MDS patients (13 RA/RARS, 5 RAEB/RAEB-T) were cultured in a clonogenic and suspension culture assay in the presence of erythropoietin (Epo) and mast cell growth factor (MGF). Burst Forming Units-Erythroid (BFU-E, 75±88/10^4 CD34$^+$/CD36$^-$ cells, x±SD) and Colony Forming Units-E (CFU-E) were observed in 62% of cases with RA/RARS and in 20% of cases with RAEB/RAEB-T. Suspension cultures with CD34$^+$/CD36$^-$ sorted cells with Epo/MGF showed an 8.9±6.5 fold expansion after 7 days in cases with >10 BFU-E 10^4 CD34$^+$/CD36$^-$ cells, while cases with <10 BFU-E 10^4 CD34$^+$/CD36$^-$ cells showed 1.0±0.8 fold expansion especially in cases with RAEB/RAEB-T. FACS and morphology analysis at day 7 of suspension culture showed partial erythroid differentiation in RA/RARS (75%) and RAEB/RAEB-T (66%) reflected by variable expression of CD36 and Glycophorin-A expression by normoblasts. In cases with erythroid colony formation 69%±24 of the cells were CD34$^-$/CD36$^+$ whereas cases with <10 BFU-E 10^4 CD34$^+$/CD36$^-$ cells 18%±16 of the cells were CD34$^-$/CD36$^+$. Iron staining showed the presence of ring sideroblasts in 2 cases with RARS indicating that the cells originate from the abnormal erythroid clone. Finally it was shown that cases with an impaired proliferative response demonstrate an enhanced binding of Annexin-V on CD34$^+$ cells during the first days of suspension culture. These results suggest that a defect in the proliferative response is most pronouncedly expressed in MDS whereas a subpopulation of cells retain the capacity to differentiate.

P-1360 CHARACTERIZATION OF THE ERYTHROPOIESIS IN MYELODYSPLASIA BY MEANS OF FERROKINETIC STUDIES, IN VITRO ERYTHROID COLONY FORMATION AND SOLUBLE TRANSFERRIN RECEPTOR

S.J.L. Brada, J.Th.M. De Wolf, D. Hendriks, H. Louwes, E. Van den Berg, E. Vellenga. *Department of Hematology, University Hospital, Department of Nuclear Medicine, Martini Hospital and Department of Medical Genetics, University of Groningen, Groningen, The Netherlands*

In refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) a discrepancy is observed between the decreased in vitro erythroid colony formation and the normal or increased number of normoblasts in the bone marrow. In vivo and in vitro erythropoiesis was studied by erythron transferrin uptake (ETU), soluble transferrin receptor (sTfR) and erythroid in vitro colony formation in 24 patients with RA and 5 patients with RARS. The results were correlated with bone marrow morphology and transfusion dependency. Increased (mean: 124.9, range 74–225 umol/l blood/d) and normal (mean: 60.6 range 50–71) ETU values were observed in 51% and 28% of the cases, whereas 21% of the cases demonstrated a diminished ETU-value (mean: 35.8 range 28–46), which correlated significantly with sTfR in cases with RA ($p < 0.05$, $r = 0.64$). A significant difference in ETU values was observed between RA (mean: 77.6, range 28–189) and RARS (mean: 144.0 range 59–225, $p < 0.05$). Most of the cases (73%) with increased ETU values showed an augmented percentage of erythroblasts in the bone marrow, which was inversely related with the serum Epo levels ($p < 0.05$, $r = 0.51$). However no correlation was found between the ETU values and the in vitro erythroid colony formation. Transfusion dependency was associated with normal to increased ETU-levels ($p < 0.05$) and cytogenetic abnormalities ($p < 0.05$). These observations demonstrate that normal increased ETU levels and the presence of cytogenetic abnormalities differentiates between cases of RA with ineffective erythropoiesis associated with regular transfusions and cases who are relatively transfusion independent.

P-1361 PROGNOSTIC VALUE OF CELL CULTURES IN 100 CASES OF MYELODYSPLASTIC SYNDROMES

M.C. Del Cañizo, A. Mota[1], M.I. Malheiro[2], E. Matos[2], J. Galende[3], S. Gil[4], F. Ortuño[5], J.F. San Miguel. *Hospital Universitáno de Salamanca; [3]Hospital Clínico Ponferrada; [4]Hospital Clinico Léon; [5]Hospital Clinico Murcia, Spain; [1]Hospital Santo António, Porto; [2]Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal*

Myelodysplastic syndromes (MDS) are a group of disorders characterized by dyshematopoiesis in bone marrow and peripheral blood cytopenias. The aim of present work was to analyze the prognostic value of *in vitro* growth in patients with MDS. A total of 31 females and 69 males, median age of 68, with diagnosis of RA in 34, RARS 7, RAEB 24, RAEBt 8 and CMML 27 were included. CFU-GM assay was performed by plating 1×10^5 mononuclear cells/ml in IMDM and 0.9% methylcellulose containing 10% PHA-LCM. Cultures were incubated at 37°C in a fully humidified atmosphere with 5% CO2 and scored at day 14. Three patterns of *in vitro* growth based on the number of CFU-GM in normal bone marrow were defined: 1 = normal (normal or elevated number of CFU-GM and cluster: colony ratio <2); 2 = hypoplastic (low number of CFU-GM and cluster: colony ratio <2); 3 = anomalous (cluster: colony ratio >2). The survival time of the patients correlated with the *in vitro* growth pattern ($p = 0.04$). The median survival time was $\gg$22 months for patients with an hypoplastic growth, 18 months for those with an anomalous one and 6 months for the normal pattern. The poorest prognosis was associated with a high number of CFU-GM ($p = 0.04$) and a cluster: colony ratio $\gg$2 ($p = 0.06$). The univariate analysis showed that FAB classification ($p = 0.003$), percentage of BM blasts ($p = 0.005$), chromosomal aberrations ($p = 0.002$), number of cytopenias ($p = 0.001$) and age ($p = 0.02$) were comparable with previously published results, as major variables predictive of outcome for survival. Using multivariate analysis, the most important variable for determining outcome were age and chromosomal aberrations ($p = 0.02$). We found no relation between FAB classification, percentage of BM blasts, chromosomal aberrations, number of cytopenias and the growth pattern. A relation was found between age (>60 years) and a CFU-GM >202 ($p = 0.05$). Our findings sugest that proliferative capacity may constitute an additional parameter with predictive value for outcome in patients with MDS.

P-1362 DEFECTIVE MYELOPOIESIS IN MYELODYSPLASTIC SYNDROMES (MDS) CAN BE PARTIALLY RESTORED IN VITRO BY N-ACETYLCYSTEINE AND ALL-*TRANS* RETINOIC ACID

A. Cortelezzi, B. Sarina, C. Cattaneo, S. Cristiani, I. Silvestris, M. Motta, M. Garatti[1], A.T. Maiolo. *Servizio Autonomo di Ematologia, IRCCS Osp. Maggiore Polichnico; [1]Istituti Clinici di Perfezionamento, Centro Trasfusionale ed Immunoematologia, Milano, Italy*

Peripheral cytopenia, together with bone marrow (BM) showing increased or normal cellularity, is a common finding in Myelodysplastic Syndromes (MDS). In this heterogeneous group of clonal disorders, haemopoietic progenitors seem to be unable to mature and differentiate and greater apoptosis in MDS BM has been demonstrated. We therefore studied the effect on BM MDS progenitor cells of an apoptosis inhibitor (N-acetyleysteine, NAC) and a differentiating agent (All-*Trans* Retinoic Acid, ATRA) alone and in association.

Methods: BM cells from sixteen MDS patients (FAB) were collected in preservative-free heparin and separated by means of density gradient centrifugation. The mononuclear cells were resuspended in IMDM and FCS 10% at a concentration of 1×10^6 cells/ml and then tested for clonogenic activity in terms of CFU-GM (Agar colony assay) and apoptosis (dUTP/tdt cytofluorimetric assay) after 24 h of incubation with and without NAC 0.5 U/ml. ATRA 5 μM was then added to an aliquot of the cells resuspended in liquid cultures with and without NAC and tested again for CFU-GM and apoptosis after 48 h.

Results: Apoptosis was significantly less ($p < 0.05$) in the BM mononuclear cells incubated with NAC for 24 h than in the control sample (3.04±2.3ES vs 7.58±3.26), and the number of CFU-GM significantly ($p < 0.05$) increased (60.3±19.5 vs 45.8±18.1). In the cells of ten patients evaluated after 48 h, ATRA alone and in association with NAC induced a marked and significant ($p < 0.05$) increase in the number of CFU-GM in comparison with the control sample (99±33.6 and 119.7±47.9 vs 79.8±39.3). Apoptosis seemed to be reduced after incubation with NAC+ATRA, but not after ATRA alone; the difference from the control sample was not significant, possibly due to the small number of tested samples.

Conclusions: Our study shows that both NAC and ATRA are capable of restoring defective myelopoiesis in vitro, possibly by acting on two of the pathways affected by the myelodysplastic defect: apoptosis (NAC) and differentiation (ATRA). Their action seems to be particularly efficacious when used together, thus confirming the multifattorial pathogenesis of MDS.

P-1363 SPECIFIC PROGNOSTIC SCORE FOR REFRACTORY ANEMIAS WITH EXCESS OF BLAST (RAEB)

S. Chevret, O. Taieb, Y. Najean, C. Chomienne. *For the French Group for Myelodysplastic syndromes; Hôpital Saint-Louis, 1 avenue Claude Vellefaux 75010 Paris, France*

Myelodysplastic syndromes which constitute a group of various hematological disorders are characterized by a high prognostic heterogeneity.

Scoring systems have allowed to identify different prognostic subgroups amongst MDS patients. Taking advantage of three homogenous cohorts of RAEB patients (n = 101, n = 115 and n = 113) entered in a low dose (3 mg/m2) ara-c trials which revealed low participation and no benefit on survival, we performed a prognosis analysis based on Cox modelling. On the first cohort of n = 101 patients we individualized prognostic factors at diagnosis: presence of fever or hemorrhages (p = 0.0002), platelet count <100. 109/L (p = 0 0002), presence of circulating blasts).

A prognostic classification was thus proposed which distinguishes 4 different sub-groups of patients related to the number of their risk factors, from 0 (median survival of 24 months) to 3 (median survival of 1 month) ($p < 0.0001$).

This classification was applied to two other prospective studies of n = 115 and n = 113 RAEB patients and its prognostic value validated ($p < 0.0001$ in both cohorts/log rank test.

P-1364 THE CLINICOPATHOLOGIC FEATURES OF MYELODYSPLASTIC SYNDROMES IN PATIENTS YOUNGER THAN 40 YEARS

T. Intragumtornchai, W. Prayoonwiwat, D. Swasdikul, N. Suwanwela, B. Chaimongkol, S. Jootar, K. Chansung, S. Chancharunee, A. Leelasiri, Y. Yoshida. *Division of Hematology, Department of Medicine, Chulalongkorn University, Pramongkutkloa Hospital, Rajvidhi Hospital, Chiangmai University, Mahidol University, Konkaen University, Thailand; Kyoto University, Japan*

Myelodysplastic syndromes (MDS) are a group of clonal hemopathy affected mainly the elderly population. The median age of the patients reported from most studies conducted among the Western population is 70 years. Recently a nationwide study had been conducted on patients newly diagnosed as MDS during 1992-1996 in the five major medical centers in various geographic region of Thailand.[1] All specimens of the patients were independently examined by the central reviewers (WP, DS, NS and YY) and were classified according to the FAB criteria. There were a total of 117 patients. The median age of the patients was 56 years (range, 16–86). Interestingly, 32% of the patients were younger than 40 years. The clinicopathologic features of these younger patients, as compared to the older patients, were as the followings:

	Age <40 yrs	Age ≥40 years	P-value
No.	37	80	
% male	59	46	0.30
% RA/RARS	59	53	0.71
% RAEB, RAEB-T	41	33	0.58
% CMML	0	14	0.03
Mean hb (g/dl)	6.0±2.1	7.4±2.3	0.005
Mean WBC ($\times 10^9$/l)	5.4±4.1	10.6±17.4	0.09
% with thrombocytopenia	86	74	0.21
% with BM blasts ≥5%	41	43	0.84
Median survival (months)	16.5	24.1	1.00
% 5-year survival	29	28	0.98
% transformation to AML	16.0	16.0	0.90
% with karyotypic aberration	43	52	1.00

The clinical feature as well as the outcome of MDS in the young patients are therefore generally similar to the older cases. The major difference is CMML which is predominantly a disease of the elderly and is very rare among those who are younger than 40 years old.

(1) Leukemia Research, accepted for publication

P-1365 REFRACTORY ANAEMIAS WITH EXCESS OF BLAST: HETEROGENEITY OF EVOLUTION AND PROGNOSIS FACTORS

N. Munteanu, T. Puscariu, C. Ursea, D. Colita. *Clinic of Haematology, Hospital Fundeni, Bucharest, Romania*

A number of 87 patients with refractory anaemia with excess of blasts (RAEB) have been studied by usual morpho-cytochemistry, immunophenotyping, CFU-GM assay, cytogenetics and a multivariate regression statistical analysis with a scoring system. Their median age was 62 years (20–84), 64% males, 36% females. Their evolution was heterogeneous regarding survival, morphologic picture and therapy response. A subgroup of 20% had a long survival over 3 years (5% over 5 years) having as main features a silent and stable abnormal clone with a low persistent percent of marrow blasts (6–10%), erythroblasts under 50%, absence of genetic anomalies and normal CFU-GM. Transition to acute leukemia (AL) was 25% and they needed a mild therapy. Another subgroup (80%) had a short survival of 14 months with 52% AL transformation. They had a rapidly evolutive clone with a high percent of blasts (15–20%), over 50% erythroblasts, trilineage involvment, chromosomes anomalies and a low or absent CFU-GM. In this subgroup, 10% fulfilled the criteria for M6; their course was similar to RAEB cases. They needed an intensive therapy. Survival was statistically influenced by the initial platelet count, the proportion of blast cell in marrow and age; it was confirmed by a score system. Leukemic transformation was connected to marrow blast - cell percent, a reduced marrow proliferative activity, a low CFU-GM and presence of clonal abnormal genetic anomalies. Identifying these subgroups has a practical importance both for prognosis and for establishing the therapeutic programms.

P-1366 MYELODYSPLASTIC SYNDROMES ASSOCIATED WITH IMMUNE THROMBOCYTOPENIA

C. Fernández-Lago, E. Romero. *Departement of Hematology, Juan Canalejo Hospital, La Coruña, Spain*

Introduction: In patients with myelodysplastic syndromes (MDS) immunological alterations have been reported, in some cases, have been associated with cytopenias of autoimmune origin, primarily in the area of the erythroid cells. Alterations in the platelets have been less studied, however recently

some literature have reported a significant incidence of antiplatelet antibodies of autoimmune origin in patients with MDS and thrombocytopenia suggests the existence of an immunological mechanism similar to the one present in thrombocytopenic purpura of autoimmune origin (PTAI) due to thrombopenia in patients with MDS. This study reports the incidence of antiplatelet antibodies in a group of 50 patients with MDS and the results of a trial steroid treatment in some of the patients with thrombocytopenia.

Materials and Methods: 50 patients were studied regardless of their cytological classification, diagnosis or platelet count at the time of the study. Patients in wich the presence of EDTA-dependent antiplatelet antibodies were detected by means of platelet count in citrated blood were excluded. The antiplatelet antibodies were studied in platelets, serum and elution using flow cytometry and a capture in solid fase method.

A small group of patients (four patients) with severe thrombocytopenia who tested positive for antiplatelet antibodies received steroid treatment

Results: The presence of antiplatelet antibodies was detected in 17 patients represents 34% of the total, corresponds to 100% of the cases with autoimmune antibodies of IgG origin. In general, their presence is associated with the groups who had the worst prognosis for FAB clasification. In every case, the presence of antibodies was related to thrombocytopenia.

In four of the patients with thrombocytopenia and positive antibodies who were treated with steroids a rapid recovery of platelet count was observed.

Conclusion: 1. Antiplatelet antibodies are relatively frequent in MDS with a negative prognosis. 2. Their presence is usually associated with thrombocytopenia improves with steroid treatment and does not affect the evolutionary course of the underlying illness. 3. Their origin, is unknown, seems to be associated with an autoimmune mechanism somehow linked to the evolutionary progress of the illness.

P-1367 SERUM LEVELS OF CYTOKINES WITH HEMATOPOIETIC ACTIVITY IN MYELODYSPLASTIC SYNDROMES (MDS)

P. Musto, G. Sanpaolo, G. D'Arena, R. Matera, M. Minervivi, M. Carotenuto. *Hematology, IRCCS "Casa Sollievo della Sofferenza", S. Giovanni Rotondo, Italy*

Several cytokines mediate proliferation, differentiation and apoptosis of hematopoietic cells. All these processes may be disrupted in MDS. In addition, treatment with growth factors, as single agents or in combination, represent a novel, but still not completely defined therapeutic approach for such patients. We determined serum levels of hematopoietic cytokines at diagnosis in 93 patients with MDS (45 RA, 15 RARS, 18 RAEB, 7 RAEB-T and 8 CMML) using ELISA assays. Overall, median values of EPO (415 +/- 104 vs 15 +/- 5 miu/ml, $p < 0.001$), G-CSF (298 +/- 154 vs 10 +/- 4 pg/ml, $p < 0.001$), GM-CSF (50 +/- 12 vs 2 +/- 0.2 pg/ml, $p < 0.02$), TNF (37 +/- 8 vs 10 +/- 3 pg/ml, $p < 0.05$), IL-1 (210 +/- 115 vs 40 +/- 6 pg/ml, $p < 0.04$), IL-8 (3200 +/- 1250 vs 50 +/- 17 pg/ml, $p < 0.0001$), and M-CSF (1010 +/- 420 vs 200 +/- 20 pg/ml, $p < 0.0001$) were significantly higher in MDS patients than in 30 normal subjects. On the contrary, serum levels of SCF were found to be lower (850 +/- 210 vs 1510 +/- 320 pg/ml, $p < 0.03$) with respect to controls, while there were no significant differences between patients and controls as far as IL-3 (27 +/- 12 vs 10 +/- 2 pg/ml) and IL-6 (79 +/- 12 vs 35 +/- 4 pg/ml) were concerned.. EPO and Hb levels evidenced an inverse reciprocal correlation, whereas a direct relationship was only found between TNF and WBC count and between IL-3 and PLT count, respectively. In comparison to other FAB subgroups, G-CSF levels were lower in RA, GM-CSF lower in RAEB-T and higher in RA, SCF lower in RARS, IL-6 and IL-3 higher in RA, IL-1 lower in RAEB-T and higher in RARS, EPO lower in RAEB-T and CMML, IL-8 higher in RAEB-T and TNF higher in CMML. Despite high levels of circulating G-CSF and GM-CSF, all patients treated in vivo with these cytokines showed a significant increase in WBC count, while no improvement of Hb was observed in patients with increased levels of TNF and IL-1 treated with EPO. Our study indicate that in MDS: a) serum levels of cytokines with "late" activity on hematopoiesis are increased, but probably unadequate for the degree of cytopenia, with the only exception of EPO; b) feed-back mechanisms of "early" growth factors appear to be even more compromised, at least in quantitative terms; c) high levels of TNF and IL-1 may exert inhibitory effects on erythropoiesis; d) leukemic evolution determines a further worsening of cytokine production.

P-1368 INTERLEUKINS, TNF-a, AND B_2-MICROGLOBULIN VARIATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

L. Christou, S.N. Tsiara, H. Kapsali, G. Alexandridis, K. Katsanos, P. Kaiafas, K.L. Bourantas. *Hematology Unit, University of Ioannina Medical School, Greece*

Patients with Myelodysplastic Syndromes (MDS), appear a great variability in their natural history and evolution that seems to be independent of the treatment modalities, so it is difficult to determine which of the patients with (MDS) will be evoluted to acute leukemia as well as the time of transformation.

The aim of the study was to define if cytokines consist a signifficant prognostic factor for patients with MDS indicating the time of transformation to acute leukemia. We determined the levels of IL-1a, IL-1b, sIL-2r, IL-6, IL-10, TNF-a, b_2-Microglobulin and (b_2-M) initially and during the course of the disease.

Material: We determined the levels of these cytokines in the serum of 20 pts with MDS. Seven were female and 13 were male. Their ages ranged between 50–78 years. Seven had RA, 4 RARS, 4 RAEB, 2 RAEBt and 3 CMML The determination of cytokines was done by an ELISA method.

Results: The levels of IL-1a, IL-1b, were within the normal range initially and during the course of the disease. The levels of TNFa and IL-6 were slightly increased at diagnosis in all patients. They were dramatically increased during the time of transformation to acute leukemia especially in the patients with CMML The levels of IL-2 and sIL-2r and b_2-M were also increased in all patients who transformed to acute leukemia regardless the subgroup they belonged. The levels of IL-10 were moderately increased in all groups of pts.

In conclusion the increased levels of the b_2-M, sIL-2r and IL-2 during the course of the disease in patients with MDS indicate bad prognosis and sooner evolution to acute leukemia. A raise of the levels of IL-6 and TNF-a in pts with CMML indicate transformation to acute leukemia.

MDS: treatment

P-1369 LONG-TERM COMPLETE REMISSIONS IN PATIENTS WITH LOW-RISK MYELODYSPLASTIC SYNDROMES DURING COMBINATION TREATMENT USING ALL-TRANS RETINOIC ACID

W.K. Hofmann, A. Ganser, O.G. Ottmann, G. Seipelt, C. Zander, J.Th. Fischer, K. Höffken, K. Hoffmann, D. Hoelzer. *Department of Hematology, Johann Wolfgang Goethe University Hospital, Theodor-Stern-Kai 7, 60596 Frankfurt/Main, Germany*

ATRA, G-CSF or IFNα used as single agents in earlier trials have shown in low-risk MDS (bone marrow blasts <5%) response rates varying from 15% up to 25%. The synergistic effect of a combination treatment of these three agents on hemoglobin (Hb), platelet and absolute neutrophil counts (ANC) as well as transfusion frequency was examined in this prospective multicentre study in 17 patients with low-risk MDS (11 RA, 4 RARS, 2 CMML). The median age was 61 years (44–81), male/female ratio was 9/8. ATRA (generously provided by Hoffmann-LaRoche, Basel, Switzerland) was given at 25 mg/m^2/day PO bimontly, IFNα at 1.5 MIU twice a week SC for continuously 52 weeks. G-CSF was administered at 100–480 µg/day SC daily with dose modifications according to ANC. The duration of therapy was scheduled for 12 months. Two patients achieved complete remissions (CR) with normalisation of peripheral blood values, one pt. with RA after 3 months and one pt. with CMML after 7 months of treatment initiation. Both pts. are in continuous CR (+24 mo. and +21 mo.). In three pts. with RA/RARS partial remissions (PR) with an increase of Hb (2 pts.) and a loss of transfusion dependency (1 pt.) were observed. In a pt. with RA and 5q- syndrome the platelet count raised from 89,000/µl to 293,000/µl after ATRA/IFNα. An additional patient with RA achieved an ongoing CR (+31 mo.) in the former study with ATRA, G-CSF and Erythropoietin (Ganser et al, Ann Hematol 1996; 72: 237–244). All the CR-patients are still on treatment without any side effects. We conclude that the combination treatment using ATRA and hematopoietic growth factors results in a response rate of 35% in low-risk MDS patients. Single long term complete remissions can be achieved in these patients.

P-1370 LOW DOSE CYTOSINE ARABINOSIDE AND ALL-*TRANS* RETINOIC ACID FOR THE TREATMENT OF SECONDARY ACUTE MYELOID LEUKAEMIA AND HIGH RISK MYELODYSPLASTIC SYNDROME

A. Venditti, A. Tamburini, F. Buccisano, G. Del Poeta, G. Aronica, A. Bruno, L. Maurillo, M. Masi, S. Amadori. *Hematology, University "Tor Vergata", St. Eugenio Hospital, Rome, Italy*

Seven patients (pts) affected by secondary acute myeloid leukaemia (sAML) and 11 by high risk myelodysplastic syndrome (5 RAEB, 2 CMMoL, 4 RAEB-t) were treated with subcutaneous low dose cytosine arabinoside (LDARAc) (20 mg twice daily for 10 days) and all-*trans* retinoic acid (ATRA) (45 mg/m^2/day for 10 days). Courses were repeated at 4-weeks interval until evidence of disease progression. The male/female ratio was 15/3, the median age was 68 yrs (range 24–79); all pts were not eligible for standard intensive therapy because of age, general conditions or concomitant medical disorders. The 24 yrs old pt was recruited while experiencing his third relapse after intensive chemotherapy and autologous BMT. Fifteen pts are currently evaluable for response to therapy. Overall, 8 pts (62%) achieved a response (6 CR and 2 PR). Among complete responders, 3 pts had a sAML, 1 CMMoL, 1 RAEB and 1 RAEB-t; 1 pt with CMMoL and another one with RAEB were partial responders. A median number of 3 cycles was required to enter a response. Five of 8 responders relapsed (response duration 20, 26, 28, 34 and 47 wks, respectively) and died of progressive disease; 3 pts are alive in remission (2 CR and 1 PR) (response duration +13, +4, +4 wks, respectively). Median duration of survival was 12 wks (range 3–68), while median duration of response (complete + partial) was 30 wks (range 12–47). Main toxicity consisted of universal myelosuppression and infections, documented in 12 pts and requiring hospitalization in 7. In conclusion, this regimen appears to be effective in inducing remission in a significant proportion of pts with high risk myeloid malignancies and warrants further investigations to better define its real efficacy. Addition of hematopoietic growth factors might be considered to ameliorate myelosuppression and reduce infectious episodes.

P-1371 COMPLETE REMISSION OF RAEB AND RAEBT WITH CONTINUOUS CYTOSINE ARABINOSIDE PLUS INTERFERON AND MITOXANTRONE

W.R. Bezwoda. *Haematology/Oncology, University of Witwatersrand, Johannesburg, South Africa*

Seventeen patients <60 years old with MDS with abnormal blast populations have been treated with an intensive chemotherapy regimen consisting of cytosine arabinoside (100 mg/m^2) plus interferon $\propto$ 2b (10 mu/m^2) by continuous IV infusion for 10 days plus bolus doses of mitoxantrone 12 mg/m^2 IV daily × 3 days (days 8, 9 & 10). 12/7 patients were classified as RAEBT and 5/7 as RAEB by the FAB criteria. All patients presented with peripheral cytopenias of variable duration (range 2 months–14 months). Cytogenetic abnormalities frequently encountered in MDS were detectable in 11 patients prior to therapy. Three patients suffered from chemotherapy related MDS. Treatment was given prior to overt leukemic transformation.

16/17 patients completed at least one induction cycle. All 17 patients were considered evaluable. The CR rate was 14/17 (82%). There was one treatment related death. Complete remission was achieved in all 11 patients who had cytogenetic changes at initial evaluation. All 11 CR's were cytogenetic CR's.

Eight patients have gone on to receive high dose chemotherapy (HDC; melphalan + VP) with autologous PSC. Hematologic recovery was not delayed in patients undergoing HDC. The median duration of remission and median duration of survival was not significantly different in patients who achieved induction plus consolidation chemotherapy alone or those undergoing autotransplantation. Response duration median 14 months. Survival duration - median 18 months in both instances. There were also no survival differences according to the presence or absence of cytogenetic changes.

MDS with abnormal blast populations is a clonal malignant disease of the marrow with residual normal stem cell populations which can be successfully treated by chemotherapy including HDC plus autologous transplantation.

P-1372 CORTICOSTEROID AND IMMUNOSUPPRESSANT THERAPY IN MYELODYSPLASTIC SYNDROMES: STUDY OF 7 PATIENTS

C. Finelli, G. Visani, P. Tosi, N. Testoni, R. Pastano, A.M. Mianulli, E. Ottaviani, A. Pellacani, M. Barassi, S. Tura. *Institute of Haematology and Clinical Oncology "Seràgnoli", Bologna University, Bologna, Italy*

From 1975, we observed in our Institution 7 pts (5 females, 2 males, median age 49, range 13–67 yrs), with otherwise typical low-risk myelodysplastic syndromes (MDS) (refractory anemia, following FAB classification), who showed a partial or complete response to a corticosteroid and/or immunosuppressant treatment. All the pts showed severe dyserythropoietic anemia, with moderate neutropenia in 2 cases. In 2 subjects a 2nd degree spleen enlargement was subsequently detected, during the follow-up. In 4/7 pts the bone marrow was hypercellular, while the other 3 pts showed a normal or decreased cellularity. In 3 cases the bone marrow aspirate or biopsy revealed a moderate lymphoid infiltration. Karyotype was abnormal (5q−) in only 1/7 pt. No ringed sideroblasts were observed. In vitro growth of myeloid or erythroid progenitors was impaired in only 1/4 subject.

5/6 pts responded to corticosteroid treatment (0.5–2 mg/Kg/die of methylprednisolone, MP) after 2–4 weeks; in 2/3 cases the remission was maintained with cyclosporine (CS-A), while in 1 pt a 2nd complete remission (C.R.), after withdrawal of MP, was achieved after 3 courses of cyclophosphamide. 1 pt, with 2nd degree splenomegaly, reached C.R. after splenectomy, and the sole pt refractory to MP showed a partial response to CS-A associated with recombinant human erythropoietin.

P-1373 LOW RESPONSE RATE TO A CONTINUOUS SCHEDULE OF AMIFOSTINE FOR LOW RISK MYELODYSPLASTIC PATIENTS

D.T. Bowen, K. Gelly, M. Hepburn, L. Kanz, W. Brugger, C. Denzlinger, D. Culligan. *Ninewells Hospital, Dundee, Scotland*

The therapeutic goal in most patients with myelodysplasia (MDS) is an improvement in cytopenias, particularly to reduce red cell transfusion requirements. Preliminary studies have suggested trilineage responses to intravenous administration of the synthetic aminothiol Amifostine on an intermittent schedule. Our previous in vitro demonstration of oxidative stress in MDS CD34+ cells provides a rationale for the use of antioxidants in MDS therapy. We treated 11 patients on a continuous schedule, administering Amifostine thrice weekly for a total of 8 weeks. Initial doses were 300 mg/m^2 for 4 weeks, escalated in non-responders to 450 mg/m^2. Patients included 5 Refractory Anaemia, 2 Sideroblastic Anaemia, 1 Chronic Myelomonocytic Leukaemia and 3 Refractory Anaemia with excess blasts. IPSS scores were: Low = 4, INT-1 = 6 and High = 1. Median age was 62 years. Median pre-treatment Hb was 9.1 g/dl (range 5.9–14.2), WBC 3.0 × 10^9/l (2–11.9) and platelets 193 × 10^9/l (17–474). Seven patients were red cell transfusion dependent. No partial or complete haematological responses were seen on treatment. One patient showed an increase in pre-transfusion Hb of 2 g/dl which developed 6 weeks post-treatment and has been maintained for 8 months. No other treatment was given during this time. A second patient had a small platelet increment with a clinically useful improvement in bleeding. Side effects included nausea (7), mild hypotension (2), pruritis (2) and sneezing (2). 5HT3 antagonist antiemetics were necessary in 8 patients and 4 patients required addition of Dexamethasone. 2 patients withdrew early, 1 with urinary tract sepsis and 1 with AML transformation (from RAEB). In 2 non-responding patients studied, in vitro blood progenitor growth was significantly stimulated by pre-incubation with Amifostine at 0.1 and 1 μM concentration (BFU-E in 1/2 and CFU-GM in 2/2). In conclusion, in our 11 MDS patients, a continuous Amifostine schedule was well tolerated but the response rate to this novel therapy was low.

P-1374 PRIMARY AND SECONDARY MYELODYSPLASTIC SYNDROMES IN CHILDHOOD

J.P. Panagiotou, S. Polychronopoulou, A. Parcharidou, V. Papadakis, D. Anagnostou[1], S. Haidas. *Dept. of Paediatric Haematology-Oncology "Aghia Sophia" Children's Hospital; [1]Dept. of Haematopathology-Evangelismos Hospital, Athens, Hellas*

Myelodysplastic syndromes (MDS) comprise a heterogenous group of clonal stem cell disorders representing less than 1% of all hematologic malignancies in childhood. Sixteen cases of primary and 4 cases of secondary MDS, 11 girls and 9 boys, aged 2 months to 14 years (mean age: 8 years), were referred to our Department during the last 8 years. Diagnosis was based on peripheral blood and bone marrow morphology, cytogenetic analysis and trephine biopsy findings. According to cytomorphologic criteria modified by Passmore et al, our patients were classified as follows: 1 RA, 2 RARS, 2 RAEB, 6 RAEB-t, 3 JCML, 3 unclassified, 2 hypoplastic, 1 combined with congenital anomaly RAEB according to FAB. Cytogenetic analysis of BM cells revealed: 4 normal karyotypes, 6 monosomy 7, 2 trisomy 8, 1 5q$^-$, 3 hypodiploid, 1 aneuploid and 3 samples failed to give metaphases. Therapeutic intervention, consisting of supportive treatment, and combinations of hemopoietic growth

factors, immunosuppressive treatment, AML type chemotherapy or allogeneic BMT, depended on the existence or not of a related compatible donor and the biologic behaviour of the MDS. Patients' characteristics, treatment and outcome are presented in the following table:

	Treatment	Outcome
RA (1)	EPO	A
RARS (2)	Splenectomy • cyclo	A
	None	A
RAEB (2)	GFs + splenectomy • BMT	D
	BMT	A
RAEB-t (3)	GFs + chemo	D
(primary)	Chemo	D
	BMT	A
RAEB-t (3)	Chemo	D
(secondary)	Chemo	D
	Chemo	D
Unclassified (3)	None	3A
Constitutional (1)	None	A
Hypoplastic (primary) (1)	BMT	D
Hypoplastic (secondary) (1)	BMT	A
JCML (3)	Chemo	3D

A = alive D = dead GFs = growth factors.

Our data are in agreement with the latest literature on Paediatric MDS: increased incidence of cytogenetic abnormalities, prevelance of aggressive subtypes, with high frequency of hypoplasia. Paediatric MDS represent unique entities, deserving their own classification, elucidation of their pathophysiology and possibly, different therapeutic approaches.

P-1375 OBSERVATIONS ON THE DIFFERENTIATION BETWEEN REFRACTORY ANEMIA AND CHRONIC APLASTIC ANEMIA BY TRIAD THERAPY

Zhang Wanggang, Cao Xingmei, Chen Yinxia, Zhang Hui, Liu Jie, Wu Wenman, Mao lijun. *Department of Haematology, The Second Affilliated Hospital of Xi'an Medical University, No 36 west 5th avenue, Xi'an 710004, Shaanxi Province, PR China*

Objectives: Presently the refractory anemia of some myelodysplastic syndromes (MDS – RA) usually has to be differentiated from chronic aplastic anemia (CAA) in clinic. We tried to approach a method of therapeutic differentiation by applying all – trans Relinoic Acid (ATRA), 1.25 dihydroxyvitamin D3 (1.25 – D3) and androgen to the therapy.

Methods: 22 patients of MDS – RA and 10 of CAA were treated by ATRA 60 mg/d, 1.25 – D3 0. 5 ug/d and danazole 0. 4 g/d.

Results: After 1 month of therapy, it had taken effect over 21 patients of MDS – RA, the total rate of efficacy was 21/22, and 3 of them attained complete remission whose cells of three lineages of peripheral blood became normal although the pathological hematopoiesis still existed in bone marrow. During the process of continual administration, the general status of patients were good, mostly they did not need transfusion and none had turned to leukemia. In contrast, 3 patients with CAA had to quit ATRA after 15 days' therapy for the notable decrease of the platelets of the peripheral blood, and the exacebation of the mucocutaneous hemarrhage. Although the remaining 7 by then showed recovery of the peripheral blood to some extent, which was faster than that of those treated only by androgen. All these 7 patients with CAA also had to quit ATRA and 1.25 – D3 after 30 days of triad therapy for the same reason. Conclusion. The results of our research showed that the combinative administration of ATRA, 1.25 – D3 and androgen had obvious effects over MDS – RA, however, poor effects of CAA, which suggests that the pathogenesis and pathologic changes of the two diseases are different essentially, as for MDS – RA, which are the disorders of the stem cell of hematopoiesis, while for CAA, which are the decrease of the amount of the stem cells. This triad therapy can differentiate the MDS – RA from CAA excellently.

Myeloma: biology

P-1376 CD44-9v EXPRESSION REPRESENTS A NEW INDEPENDENT PROGNOSTIC PARAMETER IN MULTIPLE MYELOMA

R. Stauder[1], O. Bechter[1], M. Van Driel[2], J. Thaler[1], A.C. Bloem[2], U. Günthert[3], W. Eisterer[1]. [1]*Dept. of Internal Medicine, University Innsbruck, Austria;* [2]*Dept. of Immmunology, University Hospital, Utrecht, The Netherlands;* [3]*Basel Institute for Immunology, Basel, Switzerland*

Overexpression of CD44 variant isoforms (CD44v) has been associated with an advanced disease and an unfavourable clinical course in Non-Hodgkin's lymphoma and in acute myeloid leukaemia. To evaluate the role of CD44 in multiple myeloma (MM), CD44v expression was analyzed by immunohistochemical staining of bone marrow biopsies from 10 normal persons, 11 patients with monoclonal gammopathy of undetermined significance (MGUS), four cases of smoldering myeloma (SM) and of 103 samples from 57 consecutive MM patients. Expression of variant isoforms containing the 3v, 4v or 6v domain was observed in 13, 7, 11 and 9% of cases of MM but could not be detected in normal persons, MGUS or SM. 9v expression was observed in a minor subpopulation of plasma cells in three out of then normal persons, in three out of 11 MGUS, was negative in SM and was observed in 32% of cases of MM. In MM 9v expression was associated with an advanced stage as defined by Durie and Salmon ($p < .03$), a low hemoglobin value ($p < 0.002$), a progressive disease as defined by Durie ($p < .004$) and a shorter overall survival ($p < .001$). Moreover CD44-9v emerged as an independent prognostic factor in multivariate analysis (Stage: relative risk 1.36, $p < 0.02$; v9 expression: relative risk 1.45, $p < 0.03$). These results substantiate the importance of CD44-9v as a prognostic parameter in MM and suggest a distinct role of CD44v domains in the regulation of adhesion to bone marrow stromal cells, which might influence IL-6 related autocrine and paracrine growth.

The Basel Institute for Immunology was founded and is supported by F. Hoffmann-La Roche Ltd., Basel.

P-1377 PROGNOSTIC SIGNIFICANCE OF URINARY COLLAGEN PYRIDINIUM CROSSLINKS IN MULTIPLE MYELOMA

I. Špiška, P. Špašek, P. Cieslar, B. Procházka, H. Hulejová, M. Chrz, V. Válková, P. Klener. *I. Department of Medicine, Charles University Hospital, Prague, Czech Rep*

Clear criteria to distinguish low and poor-risk myeloma patients are necessary for choosing appropriate therapeutical approach. Current prognostic factors are not sufficient to select patients who may be candidates for early intensive treatment. Bone involvement is a characteristic feature of multiple myeloma (MM) and close relationship between cytokines involved in pathogenesis of both underlying disease and bone destruction is suggested. Bone turnover could be assessed by measuring the urinary excretion of bone collagen breakdown products as pyridinolin (PYR) and deoxypyridinolin (DPYR). The value of collagen degradation products for discrimination of MM from MGUS was repeatedly observed. We investigated the significance of these markers for prediction of overall survival (OS) and event-free survival (EFS) in our group of patients with MM.

The prognostic value of various parameters was evaluated in 152 patients referred to our department from 1990–1997 with diagnosis MM (n = 120), MGUS (n = 29), EMMP (n = 2) and AL amyloidosis (n = 1). PYR, DPYR, osteocalcin (OC) and some cytokines were evaluated at the time of diagnosis and 1, 3, 6 and 12 months after diagnosis. HPLC of prepared hydrolysates was used for analysing the urinary levels of PYR and DPYR. The concentration was expressed as a ratio of the urinary creatinine concentration. Serum osteocalcin (OC) was measured using RIA method. The results of 76 patients with MM and 24 with MGUS were available for bone turnover markers analysis. According to Pearson's test the levels of PYR and DPYR correlated significantly ($p < 0.0001$). Significant difference of urine PYR and DPYR was found in MM vs. MGUS ($p < 0.001$ and $p = 0.01$, respectively). In myeloma patients the correlation between PYR and DPYR urine excretion and clinical stage was observed (III vs.I and II, $p < 0.01$). PYR was statistically significant univariate prognostic factor for OS ($p = 0.03$). However, DPYR and OC for OS and all three bone turnover markers for EFS remained insignificant. The decrease of PYR (from pathological values to normal range or by more than 50% of initial values) 12 months after diagnosis was favourable prognostic variable for OS. In contrast, the decrease after the first month of therapy was associated with the shortest survival. Patients with increase of DPYR above normal range during the course of disease had shorter time to progression ($p = 0.05$). Other statistically significant prognostic variables for OS were initial serum calcium, creatinine, LDH, ALP, Hb, subclassification A/B, B2M (at cutoff of 4 mg/mL), stage of disease (Durie-Salmon classification system). Among cytokines and their soluble receptors measured at the time of diagnosis (IL-6, sIL-6R, TNF-alpha, TGF-beta 1) only sIL-6R was significant for EFS ($p = 0.02$) and a tendency was found for OS ($p = 0.08$).

We conclude that urine PYR and DPYR could be valuable parameters for discrimination between MM and MGUS. Single evaluation was not very useful for prediction of OS and/or EFS of myeloma patients. Serial measurement of bone destruction markers may be used in predicting prognosis of MM.

P-1378 POSSIBLE INVOLVEMENT OF BCL-2/BAX IN THE PATHOGENESIS AND DRUGRESISTANCE OF MULTIPLE MYELOMA

O. De Weerdt, M. Van Vliet, D.J. Roovers, A.C. Bloem[1], H.M. Lokhorst. [1]*Depts. of Hematology and Immunology, University Hospital Utrecht, Netherlands*

The inability of a cell to undergo apoptosis after an appropriate stimulus may be an important characteristic of the malignant cell and represents a specific type of multidrug-resistance. Monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) are related plasma cell disorders. One to two percent of MGUS patients converts every year into MM. MM is well known for its resistance to chemotherapeutics. Bcl-2 (anti-apoptotic) and Bax (pro-apoptotic) are key proteins in the apoptotic pathway of B-cells, including plasma cells. Disturbed expression of these proteins may lead to ineffective apoptosis after a stimulus, e.g. chemotherapeutics. Therefore, we studied the expression of Bcl-2 and Bax in plasma cells (CD 3+++/CD19−) from normal bone marrow (NBM, n = 10), MGUS (n = 10) and MM (n = 20) patients by flow cytometry. Mean fluorescence ratio's (isotype vs. MoAb) were (+SEM)

	NBM	MGUS	MM
Bcl-2	10.9 (2.6)	36.7 (3.3)	33.4 (4.4)
Bax	20.7 (6.7)	19.8 (3.7)	8.6 (0.9)
Bc12/Bax	0.78 (0.13)	2.2 (0.3)	5.6 (1.1)

We conclude that plasma cells in MGUS and MM are characterized by changes in both Bcl-2 and Bax- expression. Upregulation of Bcl-2 is an early oncogenic event, already present in MGUS, but no additional upregulation of Bcl-2 was found in MM. Downregulation of Bax is a late oncogenic event, probably enhanced by chemotherapeutics. High Bcl-2/Bax ratio's may indicate an advanced stage of plasma cell disease and have prognostic significance for therapeutic success. From our results it can be hypothesized that restoration of the Bcl-2/Bax ratio to lower levels, e.g. by antisense gene therapy, increases chemosensitivity in MM.

P-1379 MECHANISMS OF VEROCYTOTOXIN (VT1)-INDUCED APOPTOSIS IN MYELOMA CELLS

I.D. Chant, A. Morris[1], P.E. Rose, S. Basu. *Department of Haematology, Warwick Hospital, Warwick; [1]Department of Molecular Medicine, Warwick University, Coventry, UK*

Verocytotoxin (VT1) is a bacterial toxin, abrogated by certain *E. coli*, implicated in the pathogenesis of haemolytic uraemic syndrome (HUS). The toxin has two subunits; a binding (B) unit which binds to a receptor (Gb3) on the surface of susceptible cells, and an enzymatic (A) moiety which is endocytosed and inhibits protein synthesis at 28S ribosomal subunits. The Gb3 receptor is a recognised B-cell differentiation antigen, and high levels are found on Burkitt's lymphoma cells and a sub-population of germinal centre B lymphocytes. Both cell types are notable for their susceptibility to undergo apoptosis, suggesting that Gb3 may be involved in the transduction of an apoptotic signal. VT1 has in fact been shown to induce apoptosis in B-lymphoma cell lines, e.g Ramos and Daudi, and we have shown that primary. B-CLL cells which express Gb3 undergo apoptosis *in vitro* when exposed to VT1.

We have therefore examined the myeloma cell lines, HS-Sultan, ARH-77, NCI H929, RPMI 8226, U266 B1 and JJN3 for expression of the VT receptor (Gb3). All six cell lines express detectable levels of Gb3 as revealed by flow cytometric analysis. Flow cytometric analysis of propidium iodide-stained cells revealed the appearance of a sub-G1 apoptotic peak in all six cell lines following exposure to recombinant VT1 (2–10 pg/mL). Apoptosis was confirmed and quantified by increased annexin V-FITC staining. ARH77 and RPMI 8226 were particularly susceptible to VT1-induced apoptosis; 47% and 29% apoptotic cells after only 4 hours with 10 pg/mL VT1 respectively). Co-incubation with IL-6, an anti-apoptotic factor in myeloma cells, failed to inhibit cell death. Furthermore, incubation of these cells with the binding (VT-B) subunit (1–5 μg/mL) alone resulted in apoptosis, which was similarly unaffected by the presence of IL-6. These observations imply that Gb3 is an important effector molecule involved in the control of differentiation and apoptosis in B-lineage cells and suggest a novel approach to induce cell death in myeloma cells which may have a potential therapeutic role.

P-1380 ACTIVATING *RAS*-MUTATION OCCURS IN AN IL-6 DEPENDENT MYELOMA CELL LINE

A. Guenther, M. Schmalzing, M. Karrasch, F. Bakker, R. Burger, M. Gramatzki. *Department of Medicine III, University of Erlangen-Nuernberg, Germany*

The most frequently observed transforming events in multiple myeloma (MM) are alterations of the (GTP-binding) p21 protein of the *ras* gene family. It has been shown that 10 to 67% of MM harbour activating point mutations in the N- or K-*ras* gene. The mutations are localized in codon 12, 13 and 61 of each gene and result in an amino acid substitution that leads to an accumulation of proteins in the active GTP-bound state. The *ras* gene product p21 is involved in the signaling cascade of IL-6 which is thought to be the most important cytokine in MM. We tested five human MM cell lines for mutations in the N-*ras* and K-*ras* gene. In the two IL-6 independent cell lines U266 and L363 wild type sequences were found. Three cell lines showed alterations in the investigated genes. All mutations lead to a change of the protein sequence and are assumed to be involved in the transformation process of the plasma cell. The iL-6 dependent INA-6 harbours a point mutation in a typical hot spot region of codon 12 in the N-*ras* gene (GGT to GAT, Gly to Asp). The JK-6 cell was iniatially IL-6 dependent but became after prolonged cultivation IL-6 independent. JK-6 showed a novel combination of mutations in the N- (codon

64: TAC to GAC, Tyr to Asp) and K-*ras* gene (codon 61: Gln to Leu, CAA to CTA). Although the mutation in N-*ras* gene in JK-6 is not involving a classical hot spot region, it is nevertheless located closely to hot spot codon 61 and could influence the GTP-binding of the $p21^{ras}$. The observed mutations were found in all tested subclones of JK-6 not correlating with the responsiveness to IL-6. In the IL-6 independent cell line RPMI 8226 a point mutation was detected in codon 12 of the K-*ras* gene (GGT to GCT, Gly to Ala). Our data are in contrast to the report that activating N-*ras* mutations but not K-*ras* mutations induce IL-6 independent growth in the IL6 dependent cell line ANBL-6 [Billadeau, Cancer Res., 1997]. Although there is no doubt that activating *ras* mutations play an important role, the developement of IL-6 independent growth in MM seems to be more complex than thought.

P-1381 SENSITIVITY OF MYELOMA CELLS TO CYTOTOXIC DRUGS IS DETERMINED BY INTRACELLULAR BCL-2/BAX RATIOS

D.J. Roovers, M. Van Vliet, O. De Weerdt, A.C. Bloem[1], H.M. Lokhorst. *Departments of Hematology and Immunology#, University Hospital Utrecht, The Netherlands*

The occurrence of multidrug resistance (MDR) is one of the major obstacles in the treatment of multiple myeloma (MM). MDR in MM may be caused by the presence of transport proteins like P-glycoprotein (Pgp), which lowers the intracellular drug concentration. In addition, disturbed apoptotic pathways may give rise to a similar MDR phenotype. Central in regulation of apoptosis is the anti-apoptotic Bcl-2 protein, which is highly expressed in MM plasma cells. Heterodimerization between Bcl-2 and the pro-apoptotic Bax protein is essential for the anti-apoptotic action of Bcl-2. Therefore, Bcl-2/Bax ratios may be correlated with drug resistance in MM plasma cells, which was investigated as follows:

The drug sensitivity of plasma cells from a set of 12 cell lines and a number of MM patients was determined by exposure for 72 hrs to different concentrations of the apoptosis-inducing drug daunorubicin (DNR). After incubation, MTT and annexin V assays were used to determine cell survival and induction of apoptosis, respectively. The expression levels of Pgp, Bcl-2 and Bax in plasma cells were determined by flow cytometry or by Western blotting. In general, we found a good correlation between the Bcl-2/Bax ratio in plasma cells and their resistance to DNR, while a contribution of Pgp to DNR resistance was only found in some Pgp-overexpressing cell lines. This suggests that resistance to apoptosis is important in refractory MM.

MDR in myeloma patients may be circumvented by increasing the sensitivity of cells to cytotoxic drugs by lowering the ratio between Bcl-2 and Bax. Accordingly, incubation of patient cells for 5 days with antisense oligonucleotides resulted in a greater sensitivity to DNR in 2 out of 4 patients. The oligonucleotide treatment was optimized by cotransfection with cationic lipid and studies with the MM cell line U266-B1 show a specific degradation of the Bcl-2 mRNA. From these experiments, we conclude that antisense therapy may increase chemosensitivity in myeloma patients.

P-1382 FAMILIAL MULTIPLE MYELOMA: A RETROSPECTIVE STUDY OF TWENTY FAMILIES

B. Grosbois, M. Attal, C. Payen, J.G. Fuzibet, N. Juge, R. Bataille. *For the Intergroupe Francophone du Myelome, Rennes, France*

Multiple myeloma (MM) rarely occurs in two members of the same family, but when such an event does occur, this may lead to a better understanding of the disease pathogenisis. Twenty families were identified through a retrospective study performed in the Intergroupe Francophone du Myelome (IFM). In twelve families MM occurred in siblings (two sisters: 5; two brothers: 2; brother-sister: 5), in five among parents and children (mothers-sons: 3; mother-daughter: 1; father-son: 1) and in three more distantly related (first cousins: 1; aunt-nephew: 1; uncle-nephew: 1). The immunoglobulin was identified in the two patients in twelve families: 8 times heavy chain and light chain were identical (IgG Kappa in all cases), 3 times heavy chain were identical (2 IgG, 1 IgA), once light chain was identical (Kappa). Karyotypic studies performed in two patients were normal. In two families the probands were HLA identical. Two patients have antecedent of breast cancer. Three families presented with additional cases of MGUS. Mean age at diagnosis was 62 yrs (33 to 89 yrs). The time lapsed between diagnosis ranged from 0.5 to 14 yrs with an average of 2.5 yrs. In three prospective studies of IFM frequency of familial MM was evaluated: 1/300 (3.3‰) in MY90 protocol, 1/163 (6.1‰) in CIAM protocol and 1/400 (2.5‰) in IFM 94-1 protocol. Although frequency of familial MM appears to be low such an occurrence is probably not coincidental. Our study emphasized several points: familial MM occured at the same age than non related MM, immunochemical identity is frequent, personal neoplastic antecedents and familial cases of MGUS are important. To further elucidate an eventual genetic background a prospective study of cytogenetic, immunogenetic and molecular data is needed in familial MM.

P-1383 NEW MONOCLONAL GAMMOPATHIES AFTER TRANSPLANT IN PATIENTS WITH MULTIPLE MYELOMA

A. Montuoro, D. Ingletto, M.V. Levi della Vida, M. Lalle, A. De Laurenzi. *San Camillo Hospital, Dept. of Hematology, Rome, Italy*

The clonality pattern of immunoglobulins produced in patients during immunity recovery after BMT has been recently reported in several neoplastic diseases. The evolution of Ig production after both autologous and allogeneic transplant was studied in 49 MM pts performing protein electrophoresis, quantification of Ig and light chains, immunofixation on serum and urine. The aim of this study is to characterize and define the incidence of monoclonal B-cell proliferation post-transplant, as well as clinical and laboratory correlations. Fourty-seven evaluable pts detected a monoclonal (M) gammopathy appearing 3 (range 2–12) months after transplant and persisting for 10 (range 5–21) months. The Ig gammopathies were different from the M component at the diagnosis. Only IgG and IgA gammopathies were present (80% and 20% respectively) while the κ and λ chains were identificated with equal frequency. These new post-transplant gammopathies in MM (95% of cases) was higher than we observed in transplanted pts with other malignancies. In fact only 41 (26%) out 155 autologous and 11 (19%) out of 62 allogeneic transplants showed transient M gammopathies. We have found two clinical correlations: a significative incidence of gammopathies in pts developing acute and/or chronic GVHD and a higher frequency in patients receiving marrow ablative regimens including TBI. Our results show that the developement of Ig producing cell clones is unequal and heterogeneous after transplant and it is particularly evident in MM pts. Sequential analysis of serum and urine obtained from pts at different time after transplant revealed that imbalanced clonal reconstitution was transient and evolved towards apparently normal polyclonal Ig production much slower in MM than in the other malignancies. This aberration suggests that the appearance of M gammopathy after transplant is influenced not only by the recovery of immunity but also by humoral immune dysfunctions of the patients.

P-1384 MONOCLONAL GAMMAPATHIES: NATURAL HISTORY OF 391 PATIENTS

G. Frigerio, M. Duro, G. Scognamiglio, F. Alberio, A. Beretta. *Ospedale "VALDUCE"-Ambulatorio di Oncoematologia Via Dante 11 -22100 - COMO, Italy*

Objective: Estimate of probability of malignant transformation from monoclonal gammapathy of undetermined significance (MGUS) to overt myeloma and evaluation of possible predictive factors of this event.

Results: A series of 391 consecutive patients (189 women, 202 men, aged from 29 to 87 years - mean 64) with monoclonal gammapathy were followed-up from a minimum of 6 and a maximum of 234 months (median 38; total time of observation 6334 months = 1361 years). At diagnosis 38 patients (9.7%) were classified as overt myeloma according to Durie & Salmon,; 7 patients (1.8%) as macroglobulinemia and 4 women (1%) as cryoglobulinemia. The other 342 patients (87.5%) were classified as MGUS. The transformation from MGUS to overt myeloma occurred to 21 patients after a follow-up of a range from 8 to 185 months (median 43): the estimated overall rate of transformation is 6.1% (95% C. I.: 5.04–8.81%). Only one woman evolved to macroglobulinemia and one man to amyloidosis. After the malignant transformation ten patients (48%) died because of myeloma (range of survival: 6–62 months; median 13). From the remaining 319 patients 13 (4.1%) died because of not related diseases and in 9 (2.6%) the monoclonal protein seem disappeared after a range from 7 to 46 months. A statistical analysis with stepdown method of a model of multiple logistic regression showed that Bence-Jones proteinuria ($p = 0.00009$), ERS ($p = 0.008$) and serum IgG level (only for IgG MGUS; $p = 0.03$), all evaluated at time of diagnosis, were significant predictive factors for malignant transformation; the time of follow-up was borderline ($p = 0.052$). The other variables evaluated on the model not statistical significant were: serum level of LDH, creatinine, calcemia, proteinemia, albuminemia, the heavy and light chains involved, the hemoglobin level and platelet count, the bone-marrow plasmocytosis, age and sex.

P-1385 CONVENTIONAL CYTOGENETIC IN MULTIPLE MYELOMA: DETECTION OF KARYOTYPE ABNORMALITIES DEPENDS ON TECHNICAL AND CLINICAL FACTORS

N.V. Smadja. *GFCH (Groupe Français de Cytogénétique Hématologique); Hopital St Antoine, Paris, France*

A prospective collaborative cytogenetic study of Multiple myeloma (MM) patients at diagnosis was undertaken by the GFCH to assess the type and prognostic value of complex chromosomal abnormalities (CCA) found in MM disease. Two simultaneous culture conditions were requested when possible: an unstimulated culture of 3 day duration (3d) and a second one of 4–7 day with IL-6 and GM-CSF (cytok). Presently, 121 karyotypes reviewed in plenary cytogenetic sessions have been included, and 76 (62.8%) displayed CCA in relation with MM. 89 patients benefited from the 2 culture conditions (2 cult), 22 and 10 had only one culture, 3d and cytok respectively. The percentage of CCA detected according to culture conditions and clinical stage (Durie & Salmon clinical staging system) are presented and discussed:

1. *Culture conditions:* CCA were detected in 65/111 (58.5%) karyotypes cultured with 3d and in 33/99 (33.3%) with cytok. Among the 89 patients (2 cult): the 3d. culture allows the detection of 47 CCA (52.8%), while the use of cytok allows to detect 25 CCA (28%). All abnormal clones detected using cytok were also detected in 3d unstimulated culture.
2. *Clinical stage:* detection rate of CCA on the whole series increased with the patients stage: 11/28 (39.2%), 7/15 (46.6%), 58/78 (74.3%) stage I, II and III respectively.
3. *Both culture conditions and clinical stage:* Among the 89 patients (2 cult): CCA were detected in 6/22 (27.2%) and in 3/10 (30%) in stage I and II patients respectively in the 2 culture conditions, whereas in the 57 stage III patients, 38 CCA (66.3%) were found using 3d culture and 17 CCA (29.8%) were detected in cytok. culture.

In conclusion, conventional cytogenetic (CC) using 3 day unstimulated culture is satisfactory to detect CCA in stage III MM. In contrast our data show that CC, as performed in this study remains obviously insufficiently informative in stage I and II MM patients. Therefore other culture conditions should be tested. This is particularly true if one consider that the use of FISH and plasma cells DNA content techniques allow the detection of chromosomal defects in nearly all MM.

P-1386 CELL MEDIATED IMMUNITY IN THE CONTROL OF RESIDUAL DISEASE IN PATIENTS WITH MULTIPLE MYELOMA UNDERGOING ABMT

R. Zambello, L. Trentin, E. Battistin, P. Salgarelli, E. Galloni, D. Madeo, F. Rodeghiero. *From Dept of Haematology, San Bortolo Hospital, 36100 Vicenza, Italy*

This study was undertaken to investigate the phenotype and function of the T and NK cells before and after recovery from autologous bone marrow transplantation (ABMT) in patients with multiple myeloma (MM). To this aim, in 15 patients with MM (age 42–63) we investigated: a) the percentage of cells expressing T cell markers (CD3, CD4, CD8), NK-cell related markers (CD16, CD56, CD57, CD158a and CD158b), activation antigens (HLA-DR, CD69, CD49a and CD49b), cytokine receptors (CD25, CD122 and TUGh4, to p55, p75 and p64 chains of IL-2 receptor, respectively; CD120a and CD120b, to p55 and p75 chains of TNF receptor, respectively); 2) cytotoxicity against NK-sensitive K-562 and NK-resistant Raji cells; 3) proliferative response to phytohaemoagglutinin (PHA) and interleukin-2 (IL-2). Pre-transplant phenotypic analysis showed an increase of CD8+ cells (46.3±3.5), most of them expressing CD57 antigen (41.0±4.0). NK cells were within normal range (12.1 ± 0.7), without a inbalance of subsets identified by CD158a and CD158b. A slight increase of CD69+ cells was detectable. After one/two months following ABMT, no statistically significant changes have been demonstrated on T and NK subsets comparing to pre-transplant values. Concerning functional activities, cytotoxicity against K-562 and Raji targets was within normal range (28.4±7.0 and 3.3±+1.3, at 40:1 E/T ratio, respectively). Following ABMT, NK activity to K-562 was unchanged, whereas a slight increase of cytotoxicity against Raji cells was documented (9.6±3.9 at 40:1 E/T ratio). Pre-transplant proliferative activity at optimal dose of PHA was reduced as compared to normal controls and a further reduction has been demonstrated after ABMT (25,457±9,783 vs 3,978±991). IL-2 mediated proliferation was normal before and after ABMT. Our results indicate that high dose therapy with PBPC rescue did not modify phenotype and natural killer function. The low proliferative activity to PHA is consistent with a defect of T cell compartment, whereas normal response to IL-2 suggest that NK cells are functionally active. These data may help in designing new immunotherapeutic strategies in patients with MM after ABMT.

P-1387 THREE-COLOR FLOW CYTOMETRY IN THE DIAGNOSIS OF MULTIPLE MYELOMA

V. Maisnar, M. Toušková, J. Krejsek, J. Malý, O. Kopecký. *Department of Hematology and Institute for Clinical Immunology and Allergy, Charles University Teaching Hospital Hradec Králové, Czech Republic*

The advent of new therapeutic approaches to multiple myeloma (MM) made necessary introduction of novel methods for detection of minimal residual disease. Among other approaches, residual disease can be detected by immunofluorescence using flow cytometry. The cell surface antigen expression of MM cells is diverse, and their detection in peripheral blood is complicated by the relatively low frequency.

We have examined by 3-color flow cytometry the co-expression of CD19, CD38, CD45, CD54, CD56 and CD138 in peripheral blood and marrow aspirates in patients with MM. For detection and characterization of MM cells, combinations of following antibodies were used: anti-CD19 FITC, anti-CD38 FITC, anti-CD38 PE, anti-CD54 FITC, anti-CD56 PE-Cy5, anti-CD45 PE, anti-CD45 PE-Cy5 (Immunotech) and anti CD138 PE (Serotec). The samples were analyzed using EPICS XL (Coulter) flow cytometer, and the analysis was based on at least 10000 events.

Samples from more than 30 MM patients were analyzed using this method. The percentage of MM cells ranged between 0.3% and 54.2% in bone marrow aspirates and between 0.1% and 11.8% in peripheral blood. The presence of CD138, CD38, CD54 and CD56 was expressed in 100%, 100%, 85%, and 68%, respectively. The cells were negative or dim for CD45 antigens. In our opinion, MM cells are best characterized by following combinations of antibodies: CD38 FITC/CD138 PE/CD45 PE-Cy5, CD54 FITC/CD138 PE/CD56 PE-Cy5 or CD54 FITC/CD38 PE/CD 56 PE-Cy5.

The identification of malignant clone is a first and most important step in characterization of the disease, determination of the prognosis and detection of residual disease after treatment. Three-color flow cytometry represents a method which can meet these goals.

P-1388 SERUM INTERLEUKIN-6 HAS NO DISCRIMINATORY ROLE IN PARAPROTEINEMIA NOR A PROGNOSTIC ROLE IN MULTIPLE MYELOMA

C.G. Schaar[1], U. Kaiser[2], S. Snijder[3], F. Ong[3], J. Hermans[1], J.C. Kluin-Nelemans[1]. [1]*Leiden University Medical Center, Leiden;* [3]*Comprehensive Cancer Center West, Leiden, The Netherlands;* [2]*Marburg University, Marburg, Germany*

Objective: Interleukin-6 (IL-6) is a multifunctional cytokine which serves as a growth factor in multiple myeloma (MM) and induces production of acute phase proteins during inflammation. We evaluated the discriminatory role of serum IL-6 in paraproteinemia and its prognostic value in MM.

Design and Methods: During 1991–1993 the Comprehensive Cancer Center West recorded all patients (n = 1464) with newly diagnosed paraproteinemia in a population-based prospective registry*. Upon entry patient characteristics, laboratory, bone marrow, and skeletal X-ray examinations, as well as paraprotein-related diagnoses were recorded. Serum was stored at -80°C. Based on the completeness of entry data and the presence of serum 212 patients were selected. Sixty patients had MM (indolent MM (9), stage I (13), stage II (4), stage III (34)). Other causes of paraproteinemia were: other hematological malignancies (48), paraneoplastic (35), auto-immune disease (15) and MGUS (56). We determined serum IL-6 by ELISA (Labor Diagnostika, Germany). Follow up was done for a median of 29 months (range 0–58 months).

Results: Serum IL-6 had wide and overlapping values in all categories: MM median 24 pg/ml (range 6–214), MGUS 57 pg/ml (7–474), other hematological malignancies 28 pg/ml (6–303), paraneoplastic 58 pg/ml (7–1990) and autoimmune disease 27 pg/ml (18–540). In MM increased serum IL-6 levels did not correlate with disease stage and there was no difference in survival between patients with low ($\leq$50 pg/ml) or high ($\geq$50 pg/ml) serum IL-6 levels (p = 0.23).

Conclusion: Serum IL-6 does not discriminate in patients with paraproteinemia and has no prognostic role in MM.

(1) * Ong F. J Clin Epidemiol 1997; 50: 909–915.

P-1389 BURKITT'S LYMPHOMA-ASSOCIATED TRANSLOCATIONS IN MULTIPLE MYELOMA AND PLASMA CELL LEUKEMIA

J. Dierlamm[1], L. Michaux[2,3], H.J. Weh[4], I. Wlodarska[2], M. Stefanova[1], M. Stul[2], A. Ferrant[3], J.-J. Cassiman[2], A. Hagemeijer[2], H. Van den Berghe[2], D.K. Hossfeld[1]. [1]*Department of Hematology and Oncology, University of Hamburg, Hamburg;* [4]*Franziskus Hospital, Bielefeld, Germany;* [2]*Center for Human Genetics, University of Leuven, Belgium;* [3]*Department of Hematology, Cliniques Universitaires U.C.L. St. Luc, Brussels, Belgium*

Translocations leading to rearrangement and overexpression of the *CMYC* oncogene on 8q24 by its juxtaposition to the immunoglobulin heavy or light chain loci occur in virtually all cases of Burkitt's lymphoma and occasionally in other subtypes of non-Hodgkin's lymphoma, mainly during disease progression.

We describe cytogenetic, molecular, and clinical data of 4 cases of multiple myeloma (MM) and 2 cases of plasma cell leukemia cytogenetically characterized by t(8;14)(q24;q32), t(8;22)(q24;q12), and t(2;8)(p12;q24) (2 cases each), respectively. These abnormalities were identified in complex, pseudodiploid or hyperdiploid karyotypes, which otherwise showed no common chromosomal changes. In one case t(11;14)(q13;q32) and in another der (16) t(1;16)(q11;q11), both of which represent well known recurrent chromosomal changes in MM, were additionally detected. Southern blot analysis was performed in one case and did neither reveal *CMYC* rearrangement nor EBV integration, both characteristic features of Burkitt's lymphoma.

All patients presented with stage III disease with high tumor burden as indicated by numerous osteolytic bone lesions, dense bone marrow infiltration, or elevated β2 microglobulin levels. They showed IgGκ (3 cases), IgGλ (2 cases), and IgAκ (1 case) paraproteinemia as well as Bence-Jones proteinuria in 3 of the 6 cases. Chemotherapeutic treatment resulted in temporary remissions in 3 of the 6 patients. Three patients deceased 7 to 60 months after diagnosis due to disease progression. We conclude that Burkitt's lymphoma-associated translocations in plasma cell malignancies represent recurrent chromosomal abnormalities and may be associated with disease progression.

P-1390 BONE RESORPTION IN MULTIPLE MYELOMA (MM): UTILITY OF LABORATORY INVESTIGATION

L. Arcaini, A. Corso, A. Landino[1], M. Lazzarino, C. Castagnola, M. Tajana, M. Lunghi, P. Zappasodi, C. Bernasconi. *Institute of Hematology, University of Pavia;* [1]*Department of Biochemistry, IRCCS Policlinico San Matteo, Pavia, Italy*

Neoplastic bone involvement is a common feature of MM. Prior studies have related lytic lesions to an excessive bone resorption due to activation of osteoclasts. To assess the osseous breakdown in MM we measured osteocalcin (OC) and bone-specific alkaline phosphatase (BALP) as markers of the osteoblastic activity, and urinary excretion of hydroxyproline (UHOP) as index of osteoclastic activity. The aim was: a) to investigate the correlations between laboratory findings and the clinical characteristics at presentation or the disease status; b) to assess the role of laboratory investigations in defining bone disease, especially in patients (pts) without evident lytic lesions. 49 MM pts were studied, 14 at onset and 35 pretreated: 29 males and 20 females, median age 53 yrs (31–72), IgG 27, IgA 17, IgD 1, BJ 6, non-secretory 1; 21 pts were Durie and Salmon stage 1, 4 stage II, 24 stage III; standard radiological examination did not reveal any lytic lesion in 19 pts. No pt showed an overt renal failure and only 1 had creatinine levels >2 mg/dL. Results were analyzed by Mann Whitney U test for comparison between the different groups; the various parameters evaluated were correlated by means of Spearman R test. Pts with a more extensive disease showed significantly lower levels of OC ($p < 0.02$), but no relevant differences were seen in BALP levels. A similar correlation was found when the differences between pts with or without lytic lesions were evaluated. No correlations were found when we considered the phase of the disease or when each parameter was correlated with the others. UHOP levels were higher in the advanced stages of the disease or in presence of extensive lesions, without reaching statistical significance. Stage 1 pts showed values above normal in 57% for OC, 84% for BALP, 40% for UHOP. These data are consistent with an intense bone resorption, which is present also in pts without radiological evidence of lytic bone lesions. In conclusion: 1. biochemical markers show correlation more with the extension than with the phase of the disease; 2. even in the absence of radiological evidence of bone lesions, a laboratory, evaluation can be useful to early recognize a state of bone resorbing activity.

P-1391 GLUCOCORTICOIDS AND MYELOMA: NEW DEVELOPMENTS

V. Genty, R. Genevrier, S. Brahimi, G. Dine. *Institut Biotechnologique de Troyes and Centre Hospitalier, Troyes, France*

Glucocorticoids are the mainstay of the chemotherapies used in the treatment of haematological malignancies, and mainly in multiple myeloma. But after more than 20 years of intensive research for the best cure, this family of molecules have not been totally studied, for their efficiency in antitumoral therapy was considered to be equal to their anti-inflammatory potency.

We demonstrate in this work that, in vitro, with a myeloma cell line of medium sensibility (RPMI8226), there is some differences in the antineoplastic actions of the more commonly used corticoids. By using flow cytometry technics, we showed that, depending on the concentration used, dexamethasone (DEX) potency was 1 to 10 times superior to that of methyl-prednisolone (MPL), and 20 times superior to that of prednisolone (PL). The comparison between MPL and PL gave a difference of 5 to 20 times in favour of the first. We also demonstrate that prednisone, which was totally inactive on the cellular viability or proliferation, was able to stimulate the clone's growth and induce a resistance to glucocorticoids. This resistance was materialised by a toxicity reduction of 15 to 35% of the other corticoids. We showed that this phenomenon was similar in a lesser degree to that induced by dexamethasone. The resistance was accompanied by the up-regulation of the glucocorticoids receptor as we observed in immunocytochemistry. We also detected a loss in the expression of the CD38 antigen (a plasma cell marker) in the resistant cells, that followed the same evolution than the toxicity and the intensity of these resistance. This could distort the evaluation of the residual disease.

As anticipated, we confirmed the important role that DEX could play in standard and intensive chemotherapies, for its potency at low concentrations. A second molecule emerge from this study, MPL could be as efficient as DEX for the higher concentrations, this phenomenon was not observed with PL. A second important conclusions was the resistance that prednisone was able to induce, for this molecule should not produce any effect without maturation. Such a side effect could not be minimised in the treatment of myeloma.

P-1392 COMPARISON OF MRI AND CONVENTIONAL RADIOGRAPHY IN EVALUATION OF SPINE IN MULTIPLE MYELOMA

V. Ščudla, J. Nekula, J. Bacovsky, M. Bucil, H. Sanová. *3rd Department of Medicine and Department of Radiology, University Hospital, Olomouc, Czech Republic*

The study was done to evaluate the role of MRI in the investigation of the spine in patients with multiple myeloma (MM) and to perform comparison with the conventional X-ray radiography (CR) resultes. Examinations of the lumbar and thoracic spine were performed in 50 patients (M/F ratio 1.1, age median 62 years) with MM. Nine (18%) patients were examinated at the time of MM diagnosis, 41 (82%) in various phases of the disease, 30 (60%) patients were in active, 20 (40%) in stable phase of the disease. Median myeloma duration before MRI was 29 (1–216) months. MRI examinations were performed on 0.5T Gyrex V Dex (Elscint). T_1 and T_2 weighted SE images in the saggital and selected transversal planes were obtained in all patients.

Pathological findings recorded by MRI in 45 (90%) patients while by CR (with elimination of osteoporosis findings) just in 26 (52%) patients. The heterogeneous pattern of MRI was found in 19 (38%), isolated focal lesions in 16 (32%), diffuse pattern in 10 (20%) and negative findings in 5 (10%) of our patients. Vertebral body compression was found in 28 (56%) patients (epidural spread was found in 8 e.g. 29% of these patients only) and in 23 (46%) patients examined by conventional radiography. Epidural extension was observed in 10 (20%) of patients examined by MRI but in none patients on CR. Focal lesions were observed in 35 (70%) of patients analysed by MRI but only in 4 (8%) examined by CR.

High sensitivity of MRI for examination of patients with MM was proved, namely in early detection of focal lesions and epidural extension of myeloma mass even in asymptomatic "X-ray negative" patients. MRI is very important part of the standard methods of the newly diagnosed, especially clinically asymptomatic patients with MM.

IGA MZd ČR 3447-3

P-1393 EVALUATION OF URINARY PROTEINS FOR THE ASSESSMENT OF RENAL DAMAGE IN MULTIPLE MYELOMA (MM)

A. Corso, G. Serricchio, P. Zappasodi, L. Arcaini, C. Klersy[1], T. Bosoni[2], C. Castagnola, M. Lunghi, M. Tajana, C. Bernasconi. *[1]Institute of Hematology, Biometric Unit-Scientific Direction; [2]Department of Biochemistry, IRCCS Policlinico San Matteo, Pavia, Italy*

Patients (Pts) with acute or chronic renal insufficiency have an unfavourable prognosis, but if a timely therapy is started in patients with initial damage, an high percentage of cases obtain a regression. Bence Jones as well as some low-molecular weight urinary proteins have been correlated with the tubular dysfunction. Aim of this study was to evaluate if urinary proteins could reveal an initial damage of renal function. We analyzed the follow urinary proteins in 29 MM pts, 9 at onset and 20 previously treated: α1 microglobulin (α1M);β2 microglobulin (β2M); retinol binding protein (RBP); lisozime; β-N-acetyl-D-glucosaminidase (β-NAG); α1 acid glicoprotein (α1AG); IgG; albumin; transferrine. No correlation was found between these proteins and the phase or the status of the disease. Strong correlations were present between urinary proteins and serum indicators of renal failure as reported in the table below.

PARAMETERS	s β2-M	UREMIA	s CREATININE
IgG	p: 0.035	p: 0.052	p: 0.006
Proteinuria 24 h	p: 0.0003	p: 0.121	p: 0.116
Transferrin	p: 0.0009	p: 0.072	p: 0.044
Albumine	p: 0.039	p: 0.121	p: 0.009
α1-microglobulin	p: 0.025	p: 0.385	p: 0.085
α1glicoprotein	p: 0.021	p: 0.167	p: 0.038
RBP	p: 0.00009	p: 0.004	p: 0.004
β2-microglobulin	p: 0.001	p: 0.965	p: 0.243
Lysozyme	p: 0.89	p: 0.154	p: 0.106
NAG	p: 0.016	p: 0.303	p: 0.023

In conclusion, RBP, β-NAG, albumine, transferrine, and IgG are good indicators of renal damage, even in absence of abnormality of the routinary serum parameters. They do not correlate with the severity of the disease, but they seem to be very useful in identifying a subset of patients with initial renal disfunction.

P-1394 IS ANTIGEN CD56 (NEURAL CELL ADHESION MOLECULE, N-CAM) CHARACTERISTIC OF MALIGNANT PLASMA CELL?

D. Jawniak[1], A. Dmoszyńska[1], A. Goracy[1], P. Krawczyk[2]. *[1]Department of Haematology; [2]Department of Immunology, University School of Medicine, Lublin, Poland*

In 1990 Van Camp et al. (Blood 1990, Vol 76) proved that the majority of malignant plasma cells revealed a strong expression of CD56 as opposed to normal plasma cells.

The aim of this study was to establish whether expression of CD56 can be used as a surrogate marker of malignant plasma cells. The expression of CD56 and other adhesion molecules have been studied in the bone marrow samples of 33 freshly diagnosed multiple myelomas and 10 healthy volunteers. Malignant plasma cells were identified by monoclonal antibody against Syndecan-1 (B-B4, CD138). The normal plasma cells were estimated after concentration in magnetic field (positive selection) using MoAb anti-CD19 and selection by high intensity of $CD38^{+++}$ expression. We found that CD56(+) was expressed on normal plasma cells on 10/10 healthy volunteers ranging from 77.2 to 100% and 29/33 myeloma patients ranging 0 to 99.1%. We found the following results: 69.7% patients were CD56(+)/CD19(−), 18.2% were CD56(+)/CD19(+) and 12.1% were CD56(−)/CD19(−). We didn't find the presence of couple CD56(−)/CD19(+). In the control group all patients were characterised by the presence of CD56(+)/CD19(+).

We conclude that both normal and myeloma plasma cells may have increased expression of CD56 and this antigen is not specific for malignant clone.

P-1395 ALTERATIONS IN BONE MARROW STROMAL CELLS FUNCTION IN MULTIPLE MYELOMA

M. Fernández, G. Alarcón. *INTA, University of Chile and DIPRECA Hospital, Santiago, Chile*

Plasma Cells and their malignant counterpart myeloma cells, mostly home to the bone marrow (BM) microenvironment. It is assumed that BM stromal cells play an essential role in the growth of plasma cells tumors in humans. The interaction of myeloma cells with BM stromal cells is probably mediated by adhesion and extracellular matrix molecules (ECM). Then, it may be concevaible that changes in the density or in the expresion of ECM could make posible the maintenance of myeloma plasma cells in the BM microenvironment. The aim of this study was to evaluate whether BM stromal cells of Multiple Myeloma (MM) patients can be differentiated from BM stromal cells of normal donors with regard to ECM production and proliferative capacity. For this purpose BM samples were obtained from 4 patients with newly diagnosed and untreated MM and 4 normal donors. BM mononuclear cells were isolated by Ficoll-Hypaque centrifugation, resuspended in α-MEM/10% FCS and seeded at 1×10^6 cells/cm^2 in 35 mm dishes. Quantitation of the stromal growth and cultured confluence was done at 2 weeks and morphology assesed by phase contrast microscopy. Primary adherent BM stromal cells were subcultured and used for inmunofluorescence studies performed with monoclonal antibodies anti-Collagen I (Col I) and anti-Fibronectin (FN). The stroma of MM patients was characterized by disordered growth of fibroblasts, by the presence of osteoclasts-like cells and by apoptotic figures with vacuolated cytoplasms. The proliferative capacity of BM stromal cells derived from MM patients is remarkable lower than that of control subjects. A significantly lesser area of the dishes was covered by the stroma from patients (median 50%±20%) than from controls (100%). Also MM BM stromal cells produce Col I and FN. The levels of these ECM molecules was higher in stroma of MM patients compared to control subjects. Results of this study show significant alterations in the formation and function of the MM BM stroma.

P-1396 EVALUATION OF DNA PLOIDY AND CELL CYCLE DISTRIBUTION IN PLASMA CELLS OF MULTIPLE MYELOMA PATIENTS

Isabel Lobo, Artur Paiva, Albertina Freitas, Fátima Guedes, Adriana Teixeira, Henriqueta Brêda Coimbra. *University Hospital of Coimbra, Portugal*

The analysis of plasma cells DNA content, either for the assessment of DNA ploidy status or for the study of their cycle distribution, in particular the proportion of S phase, has shown to be the most important prognostic factor for predicting survival and response duration to treatment in patients with multiple myeloma.

We studied 33 patients with multiple myeloma, 29 of the them at diagnosis and 4 at the time of relapse.

Based in a DNA/CD38 double staining technique, by flow cytometry, which allows the discrimination of the plasma cells from residual bone marrow cells, we evaluated DNA ploidy and cell cycle distribution of these cells.

Our results in untreated cases of multiple myeloma showed that DNA aneuploidy was present in 76% of the patients and the incidence of hyperdiploid and hypodiploid cases were 69% and 7%, respectively. Regarding the cell cycle distribution of plasma cells, we observed that 59% of multiple myeloma cases presented a S phase greater than 3% and 41% lower or equal to 3%.

According to the score model described by San Miguel *et al.* (Blood, 1995), our results in association with other prognostic factors, such as β_2-microglobulin serum levels, age (≤69 years) and performance status, allowed us to classify our patients into low (12.5%), intermediate (65.5%) and high risk (22%).

P-1397 CYTOGENETIC ABBERATIONS IN PRIMARY PLASMA CELL LEUKEMIA

M. Čolović, N. Suvajdžić, G. Janković, N. Kraguljac, A. Vidović, A. Novak, M. Pantić. *Institute of Haematology, Clinical Center of Serbia, Belgrade, Yugoslavia*

Objective: The aim of this study was to examine the karyotype profile in patients (pts.) fulfilling the criteria of the primary plasma cell leukemia (PPCL).

Patients: Seventeen patients [4 M/13 F, median age 56 yrs (42–78)] were diagnosed of PPCL in the period between 1978–1997. This comprises 1.3% of all acute leukemias diagnosed in the same period. The main criteria was the presence of $>2 \times 10^9$ plasma cells (blasts) in the peripheral blood. Median hemoglobin was 78 g/L, median WBC was 32×10^9/L with median of 80% plasmablasts in the formula and median platelet count of 49×10^9/L . Mean creatinine 720 μmol/L, Ca^{2+} 2.6 mmol/L and LDH 930 U/L with mean paraprotein concentration in blood of 17 g/L (range 0.3–80 g/L). In 10/17 pts. there was a $CD38^+$ clone of cells with cytoplasmic expression of λ light chains. These pts. were HLA-DR, B-Ag and My-Ag negative. Predominantly was the Bence Jones (BJ) variety of paraprotein (BJλ in 8 pts., BJ κ in 2 pts, and IgGκ,

IgAκ and IgDκ each one pt.). Three pts. displayed IgGλ paraproteinemia. This established the diagnosis of PPCL in all.

Results: The classical chromosome banding technique was performed at diagnosis in 11 pts. and valuable mitoses obtained in 9/11. The karyotype was diploid in 2 pts. [46,XX, 46,XX/ 46,XX, inv (16)]; pseudodiploid in 2 pts [46,XY,del (2p),del (6q),×1, × 6, +2mar; 46,XY/46,XY, × 1, × 2, × 5, × 16, +5mar]; hypodiploid in 4 pts. [45,XY, × 1, del (5p), 11p+, t(13;14), × 16, +2mar; 46,XY/44,XX, × 6, der (12), der (14), der (15), der (17), der (21), × X, +Dmin; 46,XY/43,XY, mar,cx, +multiple structural/numerical aberrations that can not be identified; 46,XY/40, XY,14q+, 15q+, del (11q), cx/80,XXYY) and hyperploid in 1 patient (48,XY, +multiple structural and numerical aberrations]. In accordance with previous reports our study confirmed the presence of complex numerical and structural karyotype abnormalities in pts. with PPCL.

Summary: All pts. were treated with VAD protocol. Only 2 pts achieved complete remission (still alive +1 and +3 yrs after diagnosis). The rest all experienced partial hematologic remission with median survival of 5.5 mos. It appears that karyotype changes adversely influence survival since the two long-surviving pts. had normal karyotype at presentation.

P-1398 IN VITRO EXPANSION OF MULTIPLE MYELOMA CLONALLY RELATED CELLS

J.E.J. Guikema[1,2], M.H.C. Bakkus[3], E. Vellenga[2], N.A. Bos[1]. *[1]Histology & Cell Biology, State University Groningen; [2]Hematology, University Hospital Groningen, the Netherlands; [3]Hematology & Immunology, Free University Brussels, Belgium*

The origin of the Multiple Myeloma (MM) clonogenic precursor cell is still largely unknown. The finding that MM plasma cells lack intraclonal variation regarding somatic hypermutations present in the variable region of the immunoglobulin heavy chain (IgH) gene suggests a post-germinal center origin for MM. Other studies have shown that MM clonally related (MMCR) cells expressing different Ig isotypes are detectable in peripheral blood (PB) and bone marrow (BM), suggesting a pre-Ig switch origin. Experiments to further identify the nature of MM clonogenic precursor cells are hampered by the lack of an in vitro system capable of expanding potential MM precursor populations. The recently developed 'CD40 culturing system' is capable of inducing B cell proliferation and establishing long term B cell lines. We used the 'CD40 culturing system' to culture enriched B cell fractions from BM and PB samples obtained from 7 MM patients. Proliferation was assessed by 3H-thymidine incorporation assay, phenotype of cultured cells was determined by immunogold-silver staining of cytospins and MMCR cells were detected by performing RT-PCR combining CDR3-specific oligonucleotides with IgH constant region specific oligonucleotides.

Proliferation was measured at day 7, 14 and 21. Proliferation peaks at day 7 in most cases, 3H-thymidine incorporation of BM samples was less prominent compared with PB samples. 95–100% of the cultured cells were positive for CD19 and CD40 at day 14 and 21. Cultured cells were negative for B-B4, CD38, CD3 and CD14, indicating that B cells and not plasma cells expand in the 'CD40 culturing system'. Cultured wells were screened for presence of MMCR cells by RT-PCR, in all cases MMCR cells could be detected at day 7, 14 and 21 among which MM clonogenic precursor cells might be present. In conclusion, the 'CD40 culturing system' enables expansion of potential MM precursor populations.

P-1399 ANALYSIS OF ADHESION MOLECULES EXPRESSION ON LYMPHOID CELLS IN MULTIPLE MYELOMA

R. Pogłód, M. Kraj, J. Kopec-Szlezak, B. Nasiłowska. *Institute of Haematology and Blood Transfusion, Warsaw, Poland*

The aim was to study an expression of adhesion molecules: CD18, CD11a, CD18 + CD11a+, CD11b, CD29, CD49d, CD29 + CD49d+, CD44, CD54, CD44 + 54+ and CD56 on peripheral blood (PB) and bone marrow (BM) lymhoid cells in 27 newly diagnosed multiple myeloma (m.m.) patients. The control group was 10 healthy volunteers. The percentage of lymhoid cells with expression of a given antigen was compared to the range of its normal values.

The Study Revealed: increase of percentages of CD54+ cells in BM (in 64% of cases) and in PB (51.8%), increase of CD44 + CD54+ cells in BM (57%) and PB (37%), increase of CD56+ and CD38 + 56+ cells in BM (33% and 37%, respectively) and in PB (25.9% and 22.2%). The rate of CD44+ cells in BM was decreased in 44% while in PB only in 22% of cases. A number of CD56+ cells was normal (in 63% of cases in BM and and in 70.3% in PB) or increased. A rate of CD11b was increased in PB in 42.2% of patients, while in BM was normal in 72% or decreased in 24%. A number of BM CD18+ cells was decreased in 52% of cases while in PB− in 32%. Changes in rates of CD11a+ and CD18 + 11a+ cells were much less expressed in BM and almost absent in PB. Percentages of CD29+, CD49d+, CD29 + 49d+ cells were normal in BM and PB in the majority of cases. In all patients with a massive (>90%) tumor infiltration of BM there was found an evident decrease of CD11a+, CD18+ and CD11a + CD18+ cell counts and increase in CD54+ cell count. As a dominant pattern of CD56 behavior in such cases there was seen an increase in number of CD56+ cells in coexpression with CD38 antigen, but in 2 cases the majority of CD38+ cells lacked CD56.

Conclusions: The extent of adhesion molecules alterations was more pronounced in BM than in PB and in more advanced malignancy. The most frequent findings observed in more than 50% of m.m cases were an increase of CD54+ cells both in BM and PB and CD44 + 54+ cells in PB and a decrease of those with expression of CD18 in BM. An increased rate of CD11b+ cells observed in PB in a relatively high proportion of m.m. patients did not correspond with normal or decreased CD11b+ cell rates in BM.

Myeloma: treatment

P-1400 VCMP/VBAP AT STANDARD DOSES (SD) vs. VCMP/VBAP AT HIGHER DOSES (HD) OF CYCLOPHOSPHAMIDE AND DOXORUBICIN AS INITIAL TREATMENT OF MULTIPLE MYELOMA (MM)

J. Bladé, J.F. San Miguel, M.L. Escudero, M. Fontanillas. *(On behalf of PETHEMA: Spanish Cooperative Group for Hematological Malignancies Treatment). Hospital Clínic. Barcelona, Spain*

Intermittent melphalan and prednisone (MP) has been the standard therapy for MM during the last 25 years, but the results are unsatisfactory. In a previous PETHEMA study we have shown that VCMP/VBAP increases the response irate when compared with MP. The aim of the present study was to ascertain whether treatment with VCMP/VBAP with higher doses of cyclophosphamide and doxorubicin could be superior to VCMP/VBAP at SD. From January 1, 1990 to May 31, 1994, 449 patients with symptomatic MM entered the study. All patients were prospectively randomized to receive: A) alternating cycles of VCMP (vincristine 1 mg i.v. on day 1, cyclophosphamide 500 mg/m^2 i.v. on day 1, melphalan 9 mg/m^2 p. o. on days 1–4 and prednisone 60 mg/m^2 p.o. or parenteral on days 1–4) and VBAP (vincristine 1 mg i.v.; BCNU and doxorubicin i.v., 30 mg/m^2 each on day 1; and prednisone 60 mg/m^2 p. o. or parenteral on days 1–4, or B) the same VCMP/VBAP increasing the doses of cyclophosphamide and doxorubicin from 500 to 1200 mg/m^2 and from 30 to 50 mg/m^2, respectively. There were five inelegible patients and 17 with no data. Among the 427 patients with available data there were 15 lost to follow-up and in 29 there was a protocol violation. Among the 194 evaluable patients treated with VCMP/VBAP (SD) the objective response rate was 44.8%, whereas among the 189 given VCMP/VBAP (HD) the objective response rate was 50.8% (p = NS). No significant differences were found when comparing the survival curves from both groups (median 29 vs 31 mos.). In summary, this study has not demonstrated any advantage of an intensified VCMP/VBAP regimen over VCMP/VBAP given at standard doses in MM.

P-1401 HIGH-DOSE SEQUENTIAL CHEMOTHERAPY IN THE TREATMENT OF MYELOMA

M. Canales, E. Ojeda, Ma.T. Alvárez, V. Jiménez, Ma.T. Jiménez, D. Hernández, M.J. Aguado, J.Ga. Bustos, F. Hernández-Navarro. *Haematology Department. Hospital "La Paz". Madrid, Spain*

High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) has provided tumour mass reduction and longer survival, in patients with multiple myeloma. We have analysed the experience in our centre following a sequential therapeutic approach which included high-dose chemotherapy (Cy-VAMP), high-dose melphalan, PBSC harvest and ASCT. From February 1992 until December 1997, 19 previously untreated patients and 16 patients with refractory or relapsed disease, with stage III according to the Salmon & Durie staging system, have received chemotherapy with Cy-VAMP schedule. Complete remissions after three cycles of Cy-VAMP and high-dose melphalan were 65% (according to EBMT/EORTC criteria). There no were toxic deaths during the period of aplasia following to high-dose melphalan. Sixteen patients (mean age 51 years) have undergone ASCT. The mean interval between diagnosis and ASCT was 15 months. The conditioning regimen was busulfan and cyclophosphamide in 7 patients, busulfan and melphalan in 8 patients, and 1 patient received melphalan. The mean number of MNC and $CD34^+$ cells were 8.3×10^8/kg and 4.6×10^6/kg respectively. The mean period of aplasia was 7 days (range 4–11); neutrophils $>0.5 \times 10^9$/l, platelets $>20 \times 10^9$/l and $>50 \times 10^9$/l was observed 11, 19 and 62 days postinfusion respectively. The median of days of fever was 1 day (range 0–5) and mucositis grade 3–4 was observed in four patients. One patient died due to cerebral haemorrhage. With a median follow-up of 48 months, the median survival from diagnosis was 43 months overall (range 15–86). The median survival after ASCT was 34 months for the 8 survivor patients (range 5–51) and 7 months (range 5–28) for the 8 nonsurvivors. In four patients the survival was over 4 years, and survival was close to 7 years in 2 patients. Therefore, our results confirm the feasibility of high-dose chemotherapy and ASCT as front-line or rescue therapy in patients with multiple myeloma.

P-1402 CYCLOPHOSPHAMIDE-IDARUBICIN-DEXAMETHASONE (CID) - AN EFFECTIVE ORAL COMBINATION CHEMOTHERAPY FOR MELPHALAN-PREDNISOLONE (MP) RESISTANT MULTIPLE MYELOMA

G. Majumdar, K. Ganguly, S. Kashyap, G. Bardhan. *Doncaster Royal Infirmary, Doncaster, United Kingdom*

Eleven patients (M8, F3, median age 65 years, range 44–80 years) with MP resistant multiple myeloma were treated with an oral combination of cyclophosphamide, idarubicin (Zavedos) and dexamethasone. Three patients showed primary resistance to MP and 8 others showed secondary resistance. Previous treatment included intravenous anthracycline (doxorubicin) containing combination (ABCM or ABCMP) in 5 patients. None of the patients had significant renal failure.

CID was given orally for 4 consecutive days at 4 weekly intervals for 6 such courses. The dosage used was cyclophosphamide 150 mg/m^2 daily, idarubicin 5 mg/m^2 daily and dexamethasone 40 mg daily. The dose of idarubicin (Zavedos), which comes only in 5 mg capsules, was calculated as the total dose for the course (20 mg/m^2) which was divided in 4 unequal doses and was given over 4 days. All patients also received allopurinol 300 mg daily orally and ranitidine 150 mg daily orally for a week starting from the day 1 of each course.

Median fall of paraprotein level was 55% (range 0–83%). Nine patients (82%) showed response and reached plateau phase. Seven patients (64%) had good response (>50% fall in paraprotein level) and another 2 (29%) had minor response (>25% fall in paraprotein level). Maximum response occurred after 2 or 3 courses in all but 1 patient in whom the lowest level of paraprotein was reached after 6 courses. The plateau phase lasted for a median period of 12 months (range 3 to 25).

Only 1 patient had severe neutropenia ($<0.5 \times 10^9$/l) during the treatment. One other patient had life threatening infection (septicaemia) and another one had significant thrombocytopenia ($<50 \times 10^9$/l) but no bleeding diathesis.

We conclude that oral CID is a highly effective and safe second line treatment for MP-resistant multiple myeloma.

P-1403 A PHASE II STUDY OF CID THERAPY AND EARLY PBSCT IN PATIENTS WITH DE NOVO MULTIPLE MYELOMA

A. Spencer[1], M. Seldon[1], S.F. Deveridge[1], N. McCarthy[2], P. Marlton[2], A. Enno[1], R. Cobcroft[2], D.S. Gill[2]. [1]*Mater Hospital, Newcastle;* [2]*The Princess Alexandra Hospital, Brisbane, Australia*

We have introduced a novel treatment regimen for de-novo multiple myeloma aimed at minimising myelotoxicity and facilitating effective peripheral blood stem cell mobilistation with early PBSCT. The protocol comprises 4 courses of oral CID therapy (cyclophosphamide 100 mg/m^2 d1–4, idarubicin 10 mg/m^2 d1–2 and dexamethasone 40 mg daily d1–4, d8–11 and d15–18 for course 1 and d1–4 for courses 2–4), PBSC mobilisation with IV cyclophosphamide 2 g/m^2 plus G-CSF 5 μg/kg from day +5 and PBSCT with IV melphalan 200 mg/m^2 conditioning. To date 12 consecutive patients, mean age 50 years (range 31–63 years), (Salmon & Durie stage 1, n = 3; stage 2, n = 6; stage 3, n = 3) have commenced treatment. B_2M was elevated at diagnosis in 3 patients, CRP in 6 patients and both in 2 patients. Forty two courses of CID have been administered. Haematological toxicity has been minimal (grade I, n = 6/42; grade II, n = 5/42; grade III, n = 2/42; grade IV, n = 0/42) with no instances of febrile neutropenia. Eleven patients are evaluable for response to CID (2 or more courses received) with stable disease in 2 cases, partial remission in 7 and a complete remission in 2. Six of 6 patients who have completed CID have undergone PBSC harvesting (2 collections = 4 patients, 3 collections = 1 patient, 4 collections = 1 patient) at a mean of 4.5 months post diagnosis (range 3.5 to 5.4 months) and adequate CD34 yields were obtained in all cases (mean CD34 = 4.7×10^6/kg, range $3.03–8.24 \times 10^6$/kg). So far 4 patients have proceeded to PBSCT at a mean of 5.5 months post diagnosis (range 4.5–6.0 months) with rapid and uneventful haemopoietic recovery. Overall response to date with the CID + PBSCT regimen is 90% (10 of 11 evaluable patients) with a 36% complete remission rate and a 54% partial remission rate. We conclude that CID offers an effective, low toxicity, easy to administer regimen which achieves rapid disease control and facilitates early and safe PBSCT. Patient accrual is continuing.

P-1404 High dose sequential chemotherapy and autologous peripheral stem cell transplantation in patients with previously treated or untreated multiple myeloma

S. Kuhlmann, A. Fossa, T. Wrzeciono, C. Kasper, A. Welt, S. Seeber, M.R. Nowrousian. *Department of Internal Medicine (Cancer Research), University of Essen, Germany*

Introduction: High dose chemotherapy (HDCT) and autologous peripheral stem cell transplantation (PSCT) have been reported to prolong remission duration and probably survival in patients (pts) with multiple myeloma (MM). The aim of the present study was to evaluate the toxicity and efficacy of a sequential HDCT (SHDCT) and PSCT as initial or salvage treatment in pts with MM.

Patients and Treatment: A total of 18 pts were included, 12 pts with previously untreated and 6 pts with relapsed or refractory MM. Pts characteristics were as follows: male/female 6/12; age: median 51 years (range 33–63); disease stage (Salmon and Durie): II/III 6/12, renal dysfunction 2. Monoclonal proteins: IgG 12, IgA 4, IgD 1, Bence-Jones proteinuria 4. The SHDCT started with high-dose dexamethasone (20 mg/m^2 po d 1–4 and d 8–11) given at 3 week intervals until no further reduction in tumor parameters was observed. Thereafter, treatment was continued with 2–3 cycles of HD cyclophosphamide (CPM) (3 g/m^2 1-h-infusion, d 1 + 2) + G-CSF or GM-CSF, and PSCs were collected. This was followed by 2 cycles of HD melphalan (100 mg/m^2 $\frac{1}{2}$-h-infusion, d 1 + 2) + PSCT + G-CSF. Complete remission (CR) was defined as disappearance of myeloma protein + less than 5% myeloma cells in the bone marrow, and partial remission (PR) was defined as reduction of myeloma protein of >50% from baseline and reduction of Bence-Jones proteinuria of >90%.

Results: Median follow-up: 18 months.

MM	n	CR	PR	RR	Relapse	RD/median	OS/median
Previously untreated	12	11	1	100%	2	not achieved*	not achieved*
Relapsed/ refractory	6	3	3	100%	4	10 months*	20 months*

RR = remission rate; RD = remission duration; OS = overall survival. *$p < 0.05$.

Toxicity: (WHO-grade in % of pts). leukocytopenia IV 100; thrombocytopenia IV 100, anemia III 17; pneumonia III 17; stomatitis III 11; diarrhoea III 11; bleeding III 11; OT/PT increase III 6; 1 patient died of heart failure in PR, probably related to CPM.

Conclusions: The SHDCT protocol used in this study appears to be highly effective in inducing remissions both in pts with previously treated or untreated MM. Considering RD and OS, particularly pts with untreated MM seem to benefit from this treatment.

P-1405 TANDEM AUTOLOGOUS TRANSPLANTATION (TPX) FOR MULTIPLE MYELOMA: RESULTS OF A GERMAN MULTICENTRE STUDY

H. Goldschmidt[1], H. Martin[2], S. Serke[3], U. Hegenbart[1], A. Glasmacher[4], Y. Ko[5], W. Schultze[6], A. Hänel[7], C. Rudolph[8], H. Gerhartz[9], J. Hastka[10], M. Wilhelm[11], F. Gieseler[11], M. Bornhäuser[12], G. Ehninger[12], D. Hoelzer[2], D. Huhn[3], R. Haas[1]. *For the high-dose myeloma treatment group; [1]Department of Hematology and Oncology, Univ. Heidelberg; [2]Frankfurt; [3]Berlin; [4/5]Bonn; [6]Klinikum Bad Saarow; [7]Chemnitz; [8]Cottbus; [9]Duisburg; [10]Mannheim; [11]Würzburg; [12]Dresden, Germany*

Objective: Dose-escalated therapy combined with autologous tpx improves response rates and survival in patients (pts.) with multiple myeloma (MM, Attal et al. 1996). We evaluated the toxicity of a sequential high-dose therapy including two cycles of Melphalan 200 mg/m^2 and tpx in a multicentre study.

Materials and Methods: Between 6/96 and 12/97, 136 pts. received mobilization therapy with high-dose Cyclophosphamide (CY 7 or 3 g/m^2) or Ifosfamide (IFO 12 g/m^2) + G-CSF at the time of best response to conventional treatment.

Results: 125 pts. (92%) were successfully harvested (>5.0 CD34$^+$ cells/kg). 104 pts. completed one tpx and 70 pts. two tpx. 48 pts. started IFN-alpha maintenance. 50 pts. $\geq$3 month after tpx were evaluable for remission. 44% of pts. achieved a complete remission (EBMT-Criteria). Treatment related mortality with CY (IFO) was 0%, with the first tpx. 1.0% and with the second tpx 0%. Grade IV (WHO) non-hematological toxicity was observed in 5% of pts.

Conclusion: Sequential high-dose therapy including tandem tpx is a feasable treatment option in a multicentre study. Based on these results, we plan a randomized phase-III-study to evaluate different conditioning regimens.

P-1406 ALLOGENEIC TRANSPLANTATION FOR MULTIPLE MYELOMA-EVIDENCE FOR LONG TERM MOLECULAR REMISSIONS

J.L. Byrne, G.I. Carter, C.J. Stainer, A.P. Haynes, N.H. Russell. *Nottingham City Hospital, Nottingham, UK*

The place of allogeneic transplantation in myeloma remains controversial with a reported high TRM in some series. Since 1990 we have undertaken allogeneic transplantation in 19 patients (median age 49.5 years, range 40–58 years) using either bone marrow (n = 13) or G-CSF mobilised PBSC (n = 6). 11 patients were in 1st plateau phase and 8 had more advanced or refractory disease. Conditioning was with TBI (12 Gy in 6 fractions) and melphalan 110 mg/m^2 (n = 17) or 70 mg/m^2 (n = 1) or cyclophosphamide 120 mg/kg (n = 1). 5 patients have died of transplant related causes and 1 patient transplanted with a rapidly rising paraprotein has died of relapsed disease without achieving CR. Of the 5 transplant related deaths 3 occurred in the 8 patients >50 years. Of 15 evaluable patients 14 have achieved CR and the mean time to attainment of CR post-transplant was 2.5 months. 5 patients who were not in CR when assessed at 4 months were commenced on α-interferon therapy (3 MGU × 3/week) of whom 4 subsequently achieved CR following 4–36 weeks of IFN therapy. No patient has relapsed with a median follow-up of 3.1 years (range 2–84 months). 9 informative patients have undergone post-transplant molecular monitoring using a fluorescent IgH finger-printing technique and all have remained persistently PCR negative. The DFS at 2 years for all patients is currently 67%, reaching 80% for the 11 patients transplanted in 1st response. These results suggest that allogeneic transplantation for myeloma can be carried out with an acceptable TRM, particularly in younger patients with a high response rate. The melphalan/TBI conditioning regimen may reduce TRM and allow patients to benefit from a graft versus myeloma effect not seen following autografting and which may be augmented using α-interferon.

P-1407 SURVIVAL ANALYSIS OF AN ADJUVANT THERAPY WITH ORAL ENZYMES IN MULTIPLE MYELOMA PATIENTS

A. Sakalová[1], L. Dedik[1], S. Gazová[1], J. Hanisch[2], W. Schiess[3]. *[1]Clinic of Haematology and Transfusion Medicine – Faculty Hospital, Bratislava, Slovakia; [2]IFAG, Basel, Switzerland; [3]Mucos Pharma, Geretsried, Germany*

Objective: To assess existing data on patients with Multiple Myeloma (stages 1–3) treated with different therapeutic regimens-Chemotherapy alone (VMCP/MOCCA, VAD) (CH) vs. Chemotherapy and adjuvant treatment with oral enzymes (Wobe- Mugos®E, MUCOS Pharma, Geretsried, Germany) (OE).

Design and Methods: Retrolective cohort analysis of data of all patients diagnosed and treated in the Clinic of Haematology and Transfusion Medicine, Bratislava, from January 1985 to July 97 (CH 99, OE 166 patients) to investigate the effect of OE on survival. Primary efficacy parameter was the Kaplan-Meier-estimate of survival and the median survival time for both groups. Secondary parameters were response quality and rate during the first year, duration of first remission, and the safety.

Results: Both groups were comparable for their demographic data, and also for disease specific data. In the OE group for disease stage 3 median survival was 83 months compared to 47 months in the CH group ($P_{logrank}$ = 0.0014), and also for stages 1–3 survival time was longer in the OE group; adjusted sample ($P_{logrank}$ = 0.0003). In stage 3A the resp. results were 88 vs. 49 months ($P_{logrank}$ = 0.0040), and for patients with renal insufficiency (stage 3B) 66 vs. 37 months ($P_{logrank}$ = 0.1460). Multivariate cox regression analyses confirmed these results. Response rates are higher and duration of remission is longer in the OE group. An early remission and a long duration of the first remission is an important prognostic factor for the survival of the patient.

Oral enzymes were well tolerated with 3.6% of the patients experiencing mild to moderate gastrointestinal symptoms.

Conclusions: The long term adjuvant therapy with oral enzymes in patients with multiple myeloma receiving optimised chemotherapy regimens considerably prolongs survival. In our group of 265 patients median survival prolongation depended on disease stage, and on therapy with/without OE. Progression of disease stage increases the estimated mortality risk 5 to 6 fold, whereas OE decrease the estimated mortality risk by 50 to 60%.

P-1408 ENZYME THERAPY IMPROVED REMISSION TIME, SOLUBLE TNF- RECEPTORS AND ß2-MICROGLOBULIN CONCENTRATION IN CHEMOTHERAPY TREATED MULTIPLE MYELOMA PATIENTS

Adriena Sakalova[1], Lucia Desser[2], Eva Zavadova[2], Dagmar Holomanova[1], Thomas Mohr[2]. *[1]Clinic of Hematology and Transfusiology, University of Bratislava, Slovakia; [2]Institute of Tumorbiology/Cancer Research, Applied and Experimental Oncology Department, University of Vienna, Austria*

We determined soluble TNF-Receptors (sTNF-R) p55 and p75, β2-Microglobulin (β2M), Interleukin-6 (IL-6) and Tumor Necrosis Factor (TNF) in the sera of 198 patients with Multiple Myeloma (MM) stage I–III: before therapy, after chemotherapy (MOCCA/VMCP) or after enzyme (WOBE-MUGOS: chymotrypsin, trypsin, papain) -+chemotherapy and in 67 age matched healthy volunteers.

The remission time of MM patients after chemotherapy and after enzyme-+chemotherapy were compared retrospectively. The remission time was significant ($p < 0.001$) longer in enzyme treated patients stage II. The serum concentrations of sTNF-Rs and β2M were significantly ($p < 0.05$) elevated in patients stage II and III before therapy. STNF-Rs and β2M correlate ($r = 0.88$ $p < 0.001$ for sTNF-R p55 vs. β2M and $r = 0.835$ $p < 0.001$ for sTNF-R p75 vs. β2M). The levels of these serum markers (sTNF-R p55, sTNF-R p75 and β2M) were lower after chemotherapy and significant lower after chemo-+enzymetherapy. During the time course over 17 months the β2M, p55 and p75 levels in 52 MM patients stage II (chemo- or chemo-+enzymetherapy) were compared. A significant reduction of these marker concentration in MM patients treated with enzyme-+chemotherapy ($p < 0.001$) in comparison to the chemotherapy group was observed. No differences in IL-6 concentrations were detected between the treatment groups. The concentration of bioactive TNF was elevated in stage I (all therapies) only, the immunoreactive TNF was elevated in all three stage and treatment groups. Treatment with WOBE-MUGOS® in addition to conventional chemotherapy prolongs remission times in stage II MM patients and reduces the concentration of progression markers.

P-1409 PROGNOSTIC FACTORS AND SURVIVAL IN ELDERLY PATIENTS WITH MULTIPLE MYELOMA

Ph. Rodon, J.B. Gauvain, C. Linassier, L. Benboubker, D. Levallois, M. Maigre, Ph. Goupille, F. Luthier, V. Lucas, J. Dugay, Ph. Colombat. *Service de Médecine Interne et d'Hématologie, CHG Blois; CHR Orléans; CHU Tours; CHG Saumur; CHG Le Mans, France*

Although the incidence of multiple myeloma (MM) is increasing with age, data specifically regarding elderly patients (pts) are lacking. We retrospectively reviewed pts with MM aged 75 years or more at diagnosis hospitalized in our institutions between january 1985 and december 1996. The study was closed in december 1997. 130 pts were included (male: 53, female: 77). The median age at diagnosis was 81 years (range: 75 to 97). According to the Durie and Salmon clinical staging system, 38 pts were in stage I, 30 pts in stage II and 62 pts in stage III. 85 pts received chemotherapy: 7 stage I pts for progression or amyloidosis, 22 stage II pts and 56 stage III pts. The initial protocol regimen was the association of an alkylating agent (melphalan or cyclophosphamide) and steroids in 66 cases, VAD in 11 cases, VMCP in 7 cases and CEP in 1. At the end of the study, 100 pts had died, 22 were alive and 8 lost of follow-up. The median survival was 22 months. Univariate analysis showed that age 85 years or more ($p < 0.0001$), performance status 3–4 ($p < 0.0001$), beta 2 microglobulin serum level ≥ 4 mg/l ($p = 0.001$), creatinin level >120 mcmol/l ($p = 0.002$) and serum albumin level <30 g/l ($p = 0.02$) were adverse prognostic factors for overall survival. Durie and Salmon clinical staging, hemoglobin, calcium, LDH and CRP serum levels and plasmablastic cells were not significant predictors of outcome. A Cox regression analysis identified age 85 years or more ($p < 0.0001$) and performance status 3–4 ($p < 0.0001$) as independent prognosis variables for overall survival. Among 78 treated pts evaluable for response, 49 (62.8%) were responders, 16 (20.5%) were stable or progressed and 13 (16.7%) early deaths (≤ 3 months after initiation of treatment) occured. The median survival for pts achieving a response was 31 months as compared to 12 months for stable or progressive pts ($p = 0.002$). Cause of death was related to MM in 8 stage I and 54 stage II–III pts, unrelated to MM in 13 stage I and 11 stage II–III pts and unknown in 4 stage I and 10 stage II–III pts ($p < 0.0001$). We conclude that age and performance status are the main prognostic factors in elderly pts with MM and need to be considered when discussing therapeutic options. A high rate of death unrelated to MM in stage I pts reflects heavy comorbidity. Response rate and survival of pts responding to chemotherapy appear to be quite comparable to those usually reported for younger pts. A study of quality of life should be undertaken.

P-1410 IDARUBICIN OVERCOMES P-GLYCOPROTEIN-RELATED MULTIDRUG RESISTANCE: COMPARISON WITH DOXORUBICIN AND DAUNORUBICIN IN HUMAN MULTIPLE MYELOMA CELL LINES

D.J. Roovers, M. Van Vliet, A. Martinotti[1], P. De' Besi[1], J. Smeels[2], A.C. Bloem[3], H.M. Lokhorst. [2]*Depts. of Hematology and Immunology University Hospital Utrecht, Utrecht;* [2]*Pharmacia & Upjohn Woerden, The Netherlands;* [1]*Pharmacia & Upjohn Milan, Italy*

The clinical use of anthracyclines like Doxorubicin (DOX) or Daunorubicin (DNR) for treatment of MM is limited due to the emergence of cell populations resistant to multidrug chemotherapy. Idarubicin (IDA) shows a higher efficacy than DOX or DNR in in vitro-killing of myeloma cells even overexpressing Pgp. This could be due to higher lipophilicity probably enhancing IDA influx, while IDA appears to behave as a Pgp substrate to an extent comparable to DOX or DNR. The cytotoxicity of IDA, DNR, DOX, IDAol and DOXol has been determined in the RPMI 8226 parental cell line and in the sublines with MDR phenotype overexpressing Pgp (8226-R7 and 8226-Dox40). The MTT assay (viability) as well as the annexin V flow cytometry assay (apoptosis) showed that the MDR variants of RPMI 8226 are more resistant to DNR and DOX than the parental cell line. In the MDR cell lines the ability to induce apoptosis and cytotoxicity of IDA was 8–32 times higher than DOX and DNR. The difference in cytotoxicity was lower in the parental cell line. The IDA uniquely cytotoxic 13-OH metabolite, IDAol, was at least 32 times more active than DOXol, and even 2–4 fold more cytotoxic than DOX. Cytotoxicity of DNR and DOX in MDR cell lines was clearly enhanced by the MDR-modifier Verapamil, while cytotoxicity of IDA was only slightly affected. These results can be explained based on IDA intracellular pharmacokinetics. The rate of intracellular uptake for IDA was about 7 times that of DNR both in the parental 8226 cell line and in the MDR 8226-R7, and was not significantly increased after incubation with Verapamil, while on the contrary the addition of Verapamil to 8226-R7 increased DNR uptake approximately 4-fold. However, in the highly resistant variant 8226-Dox40 a definitely lower IDA accumulation was enhanced by the addition of Verapamil (uptake increased about 3.5 fold). This trial demonstrates that IDA is less sensitive than other less lipophilic anthracyclines to Pgp/mdr-1-mediated MDR. Accordingly, IDA might play a significant role in treatment of MM, especially in refractory or anthracyclines-based chemotherapy relapsed patients. Its efficacy can be further increased by combination with MDR modifiers such as Verapamil or cyclosporines.

P-1411 STUDIES ON THE EBV, B7 AND ACTIVE IMMUNOTHERAPY OF MULTIPLE MYELOMA PATIENTS

Zhang Wanggang[1], Cao Xingmei[1], Wang Xiangling[2], Wang Jianli[1], Ma Xiaorong[1], Zhao Wanhong[1], Liu Jie[1], Wang Yili[2], Mao Lijun. [1]*Department of Haematology;* [2]*Institute of Immunopathology, The Second Affiliated Hospital of Xi'an Medical University, No 36 west 5th avenue, Xi'an 710004, ShannXi Province, P.R, China*

Background: The etiopathology of multiple myeloma (MM) is still unclear. The mortality of MM patients treated only by chemotherapeutics has been rather high. So it is important to study the etiology, pathogenesis and new therapeutic methods of MM. In this study the presence of EBV and expression of costimulatory molecule B7 in myeloma cells were detected. Meanwhile, the efficiency of a novel active immunotherapy in MM patients was observed.

Methods: 1) The presence of EBV-DNA were examined in myeloma cells of 21 MM patients who were HIV-negative and not posttransplanted by polymerase chain reaction. 2) The expression of B7 on myeloma cells membrance of 4 MM patients were examined by immunofluorescence. 3) A novel myeloma vaccine was made by using myeloma cells and Freund's adjuvant added with IL-2, IL-6, GM-CSF. 3 MM patients who showed no any improvement to chemotheraty, 20 days later after chemotherapy ended, received 1 ml vaccine by subcutaneous injection in bilateral upper arms' triangular muscle areas weekly for 4 weeks. Then all treatment were stopped. Effect and adverse reaction were estimated.

Results: EBV-DNA of bone marrow cell were detected in 14 of 21 MM patients (positive rate:66.7%), which was significantly higher than that of acute myeloid leukemia patients (4/26) and normal pepole (0/25) ($p < 0.05$). B7 were expressed on myeloma cells membrance in 3 of 4 MM patients. After immunotherapy for 1 month, the mumber of myeloma cells of 3 MM patients dropped from begining 9%, 23%. 40%. to 3%, 9%, 20% respectively. The median maintaining period covered about 65 days. Then the myeloma cells began to increase. Except inflammatory hyperemia and swelling appeared in injected areas, no other adverse reactions were observed.

Conclusions: The present study revealed EBV-DNA were positive in myeloma cells of most MM patients. EBV is maybe the important pathogeny of MM. B7 were expressed in most myeloma cells membrance, which perphaps was key cause for effective immunotherapy in these MM patients. Vaccine is of much high value in MM patient's therapy.

P-1412 SURVIVAL IN CONVENTIONALLY TREATED YOUNGER (<60 YEARS) MULTIPLE MYELOMA PATIENTS - NO PROGRESS DURING TWO DECADES

M. Hjorth[1], E. Holmberg[2], S. Rödjer[3], I. Turesson[4], J. Westin[5], F. Wislöff[6]. *for the Nordic Myeloma Study Group (NMSG);* [1]*Department of Medicine, Lidköping Hospital;* [2]*Oncology Center, Sahlgrenska Hospital and* [3]*Department of Medicine, Östra Hospital, University of Göteborg,* [4]*Department of Medicine, Malmö University Hospital;* [5]*Department of Medicine, Lund University Hospital, University of Lund, Sweden*

; [6]Department of Medicine, Ullevål Hospital, University of Oslo, Norway

Objective: The purpose of this study was to describe a population of conventionally treated younger (<60 years) patients with multiple myeloma, with respect to clinical characteristics, prognostic factors, and survival. A second purpose was to create a patient cohort for use as historical control to a similar group of patients treated with high dose chemotherapy.

Material and Methods: The patient registers of 5 Nordic studies were reviewed for patients <60 years. A total of 317 patients with symptomatic multiple myeloma were identified. 39 were retrospectively judged to be uneligible for intensive chemotherapy regimens because of heart disease (9), psychiatric reasons (15), unwillingness (5), terminal illness (6), refractory end stage uremia (3), or other malignancy (1). The remaining 278 patients were considered appropriate for a historical control material. 32 cases were diagnosed 1970–83, 101 1984–89 and 145 1990–92. The median age was 52 years, range 27–59 years. 6% were Durie/Salmon stage I, 38% stage II and 56% stage III. Melphalan-prednisone was used for initial therapy in about

90%. 27 patients were treated with high-dose chemotherapy with allogeneic BMT (12), autologous BMT (10), or autologous blood stem cell rescue (5).

Results: The median survival was 44 months. There were no differences in survival between the 5 different studies, nor between patients diagnosed 1970–83, 84–89 or 90–92.

Conclusions: The expected median survival for myeloma patients <60 years who are considered for high dose therapy protocols is 44 months. Progress in chemotherapy and supportive therapy has not changed the overall prognosis in multiple myeloma during the last 2 decades preceding the use of high-dose chemotherapy with stem cell rescue.

P-1413 PLATEAU PHASE IN MULTIPLE MYELOMA (MM): AN END-POINT OF CONVENTIONAL-DOSE CHEMOTHERAPY?

P. Zappasodi, A. Corso, A. Nozza, L. Arcaini, C. Klersy[1], C. Castagnola, M. Tajana, M. Lunghi, C. Bernasconi. [1]*Institute of Hematology, University of Pavia, Biometric Unit-Scientific Direction; IRCCS Policlinico San Matteo, Pavia, Italy*

Polichemotherapeutic protocols available for treatment of MM, allow an higher percentage of response than conventional therapy with melphalan and prednisone even though an improvement of survival is rarely obtained. Better survival is reported with the association of high-dose chemotherapy and autologous stem cell transplantation. On the other hand, since conventional-dose chemotherapy more often gives a stable state of the disease, recent evidences suggest to consider it as an end-point of therapy instead of the degree of praprotein fall.

Aims of this study were: 1) to analyze within a cohort of 177 previously untreated myeloma patients (pts) the incidence and the duration of plateau; 2) to correlate it with the presenting features; 3) to assess its impact on survival. Among 146 valuable pts a response was obtained in 70% of pts with a median survival of 46 months, while it was 18.8 months for not responders (p = 0.0001). Time to the best response (TBR) was 8.8 months. The type of response was not correlated with the duration of plateau phase. An analysis of presenting features showed that age, sex, and skeletal lesions were correlated in multivariate analysis to the achievement of a stable state. The duration of plateau phase (median 21 months), considered as a time dependent variable, had an higher prognostic value for survival (p = 0.0001). To analyze the prognostic impact of presenting features, response to therapy and occurrence of plateau we used an hierarchical model for survival. The analysis showed that response to therapy and duration of plateau have an higher influence on survival.

In conclusion, the duration of plateau phase is a stronger predictor of survival than the degree of response after conventional-dose chemotherapy in MM.

P-1414 THE PROGNOSIS OF RENAL FAILURE IN THE SURVIVAL OF MYELOMA PATIENTS

V. Grigoraki[1], A. Tsakri[1], S.C. Georgakopoulou[2], G. Tsoufakis[2], H. Roumeliotis[1], K. Matsis[1], T. Kalmantis[1], P. Zirogiannis[2], N.I. Anagnostopoulos[1]. [1]*Dept. Of Hematology, District General Hospital of Athens;* [2]*Dept. Of Nephrology, District General Hospital of Athens, Greece*

Purpose: To evaluate the prognostic significance of acute or chronic renal failure in patients with newly diagnosed symptomatic multiple myeloma (MM).

Patients and Methods: This retrospective study included 81 patients diagnosed with MM between 1989 and 1995. Inclusion in this study was MM according to those criteria specified by the South West Oncology group. Plasma cell tumor load was defined as high, intermediate or low (Durie, 1975). Clinical response was explained by a greater rather than 75% reduction in tumor mass with disappearance of Bence- Jones protein excretion. Survival curves were calculated by the Kaplan Meier- method.

Results: Among 81 patients, 74 were evaluated. Acute or chronic renal failure was presented in 26 patients (35.1%): Group 1; the remaining 48 patients (64.9%) had normal renal function: Group II. There were 51 males and 23 females with an average age 65.8±10 yrs. Out of 26 patients with renal failure, 9 reversed completely their abnormal renal function while 9 were partially. Among 8 patients with persisting renal failure, 3 patients required hemodialysis. In univariate analysis, the comparison of two groups with the respective parameters Hb, albumin, CRP, β_2-microglobulin, LDH, Ca^{++}, percentage of bone marrow plasma cell - infiltration and number of lytic bone lesions, showed that were statistically significant only for Hb, Ca^{++}, β_2, - microglobulin. However, the level of Hb was statistically lower in the Group I, while the levels of Ca^{++} and β_2 - microglobulin were statistically higher in the Group I. Furthermore, there was a statistically significant difference in the response rates (p 0.0067) between these two groups of patients. But there was no statistically significant difference in the survival rates (p 0.2546) with a median survival of 24 months for the Group I and 48 months for the Group II.

Conclusion: The presence of acute or chronic renal failure did not adversely affect survival for the multiple myeloma patients.[1]

(1) Archives Internal Medicine 1990, 150: 1693–695

P-1415 HIGH INCIDENCE OF THROMBOTIC EVENTS IN MYELOMA PATIENTS

M.C. Perrin, J. Jaubert, J. Reynaud, C. Mounier, P. Oriol, D. Guyotat. *Hopital Nord, Saint-Etienne, France*

To estimate the effective incidence of thrombotic events (TE) in multiple myeloma (MM) patients, we analysed a total of 51 consecutive patients admitted to the Service d'Hématologie of the CHU of St Etienne for chemotherapy or treatment of an intercurrent episode. Deep vein thrombophlebitis (DVT) were diagnosed by Doppler ultrasonography or phlebography. Pulmonary embolism (PE) were diagnosed by perfusion-ventilation technetium scan. Fifty-one patients with MM were considered evaluable with a median follow-up of 32 months (12–210). Twelve patients (23.5%) suffered a TE at diagnosis or during MM evolution. For this population, the median follow-up was 39 months (16–91) and the median age was 59 years (45–73). Only 2 patients had a personal history of varicosities, 2 were bed-rested and 1 had a family history of PE. There were 19 TE for 12 patients: DVT in 10 cases (53%), DVT and PE in 7 cases (37%), PE in one case (5%) and mesenteric thrombosis documented at surgery in 1 case (5%). Only 4 TE occured when no chimiotherapy was being administred. The different lines were: first line in 7, 2nd line in 3, 3rd line in 4 and 4th line in 1 case. The stage of the MM at the time of the TE was: diagnosis = 3, partial response = 9, failure and progressive disease = 7. Two TE conducted to death, 2 required a modication and 15 allowed the continuation of therapy as sheduled. The biologic data at the time of the TE were: Anaemia, high level of protidemia and low level of albumin. The monoclonal globulin concentration was low in 72% of the cases (IgG < 50 g/l, IgA < 30 g/l or BJ < 4 g/24 h). In our study, only 10% of these events occured in hospitalised patients but many presented bone pain with probably a reduction of activity. TE appeared in patients receiving chemotherapy (79%) or continuous or intermitent cortocosteroid therapy (68.4%). This 23.5% incidence of TE suggests that incidence of thrombotic events in MM is largely underestimated. The analysis of a larger number of patients, clotting activities at diagnosis and during MM evolution, and the role of corticosteroid treatment in myeloma thrombosis need to be evaluated.

P-1416 THE INCIDENCE OF ACUTE MYELOID LEUKEMIA IN MULTIPLE MYELOMA PATIENTS

M. Kraj, R. Pogłód, B. Nasiłowska, S. Maj. *Institute of Haematology and Blood Transfusion, Warsaw, Poland*

The analysis of acute myeloid leukemia incidence, correlation between plasma cell and myeloid proliferation and effect of applied anti-myeloma treatment on the occurrence of myeloid proliferation was carried out in 600 multiple myeloma (m.m.) patients treated in the Department of Haematology of IH&BT in the years 1960–1997. Acute meloid leukemia was diagnosed in 7 patients (in 5 - myeloblastic, in one - myelomonocytic and in one -erythroleukemia). The study revealed that the incidence of myeloid leukemia in m.m. patients increases with an elongation of their survival time and ranges from 1% in patients with survival time up to 6 years from the onset of antitumor treatment to 10% in those with survival duration exceeding 10 years. Myeloblastic leukemia occurred after 63 to 142 months and erythroleukemia after 129 months from the m.m. diagnosis and the beginning of melphalan treatment. Myelomonocytic leukemia was diagnosed after 20 years from the occurrence of proteinuria and bone destructive lesions and 10 years from the diagnosis of m.m. in form of λ light chain disease. It is necessary to stress that during last 10 years when polychemotherapy programs with a limited use of melphalan prevailed in the treatment of m.m. acute myeloid leukemia was observed only in one case.

Among 7 m.m. patients in whom acute myeloid leukemia was revealed in 3 no plasma cell proliferation features were found at the diagnosis of acute leukemia; in 2 of them the absence of plasma cell proliferation was confirmed at autopsy. It proves curing multiple myeloma in those patients. In the review of available literature there was found only one case of the cure of m.m. confirmed at autopsy after 14 years from m.m. diagnosis and the onset of treatment according to M-2 program. The cause of death in that case was adenocarcinoma of the lung.

P-1417 SECONDARY MYELODYSPLASTIC SYNDROME (MDS) IN PREVIOUSLY TREATED MYELOMA PATIENTS

D. Boskovic, D. Marisavljevic, N. Radosevic, I. Elezovic, A. Mijovic, D. Tomin, M. Gotic. *Institute of Hematology, Clinical Center of Serbia, Belgarde, Yugoslavia*

Development of secondary MDS in myeloma patients (pts) is mostly caused through leucemogenic effects of alkylating agents, particularly melphalan, commonly used in myeloma induction therapy. However, neither the incidence nor the clinical features of this complication are well established.

In 92 myeloma patients (pts) treated uniformly with VMCP combination therapy (1986–1997 period), and followed for at least 2.5 years, secondary MDS developed in 8 pts (9%). All pts were female (mean age 57 yr.). MDS developed after 24–71 months after diagnosis of myeloma (median 58 months). Most of them had anemia (7/8, Hb 83–117 g/L), elevated MCV (6/8, median 108 fl) and thrombocytopenia (6/8, platelets 30–120 $\times$ 10^9/l), while leukopenia was noticed in 3/8 pts (WBC 2.4–3.3 $\times$ 10^9/l). Bone marrow (bm) dyshematopoiesis was prominent in erythroid and megakaryocytic series (7/8 pts) while dysgranulopoiesis was noticed in 3/8 pts. Five pts had RA, 2 RAEB, and one RARS. Cytogenetic analysis was done in 5/8 pts, and disclosed chromosome abnormalities in 4/5 pts (del (5q), -7, -X, +21). The most interesting feature was that 7/8 pts were in a " plateau-phase" of myeloma with low/moderate concentration of paraprotein in 2 pts. At the time of MDS diagnosis, all pts had less than 2% plasma cell in their b.m. In 6/8 pts alkylating agents were used for prolonged maintenance therapy, after reaching the "plateau-phase" of myeloma. The course of sec. MDS in was progressive: either through accelerated worsening of cytopenia, evolution in RAEB (3 pts with RA), or transformation to AML (2 pts with RAEB, and pts with RARS). Median survival after diagnosis of sec. MDS was 16 months.

Conclusion: Persistent cytopenia in myeloma "plateau-phase" is highly suspective for secondary MDS, especially in pts maintained with alkylating agents. Thus, maintenance interferon therapy although costly, seems more than reasonable. Alternative is discontinuation of any further therapy after reaching the "plateau", however with expanse risk for the earlier relapse.

P-1418 PLASMA CELL LEUKEMIA (PCL): A MULTICENTRIC RETROSPECTIVE STUDY

R. Costello, D. Sainty, J.P. Fermand, A. Delmer, M. Divine, J.P. Marolleau, J.A. Gastaut, H. Dombret, P. Chaïbi. *The CRH; Institut Paoli Calmettes, Marseille, France*

PCL is considered as the leukemic variant of multiple myeloma, with circulating plasma cells accounting for more than 2,000/mm^3 and/or more than 20% of the differential WBC. In order to analyse clinical and biological features of PCL, as well as response to therapy, we have retrospectively reviewed all PCL cases diagnosed in our centers between 90 and 97. Twenty-one patients (pts) with a median age of 53 years (30–71) and a sex ratio of 9 women/12 men were studied. Fifteen pts had primary PCL and 6 pts had secondary PCL with a median time between myeloma diagnosis and PCL onset of 14 months (3–96). At PCL diagnosis, clinical symptoms were mainly asthenia (10 pts) and bone pain (9 pts); the diagnosis was made on blood routine examinations in two asymptomatic pts. Five pts had splenomegaly and/or hepatomegaly; 13 pts had radiological bone lesions; 2 pts had pleural effusion; 1 pt had meningeal myelomatosis. Mean hemoglobin value was 92 g/L; mean leukocyte count was 23,000/mm^3, with a mean PC percentage of 50%. Ten pts had renal insufficiency and 9 pts had hypercalcemia. Cytogenetic study was performed in 5 pts, with complex abnormalities in all cases. In the 10 pts tested for immunophenotype, PC were found positive for mature B-cell markers (PCA-1, CD38) in 7/8 cases, for early B-lineage markers (CD19, CD20) in 3/9 cases, and for CD56 in 2/7 cases. First line therapy was an anthracycline-containing regimen in 13 pts, high-dose cyclophosphamide in 3 pts, high-dose IV dexamethasone in 3 pts, while 1 pt with secondary PCL was treated with a double intensification procedure (high-dose melphalan and PBSC autologous transplantation). The last remainig pt received only palliative therapy. Response to therapy was: 3 CR, 9 PR, 1 resistant disease, and 8 early deaths. Further therapy comprised single or double intensification with autologous PBSC transplantation in 7 pts and secondary allogeneic BMT in 2 pts. Median overall survival was 7 months (7 days to 26 months). Only 4 pts survived more than 1 year after PCL diagnosis. In conclusion, PCL is a very agressive form of multiple myeloma with a rapid fatal outcome even in pts treated intensively, despite a quite good initial response rate.

Vessels and platelets

P-1423 ENDOTHELIAL CELL MARKERS AND THE PREDICTION OF ARTERIAL STENOSIS IN PERIPHERAL AND CAROTID ARTERY DISEASE

A.D. Blann[1], A. Farrell, A. Picton, C.N. McCollum. [1]*University Department of Medicine, City Hospital, Birmingham; University Department of Surgery, Withington Hospital, Manchester, UK*

Damage to the endothelium is believed to be important in the progression of atherosclerosis and can be assessed by measuring levels of markers such as von Willebrand factor, soluble thrombomodulin, intercellular adhesion molecule-1 (ICAM-1) and soluble E-selectin. We tested the hypothesis that these markers would be related to objectively-defined arterial stenosis in peripheral artery disease and in carotid artery disease. Accordingly, we measuring levels of these markers by commercial ELISA in 43 new patients presenting with intermittent claudication, and in 43 new patients presenting with transient ischaemic attack (TIA). Disease severity was estimated by means of the ankle to brachial pressure index (ABPI, range 0.33 to 1.00) of the affected leg in intermittent claudication and by ultrasound/Doppler defined stenosis of the carotid artery (range 50% to 95%) in patients presenting with TIAs. Data was correlated according to Spearman's method (r = correlation coefficient).

Results:

	Peripheral disease		Carotid disease	
Marker	r	p	r	p
von Willebrand factor (IU/dL)	-0.395	0.009	0.054	0.961
soluble thrombomodulin (ng/mL)	0.011	0.740	0.035	0.970
soluble E selectin (ng/mL)	0.064	0.952	0.043	0.966
sICAM-1 (ng/mL)	-0.274	0.080	-0.262	0.109

None of the markers were related to the degree of stenosis in the carotid artery. However, our data suggest that von Willebrand factor is the most sensitive marker of the degree of peripheral artery stenosis among patients with intermittent claudication. This implies that peripheral and carotid disease may not share the same degree of endothelial perturbation, and this may have pathophysiological significance.

P-1425 SUBCUTANEOUS CONCENTRATED DESMOPRESSIN INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1, CD54) PLASMA OVER-EXPRESSION

R. Musso, S. Cabibbo, D. Cultrera, M. Russo, G. Giuffrida, C. Ferlito, S. Salemi, R. Giustolisi. *Institute of Hematology, Haemophilia Centre, University of Catania, Italy*

Desmopressin (DDAVP) is a synthetic analogue of the natural hormone vasopressin with several effects on the hemostatic system which notoriously causes endogenous release of coagulation factor VIII (FVIII:C), von Willebrand factor (FVIII:vWF), tissue plasminogen activator (t-PA) and also increases platelet adhesiveness and shortens bleeding time (BT) without affecting vascular tone. Owing to this hemostatic effect, DDAVP is currently used in various clinical conditions with increased congenital or acquired bleeding tendency as well in heathy volunteers (blood donors) and in patients undergoing to elective surgical operations. Even if DDAVP does not appear to act directly on endothelial cells (EC) 'in vitro', we assumed that platelet mediated events 'in vivo' and additional factors which are present in the vascular endothelium and released in the circulation would be important elements in the bleeding's control and/or stopping. We firstly report that the subcutaneous injection of concentrated DDAVP (EMOSINT Sclavo, Siena, Italy) in 1 ml vial (20 μg) at the dosage of 0.3 μg/kg also promotes the over-expression of the Intercellular Adhesion Molecule-1 (ICAM-1, CD54), a novel member of the immunoglobulin superfamily, which is notoriously expressed on the surface of blood cells, epitelial and endothelial cells and also circulates in soluble form (sICAM-1). In 10 patients (4 females, 6 males), age ranging 19–55 years, recently diagnosed having moderate/mild haemophilia A (n = 5), type I vWD (n = 5) we performed the preliminary test with EMOSINT. In all patients responders to they hormonal analogue sICAM-1 (ELISA method, HyCult byotechnology, Uden-the Nederlands) was assayed before and after (30 min, 2, 4, 12 h) therapy. After 30 min we observed a significant ($p < 0.001$) increase of sICAM -1 (131.6±23.3 pg/ml vs 87±18.4 pg/ml baseline). This parameter lasted higher after 2-4 hour (116±19.4 pg/ml, 99.6±21 pg/ml) retourning to baseline at 12 hour in all subjects. The factor VIII-related plasma activities were increased, as expected. Facial flush and transient/mild headache were noted; no decrease in blood pressure was seen. Our observation would suggest that ICAM-1, CD 54 and/or the soluble form of this molecule is involved and over-expressed in the vascular endothelium responses which certainly occur after DDAVP subcutaneous administration.

P-1426 HIGH DERMATAN SULPHATE PLASMATIC LEVELS IN ELDERLY INDIVIDUALS

I. Quintana[1], C. Duboscq[1], A. Murúa[2], C. Galarza[2], M. Cámera[2], L. Kordich[1]. [1]- *Laboratorio de Hemostasia y Trombosis. Dpto de Química Biológica. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Buenos Aires;* [2]- *Sociedad Argentina de Medicina Vascular, Buenos Aires*

Dermatan sulphate (DS) is a natural glycosaminoglycan sinthetized by subendothelium fibroblasts. DS plasmatic levels are not detected in normal subjects and they are increased in pregnancy and in chronic renal patients.

DS plasmatic levels were measured by a functional method (STAGO) in 37 individuals (group A) over the age of 65 (67–92) years, median and range; 23 females and 14 males; 12/37 patients showed vascular disease history (peripheral arterial occlusive disease, ischemic stroke, coronary artery disease).

A control group was studied (group B): 15 healthy subjects under 35 years old, 8 females and 7 males.

DS plasma concentration in group A was: mean 1.47±1.02 μg/ml, median 1.30 μg/ml, range (0.00–3.00) μg/ml DS plasm level in group B was: median 0.00 μg/ml, range (0.00–0.05) μg/ml.

Total Homocysteine in plasma of group A was determinated by ELISA (AXIS Biochemicals ASA): mean 19±9 μmol/l, median 18 μmol/l, range (6–43) μmol/l (reference values: 5–20 μmol/1).

von Willebrand factor (vWf) was measured by electro-immunodifussion (STAGO): mean 289±87%, median 328%, range (60–368) % (reference values 60–150%).

DS levels were significantly higher in group A as compared to group B ($p < 0.001$). Neither homocysteine nor vWf levels correlated with DS values.

We found no evidence for a dependent association between age, sex, vascular disease and DS plasma levels.

Although the group size is not so large, this is the only reported study of increased OS plasma levels in elderly subjects so far. These results would show a different endothelial function in ancients.

P-1427 ENDOTHELIAL DYSFUNCTION IN INTENSIVE CARE PATIENTS: THE EFFECT OF HAEMOFILTRATION

R.A. Cardigan, M. Singer[1], H. McGloin[1], I.J. Mackie, S.J. Machin. *Department of Haematology and* [1]*Intensive Care, UCL, London, UK*

Continuous veno-venous haemofiltration (CVVH) is an established supportive treatment for acute renal failure in intensive care patients. We sought to examine the effect of CVVH on endothelial dysfunction and the protein C (PC) and S (PS) system in intensive care patients. Samples were collected from 12 critically ill patients in acute renal failure requiring CVVH prior to commencement of CVVH and at sequential time points (15 minutes, 1 hour, 3–4 hours, 8–12 hours, 24 hours, 48 hours) during the first episode of CVVH until the polyacrylonitrile filter clotted. Levels of soluble tissue factor (sTF), thrombomodulin (sTM), E-selectin (E-sel) and endothelin-1 (ET-1) were measured by ELISA as were PC and both free and total PS. Data are expressed as median with first and third quartiles. PC (0.30, 0.10–0.98 IU/ml) and both free (0.62, 0.11–1.21 U/ml) and total PS (0.66, 0.11–1.25 U/ml) levels were below the reference range in most patients prior to CVVH, but there were no further changes during CVVH. Levels of all four markers of endothelial dysfunction were increased in most patients prior to CVVH [sTF 336, 133–560 pg/ml (normal = 64–129); sTM 123, 72–250 ng/ml (normal = 5–55); E-sel 103, 59–186 ng/ml (normal = 29–64); ET-1 1.80, 1.04–2.18 pg/ml (normal = 0.3–0.9)]. There were further increases during CVVH in some but not all patients and in one or other of the markers of endothelial injury. These data suggest that CVVH has little effect on the PC/PS system and that the increased endothelial dysfunction seen during CVVH is more likely to be related to the underlying condition of the patient rather than an effect of the haemofilter circuit.

P-1428 ENDOTHELIAL DAMAGE IN SICKLE CELL DISEASE

S.J. McDonald, A.S. Lawrie, G. Purdy, I.J. Mackie, J.B. Porter, S.J. Machin. *Haemostasis Research Unit, Haematology Dept, University College London, UK*

Patients with sickle cell disorders are hypercoagulable. We have previously reported increased thrombin generation against a background of reduced coagulation inhibitor levels in this group of patients. Circulating sickled erythrocytes present the potential for disruption of vascular integrity and may therefore exacerbate disordered haemostasis even in steady-state sickle disorders. In this study we examined markers of thrombin generation and endothelial activation to assess the contribution of endothelial cell damage to hypercoagulability. Prothrombin Fragment 1 + 2 (F1 + 2), Thrombin:antithrombin complexes (TAT), E-selectin (E-s) and Thrombomodulin (TM) levels were measured in 31 steady-state HbSS (including 3 HbS/β^{o}Tha1) and 12 HbSC subjects and compared to 10 HbAA controls of African descent. Seven of the HbSS group were regularly transfused. F1 + 2, TAT, E-s and TM levels were significantly higher in non-transfused HbSS and HbSC than the control group: (F1 + 2: 0.88 [$p < 0.01$] and 0.91 [$p < 0.051$ vs 0.58 nmol/L, TAT: 7.2 [$p < 0.001$] and 5.2 [$p < 0.05$] vs 2.7 μg/L, E-s: 123.2 [$p < 0.0005$] and 67.4 [$p < 0.005$] vs 48.0 ng/mL, TM: 48.4 [$n = 16$, $p < 0.005$] and 49.3 [$n = 7$, $p < 0.005$] vs 26.9 ng/mL). Enhanced thrombin generation was demonstrated by elevated F1 + 2 and TAT levels in both HbSS and HbSC patients. Evidence of endothelial activation or damage was indicated by elevated TM and E-s levels in both patient groups. Lower E-s levels in HbSC relative to HbSS ($p < 0.05$) reflected the milder clinical course of HbSC. Interestingly, E-selectin levels were highest in non -transfused HbSS patients (123.2 ng/mL), but approximated those of the race matched controls in the transfused HbSS group (55.0 vs 48.0 ng/ml, p = NS) suggesting endothelial damage may be ameliorated by chronic transfusion. Levels of E-s did not correlate with TM, F1 + 2 or TAT levels, indicating that while sickle-cell induced endothelial damage may contribute to hypercoagulability, it is clearly not the only factor responsible for thrombin generation in sickle cell disorders.

P-1429 CIRCULATING COLONY-FORMING UNITS FOR FIBROBLASTS (CFU-F) AND HYPERLIPIDEMIA

E.L. Soboleva, O.S. Saburova, V.N. Smirnov, R.S. Akchurin. *Cardiology Research Center, Moscow, Russia*

In our previous studies *in vitro* and *in situ* [1] colony-forming units (CFU) for granulocytes/macrophages, basophil/mast cells and stromal CFU-F were found among intimal cells of atheromatous human aorta. Later, peripheral blood of coronary patients with hypercholesterolemia (HCL) was examined for the presence of CFU. Mononuclear cells from 20 coronary patients with type IIa or IIb HCL were cultured in semisolid and liquid test-systems. 14-day-old cultures were analyzed by histochemical, immunomorphological and electron microscopy methods. Precursors with stromal potencies forming colonies of fibroblast-like cells (CFU-F) were identified as well as hemopoietic CFU-monocytes/macrophages and CFU-granulocytes. In contrast to hemopoietic colonies, stromal colonies did not stain for nonspecific esterase and myeloperoxidase but were positively stained with antibodies to type III collagen and osteonectin. Cells from stromal colonies had heterogeneous differentiation potencies and multiple phenotypes. Fibroblast-like cells were able to produce fibrillar extracellular matrix in some colonies, while osteoid matrix was synthesized in others colonies. CFU-F were absent in peripheral blood of normolipidemic donors. Thus, it is HCL that induces the appearance of CFU-F in peripheral blood due to significant changes in myeloid tissue resulting in CFU migration into circulation. Morphological examination of bone marrow aspirates from HCL patients undergoing bypass surgery demonstrated myeloid tissue aplasia and bone marrow myelofibrosis of varying severity. The presence of CFU-F in the intima of atheromatous vessels and in peripheral blood of patients with hypercholesterolemia suggest a link between the presence of CFU-F in blood and progression of atherosclerosis. CFU-F harbored in intima may create microenvironment necessary for ectopic hemopoiesis producing factors required to maintain local plaque formation.

(1) Soboleva E. et al, Lectures XIIIth ISH Meeting, Turkey, 1995, p. 134–139.

P-1430 LOCALIZATION OF CONTRACTILE PROTEINS IN HUMAN PLATELETS

T. Takubo, N. Tatsumi. *Osaka City University Medical School, Osaka, Japan*

It is known that the contractile proteins play an essential role during platelet activation by producing mechanical energy from adenosine triphosphate hydrolysis. We observed the localization of contractile proteins (myosin, filamentous actin, α-actinin, tropomyosin, and vinculin) in surface-activated, spreading human platelets using a single-immunofluorescence staining procedure and conventional fluorescence microscopy. Myosin was distributed in a speckled pattern that extended radially from the granulomere. F-actin demonstrated cable-networks. α-actinin and tropomyosin occurred in a punctate distribution, and vinculin was localized at adhesion sites. Our results suggest that myosin, F-actin, α-actinin, tropomyosin, and vinculin are reorganized and assembled in activated platelets during platelet function. Further morphologic studies would clarify the structures and roles of each contractile protein in platelet shape change, pseudopod formation, internal contraction, secretion of cytoplasmic granules, and clot retraction after activation of platelets.

P-1431 THE SIZE EXTENTS OF NATIVE MICROAGGREGATES EXISTING *IN VIVO* BY A NOVEL PLATELET AGGREGOMETER USING LASER LIGHT SCATTER

Y. Tomida, S. Iino[1], H. Hidaka[1]. *Aichi Prefectural Owari Hospital; [1]Nagoya University School of Medicine, Nagoya, Japan*

Circulating platelets may be susceptible due to various stimuli in vessels. A certain division of platelets may form "native microaggregates (NMAs)" of which size extent has not been clarified. A novel platelet aggregometer, PA-200 (Kowa, Japan) employing a laser light scattering can sensitively detect small aggregates consisted of only a little number of platelets. We have developed an integrated system with PA-200, to distinguish the NMAs from subsequent spontaneous platelet aggregation (SPA) induced by not agonists but stirring force. In this study, we evaluated the size extents of NMAs with the system in varieties of thresholds of the light scatter from microaggregates.

Methods and Results: PRP prepared from citrated blood from healthy volunteers was applied. In the default thresholds of light scatter intensity; 25 (ϕ 9 μm) <[S] <400 (ϕ 25 μm) <[M] <1000 <[L], only the [S] division could be detected. However, the size extent of NMAs should be smaller sustained by the results of scanning electron microscopy. With the modified cut-off value for light scatter from 25 to 5 (ϕ 3.38 μm) and the modified thresholds, an evident peak was found at estimated diameters of ϕ 3.88–4.74 μm. The size should be considered precise enough to detect NMAs composed with a couple of platelets. Meanwhile, the size extents of SPA was mostly recognized within light scatter intensities of 25 to 100 (ϕ 16 μm). The existence of NMAs and the extent of SPA generation, in aliquot platelets respectively, revealed no correlation.

Conclusion: These results showed that NMAs and SPA might be mutually different cluster and meanings. Further studies should be expected for analyses of diversities of NMAs and SPA in various pathological states, and hopefully to become valuable indexes to elucidate and investigate platelet activation.

P-1432 NITRIC OXIDE INHIBITION OF PLATELET ACTIVATION DURING CARDIOPULMONARY BYPASS

M.D. Prager, R.C. Eberhart, M. Jessen, M.K. Sly. *University of Texas Southwestern Medical Center, Dallas, Texas, USA*

When blood contacts the synthetic polymer and metal surfaces of cardiopulmonary bypass (CPB) circuits, there is an ensuing activation of platelets, neutrophils, and the coagulation-fibrinolytic, and complement cascades. Postoperative complications of these processes include hemorrhage, thromboembolism, and organ damage. This report focuses on ability of NO to inhibit the reduced platelet survival and function in a porcine model of CPB. Gamma scintigraphy was used to evaluate the effect of NO on (^{111}In-labeled) platelet deposition in the oxygenator. NO gas was infused at 500 ppm for t = 0–60 min; 1000 ppm, t = 60–80 min; and stopped for the final 10 min of CB. NO did not affect blood flow, pressure, hematocrit, or required replacement volume. There was a significant decrease in platelet adherence to the oxygenator: 4% of the prebypass circulating platelet number in NO treated pigs compared to 25% in controls ($p < 0.05$). The former group exhibited a concomitant increase in circulating platelet levels. Whole blood ADP-induced platelet aggregation, measured by impedance aggregometry, was reduced in the NO treated animals *in vitro* tests ($p < 0.05$). These results indicate that NO diminished platelet activation in this CPB model. Additional investigation explored use of selective phosphodiesterase (PDE) inhibitors to enhance the NO effect. Of the 5 PDE families, inhibitors of types III and V were attractive. PDE III which selectively hydrolyzes cAMP is inhibited by milrinone, and PDE V which specifically acts on cGMP is inhibited by zaprinast. Milrinone alone inhibited platelet aggregation and also synergized with NO. Zaprinast, which had no effect by itself, produced a dramatic increase in the inhibitory activity of NO. The latter effect is consistent with the known actions of NO to increase cGMP and zaprinast to prevent its degradation.

P-1433 ERYTHROPOIETIN STOPS CHRONIC, DIFFUSE, TRANSFUSION DEPENDENT GASTRO-INTESTINAL BLEEDING

V.L. Czeizler-Zaharia. *Cabrini Medical Center, New York, USA*

Objective: Recombinant human erythropoietin (rHuEPO) is currently indicated in the treatment of anemia of chronic renal failure, anemia in cancer patients and HIV positive patients. This report shows that rHuEPO can stop diffuse chronic gastrointestinal (GI) blood loss. Patients previously dependent on large, frequent blood transfusions are rendered completely transfusion independent, and thus hospitalization and surgery can be avoided.

Method: Two patients were studied. Patient #1 had radiation proctitis due to radiotherapy for prostate cancer. He had frequent rectal bleeding requiring 20 units of blood to be transfused over 6 months. His bone marrow was hypoplastic. RHuEPO was started at 5000 U subcutaneously, 3x per week. The bleeding stopped and Hg increased to 12g/dL. When rHuEPO was stopped, the rectal bleeding recurred and the Hg dropped. When rHuEPO was restarted the bleeding stopped again and the Hg increased to above 12 g/dL. To this date (one year later), no transfusion was needed. Patient #2 had angiodysplasia of the colon and was transfused eve 2 months for two years prior to starting rHu-EPO. After rHuEPO was started at 5000 U subcutaneously 3x per week, Hg increased to 12 g/dL. On a regular maintenance dose of 3000 U weekly, the Hg is stable at 12 g/dL. No further transfusions were required within the following year.

Conclusion: The above clinical observations point towards the potential use of rHuEPO in chronic diffuse, transfusion dependent, GI bleeding. The shortening of the bleeding time in uremic patients treated with rHuEPO (due to improved platelet/vessel wall interaction) is one possible explanation of the above findings. In the compliant patient, regularly administered maintenance

doses lead to a sustained response; in the non-compliant patients the irregular administration of rHuEPO leads to intermittent bleeding and spikes and dips in the Hg level. This treatment, which is given in an outpatient setting, may save the patient: (1.) frequent transfusions, (2.) abdominal surgery, (3.) hospitalization; it also (4.) allows the patient a normal quality of life and (5.) has the potential for substantial healthcare cost savings.

P-1434 SPONTANEOUS PLATELET ACTIVATION IN VIVO AS A CAUSE OF PLATELET MEDIATED THROMBOSIS IN THROMBOCYTHEMIA: RATIONALE FOR USING LOW-DOSE ASPIRIN AS A SAFE AND EFFECTIVE ANTITHROMBOTIC AGENT

J.J. Michiels, P.J.J. Van Genderen, K. Schrör. *Goodheart Institute, Clinical and Applied Thrombosis and Hemostasis Research and Development Center, Department of Hematology Erasmus University Hospital Rotterdam, Heinrich-Heine University Düsseldorf, The Netherlands*

Microvascular ischemic complications in essential thrombocythemia (ET) and polycythemia vera (PV) are caused by platelet-mediated thrombotic processes in the endartarial circulation. We measured excretion of urinary thromboxane (TX) B_2 in relation to clinical outcome as an established estimate for platelet cyclooxygenase reflecting platelet activation in vivo in 1 PV and 6 ET patients. Within 10 days after discontinuation of low-dose aspirin 3 ET patients spontaneously developed erythromelalgia, which was preceded by a 3 to 30-fold increase in urinary TxB_2 excretion. The enhanced TxB_2 formation and clinical signs could be inhibited by a subsequent regimen of 50 mg aspirin selectively inhibiting platelet COX1 cyclooxygenase. The enhanced TxB_2 production reflects spontaneous platelet activation in vivo preceding the development of platelet-rich thrombi and thus not only provides a biological rationale for using aspirin as an effective and safe antithrombotic agent in ET and PV, but also confirms the existence of a platelet-dependent arterial thrombophilia in patients with thrombocythemia in myeloproliferative disorders

P-1435 SEQUENTIAL AND PARADOXICAL OCCURRENCE OF THROMBOSIS AND BLEEDING AND THE ACQUISITION OF VON WILLEBRAND FACTOR DEFICIENCY AT INCREASING PLATELET COUNT IN THROMBOCYTHEMIA

J.J. Michiels, H.H.D.M. Van Vliet. *Goodheart Institute, Clinical and Applied Thrombosis/Hemostasis Research and Development Center and Department of Hematology, University Hospital Dijkzigt Rotterdam, The Netherlands*

The arterial microvacular thrombotic events in thrombocythemia already occur at platelet counts in excess of 400 $\times$ 10^9/l and are exquisitely sensitive to low dose aspirin, which does not elicit bleedings at platelet counts below 1000 $\times$ 10^9/l. Increasing platelet counts to above 1000 $\times$ 10^9/l is accompanied by the acquisition of a von Willebrand factor (vWF) deficiency due to the loss of large vWF-multimers by proteolytic degradation. The arterial thrombotic condition changes into an overt spontaneous bleeding tendency at mean platelet counts of 2417 to 2483 $\times$ 10^9/l (range 1285–5680), which unseparately appears to be associated with an acquired von Willebrand disease type II as demonstrated by the absence of intermediate and large vWF-multimers in plasma. At platelet counts between 1000 and 2000 $\times$ 10^9/l, thrombosis and bleeding occur in sequence or paradoxically and low dose aspirin for effective thrombosis prophylaxis may elicit or aggravate bleeding symptoms. Reduction of the platelet count to below 1000 $\times$ 10^9/l significantly restores the von Willebrand factor deficiency with the reappearance of the intermediate and some of the large vWF-multimers and the disappearance of the bleeding tendency, but the thrombotic tendency persists as long as platelet count are above the upper limit of normal.

The acquisition of von Willebrand factor deficiency at increasing platelet counts can readily explain the paradox of thrombosis and bleeding in thrombocythemia and has important clinical implications.

P-1436 ALTERATIONS IN PLATELET FUNCTION FOLLOWING PERIPHERAL BLOOD STEM CELL COLLECTION

G. Rock, L. Huebsch, S. Nagib, C.A. Jodouin, A. Giulivi. *Ottawa Civic Hospital, Ottawa General Hospital, Canadian Red Cross, Ottawa, Ontario, Canada*

Following collection of peripheral blood stem cell collection (PBSC) we noted a decrease in the platelet count in many patients. To determine if this was related to the mechanics of the procedure we evaluated the platelet count and activation state before, during (10 minutes) and after (1 hour), a first apheresis procedure in a series of nine patients (age 24–53 years) who had received G-CSF and were undergoing PBSC for autologous transplantation. The Cobe Spectra were used for all procedures with an ACD:blood ratio of 1:15. The platelet count decreased an average of 30% after the single procedure with 8 of 9 patients having a platelet count of less than 70,000 pre procedure. All stem cell preparation contained a considerable number of platelets (range 53.0–1,483 $\times$ 10_9/L) in the collection bag. However, this did not totally account for the degree of loss. The MPV increased considerably in 50% of the patients. Electron microscopy showed normal granule content during and after leukopheresis. Analysis by flow cytometry showed an up- regulation of CD62 (P-selectin) in 7 of 9 patients although GP1b and GPIIb/IIa levels were not changed. The data indicates that platelet activation occurs during some stem cell collection procedures and may contribute to the reduction in circulating platelet count.

P-1437 INCREASED CALCIUM RESPONSES IN PLATELETS FROM PATIENTS WITH ARTERIAL VASCULAR DISEASE

E.C.M. Van Pampus, M.A.H. Feijge, K. Hamulyàk, J.W.M. Heemskerk. *Dept. Haematology, University Hospital of Maastricht, and Depts. Biochemistryl Human Biology, University of Maastricht, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands*

Calcium is an essential second messenger for platelet functions such as shape change, aggregation, secretion and procoagulant activity. A mild bleeding disorder has been observed in various subjects with defective Ca^{2+} signal generation. It can be envisioned therefore that high Ca^{2+} responses may contribute to the risk for thrombosis in atherosclerotic arteries. To measure cytosolic $[Ca^{2+}]_i$ during platelet activation, we used the Ca^{2+}-probe fura-2 and the agonists thrombin, collagen, or thapsigargin (an inhibitor of endomembrane Ca^{2+}-ATPases). We firstly determined the agonist-evoked Ca^{2+} reponses in platelets from 51 healthy volunteers both before and after intake of 500 mg aspirin. For each agonist, the normal Ca^{2+} response was arbitrarily defined as the mean$\pm$1 *SD*. Following this definition, without aspirin 15% and with aspirin 10% of the volunteers were identified as high Ca^{2+} responders. With the exception of one subject, high responsiveness was confirmed for all tested volunteers in new blood samples taken a half year later. To determine the clinical significance of these findings, we subsequently studied the Ca^{2+} responses of platelets from two groups of patients with arterial vascular disease: symptomatic peripheral vascular disease (n = 23) and young stroke (n = 21). For both groups we found that, in aspirinated platelets, about 40% of the patients had a high Ca^{2+} response with thrombin. However, with thapsigargin as agonist, there was no difference in responsiveness between platelets from patients and healthy controls. The procoagulant response of patients' platelets (*i.e.* the capability to support prothrombinase activity) was measured as a Ca^{2+}-linked, functional parameter defining platelet hyper-reactivity. In platelets from 10 out of 13 patients with high Ca^{2+} responses, this response was elevated. In conclusion we found that: *1)* high Ca^{2+}-responsiveness is a reproducible property of platelets; *2)* high Ca^{2+} responses are detectable in about 40% of patients with arterial vascular disease; *3)* platelets from most of the high Ca^{2+} responders are also increased in procoagulant responses.

P-1438 PREVALENCE OF ANTI-PLATELET ANTIBODIES IN PATIENTS WITH CHRONIC LIVER DISEASES: A PRELIMINARY STUDY

D. Christodoulou[1], E. Zervou[2], K. Kistis[1], G.N. Dalekos[1], E.V. Tsianos[1]. [1]*Department of Internal Medicine, Medical School, University of Ioannina;* [2]*Blood Bank at the University Hospital of Ioannina, Greece*

Background/Aims: It is already known that anti-platelet antibodies are responsible for thrombocytopenia in idiopathic autoimmune thrombocytopenic purpura. The aim of this preliminary study was to investigate the presence of anti-platelet IgG antibodies in patients with chronic liver diseases of diverse but well defined etiology since so far there are no well established data in regard with the prevalence of these antibodies among patients with liver diseases.

Methods: One hundred and fifty seven consecutive patients with several chronic liver diseases (43 with chronic hepatitis C, 55 with chronic hepatitis B, 23 with alcoholic liver disease, 11 with autoimmune liver disease, and 25 with cirrhosis due to hepatitis B or C viruses) and 144 healthy blood donors matched for age and sex with the patients were investigated. The method used was the solid phase red cell adherence test-Immucor, which is accepted in general for its high specifity and sensitivity. Further incubation of the positive serum samples samples with chloroquine contributed to the exclussion of anti-platelet antibodies related to HLA-incompatibility.

Results: In overall, we found that IgG anti-platelet antibodies were present in 30.5 of patients with chronic liver diseases. In more detail, anti-platelet antibodies were detected in 29% of patients with chronic active hepatitis B, 18.6% of patients with chronic hepatitis C, 34.7% of patients with alcoholic liver disease, 54.5% of patients with autoimmune liver disease and 40% of patients suffering from cirrhosis due to hepatitis viruses. By contrast, anti-platelet antibodies were present in only 6 healthy blood donors. (30.5% vs 4.1%, $p < 0.001$).

Conclusions: This preliminary study demonstrated the presence of a high prevalence of anti-platelet antibodies in patients with chronic liver diseases. The latter prevalence was higher in certain groups of liver disease, especially in the presence of autoimmunity or cirrhosis. These antibodies may contribute to the induction or the aggravation of thrombocytopenia observed in cirrhotic patients. However, more studies are needed in order to address the latter speculation.

P-1439 BLOOD COMPATIBILITY OF NEW BIOMATERIALS

J.E. Dyr, M. Jiroušková, J. Suttnar. *Institute of Hematology and Blood Transfusion, Prague, Czech Republic*

All blood-contacting biomaterials must be evaluated for their effects on blood. Unlike standard toxicity testing, there are not standard methods for testing hemocompatibility. The first step to improve thrombogenicity of materials is to establish a reliable strategy for thrombogenicity screening. Based on the literature data and on our preliminary results, a multiparametr approach was taken. The biomaterials were exposed to protein solutions, plasma, citrated whole blood, and platelet rich plasma and the resulting activation was measured both in solution and on the material surface and under controlled flow. The approach is superior to analysis limited to either the fluid-phase or solid-phase, since certain materials can adsorb activation products whereas other not. Also platelet activation (and refractoriness) was measured in suspension by flow cytometry as well as on the surface (image analysis). *In vitro* tests were correlated with *in vivo* results obtained by implantations of different knitted prosthetic grafts with different surface modifications and using as a reference the biomaterials currently in clinical use. The most thrombogenic material had the highest fibrinogen and platelet retention rate. The difference in platelet adhesion was not only due to the surface distribution of fibrinogen but also depended on its conformation and arrangement on the surface. In addition to overall properties of biomaterials, such as a hydrophobic or hydrophilic surface and the presence of charged groups, the morphological structure of the surface and its structuring into sequestered domains played an important role. Platelets in contact with a surface can: adhere and activate which results in thrombus formation, adhere only, then the adherent platelets may be passivating the surface, be activated without adhesion that may later be followed by their refractoriness, adhere loosely, transient contact may be still sufficient for activation and premature consumption, or form microparticles. Based on our results, in a screening method to evaluate thrombogenicity, we especially recommend measuring platelet and fibrinogen adhesion (static, dynamic system), platelet count, platelet activation and refractoriness, and prothrombin fragment F (1 + 2) determination.

P-1440 ADHESION AND THROMBUS FORMATION ON COLLAGENS TYPE I, III AND IV IS CORRELATED WITH THE SIMPLATE BLEEDING TIME IN NORMALS

Henrita Van Zanten, Mark Roest, Yaping Wu, Philip G. De Groot, Jan J. Sixma. *University Hospital, Dept of Haematology, Utrecht, The Netherlands*

Recently GPIa expression on the platelet plasma membrane was found to be a good predictor of collagen induced platelet aggregation. This expression was correlated to a polymorphism in the GPIa gene (Kunicki T.J. et al Blood 89, 1939, 1997). We have investigated whether collagen induced thrombus formation on collagen correlates with the bleeding time and with other parameters such as plasma and platelet vWF, and GpIa expression by FACS scanning. For the study of thrombus formation, a small perfusion chamber (5 × 0.1 mm) was used through which blood anticoagulated with Orgaran was drawn with a syringe pump at a shear rate of 1600/sec for 5 min. Collagen type I, III and IV were used as surface. Aggregation in PRP with collagen type I and III and arachidonic acid were also studied. Platelet ADP, and ATP were measured as well. Blood was obtained from 34 normal volunteers, 19 males and 15 females. Subjects with high platelet thrombus formation on collagen type I and III had significantly shorter bleeding times than subjects with low thrombus formation ($P = 0.01$ and 0.03, respectively). Platelet thrombus formation on collagen type IV was not associated with the bleeding time. Plasma vWF was significantly associated with thrombus formation on collagen type I and III ($p = 0.005$ and 0.05, respectively), whereas GPIa expression on the platelet membrane was associated with thrombus formation on collagen type IV ($p = 0.005$). No association was found in this study with platelet vWF.

These results indicate that the bleeding time is determined to a large extent by thrombus formation on collagen which shows a great deal of variation between normals and which is determined by GPIa and plasma vWF.

P-1441 WHITE BLOOD CELL AND PLATELET FUNCTION IN PATIENTS SUFFERING FROM PERIPHERAL OCCLUSIVE ARTERIAL DISEASE

K. Krupiński, J. Giedrojć, A. Szpak. *Department of Haematology, Medical School, Białystok, Poland*

Evidence that platelets play a decisive role in the development of atherosclerosis has been obtained by a number of experimental studies, but the role of other blood cells mainly leukocytes is still discussed. It has been recently reported that leukocytes play an important role in the formation in situ. The aim of our study was to investigate the behaviour of the platelets spraeding, schape change, platelets adhesion to the siliconised glass and extracellular matrix in 38 patients suffering from peripheral occlusive arterial diseases (POAD) (II or III stage according to Fontaine) and 30 healthy subjects. Also platelet-leukocytes aggregates, leukocyte adherence to the nylon fibres and leukocyte adhesion to the subendothelial extracellular matrix have been studied in these patients and healthy volunteers. It was found that patients with POAD: revealed increased the mean numbers of platelet-leukocyte aggregates /132.5±44.3/ increased platelet adhesion to the siliconesed glass and subendothelial bovine extracellular matrix /+24.4±6.7%/. Platelet spraeding and schape change in patients suffering from POAD was significant inhibited, compared to the control however leukocytes adherence to the nylon fibres and also leukocyte adhesion to the bovine extracellular matrix were changed but not significant. Futher studies are needed to clarify leukocytes and platelets interaction in pathogenesis of peripheral occlusive arterial diseases.

P-1442 LOW POWER LASER IRRADIATION DECREASES PLATELET DEPOSITION ON EXTRACELLULAR MATRIX UNDER SHEAR CONDITIONS

A.G. Brill, B. Shenkman, N. Savion, G.E. Brill, I. Tamarin, R. Dardik, V.F. Kirichuk, D. Varon. *Saratov State Medical University, Russia; Institute of Thrombosis and Hemostasis, Sheba Medical Center, Tel-Aviv University, Israel*

The effect of low power laser irradiation (LPLI) on platelet function *in vitro* was studied. Citrated whole blood (WB) was exposed to He-Ne laser (632.8 nm, 7 mW) for 1, 2 and 10 min, and then subjected to shear stress (1300 s^{-1}) for 2 min on extracellular matrix (ECM)-coated plates. LPLI, in a time-dependent manner, caused a decrease in both surface coverage (SC) and average size (AS) of platelet aggregates deposited on ECM. The LPLI effect on platelet function was detectable for up to 1 h after ceasing of irradiation. Similar decrease was observed when LPLI-treated WB was applied to collagen type I

or to von Willebrand factor coated plates. In conventional aggregometry, LPLI-treated platelet-rich plasma (PRP) demonstrated a decrease in response of platelets to either thrombin receptor activating peptide (TRAP) or phorbol-miristate acetate (protein kinase C activator). In flow cytometry analysis, irradiated WB platelets presented lower P-selectin expression and fibrinogen binding in response to activation with suboptimal concentrations (5 μM) of TRAP. LPLI treatment of either PRP, gel-filtered platelets, platelet-poor plasma or red blood cells followed by WB reconstitution revealed marked decrease in platelet deposition on ECM only in the case of PRP or gel-filtered platelet irradiation. Additionally, increased level of cGMP was observed in LPLI-treated gel-filtered platelets.

In conclusion, He-Ne laser irradiation reduces platelet function. Primary acceptor of laser energy which modulates platelet activity is located in platelets but not in plasma or red blood cells. Guanylate cyclase which is able to absorb the light photons at 633 nm wavelength may mediate LPLI effect on platelet function.

Disorders of platelets

P-1443 THERAPY WITH α-INTERFERON INDUCES IMPROVEMENT OF PLATELET COUNTS IN CHILDREN WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA

H. Donato, A. Picón, A. Rovó, R. Kohan, M.C. Rapetti, G. Schvartzman, M. Laverne, A. De Galvagni, P. Rendo. *Policlinico Bancario, Hospital A. Posadas, Hospital R. Mejía, Hospital P. de Elizalde, Hospital del Niño de S. Justo, Laboratorio Bio Sidus, Buenos Aires, Argentina*

The aim of this trial was to investigate α-Interferon (IFN) as an alternative for the treatment of children with chronic idiopathic thrombocytopenic purpura (ITP) (thrombocytopenia; normal or increased megakaryocytes; exclusion of systemic disease (SLE, HIV); lasting more than 12 months from the date of diagnosis). Inclusion criteria: patients with platelet counts (PC) $<50 \times 10^9$/liter and without treatment during the last month. Children received IFN (3×10^6 units/Kg/dose), SC, 3 times a week, during 4 weeks; if partial ($<150 \times 10^9$/liter) or no response was obtained, the same dose was administered for other 8 weeks. In patients with favorable responses and posterior drop to pretreatment values, additional 4 weeks of treatment could be administrated. Eleven patients (aged 4 to 20 years), receiving 14 IFN courses, were included; all had received previous treatment with steroids and/or IgG, and one was splenectomized. Mean initial PC was $32\pm15 \times 10^9$/liter (10–50). A significant increase was achieved by 9 patients (81.81/6), 6 of them reaching values $>150 \times 10^9$/liter. A PC $> 50 \times 10^9$/liter was rapidly achieved (mean 12±7 days); the time required to reach the maximum PC was 38±38 days. All the responses were transitory: PC remained elevated throughout the treatment, but returned to initial values shortly after IFN discontinuation (mean 30±22 days). One patient had no change in PC, and another presented a drop of PC under basal values, but without bleeding. The "flu-like" syndrome was present in all the children during the first days. Neutrophil count decreased from 5.6 ± 3.4 to $3.4\pm1.8 \times 10^9$/liter (*p: NS*) throughout the treatment; no count $<1.0 \times 10^9$/liter was seen. No other adverse effect was observed.

IFN therapy induces a significant increase of PC in most of children and seems to be a valid alternative therapy during bleeding periods, as well as to improve PC prior to splenectomy.

P-1444 EVALUATION OF PROGNOSTIC FACTORS IN CHILDHOOD IDIOPATHIC THROMBOCYTOPENIC PURPURA

H. Donato, A. Picón, M.C. Rapetti, G. Schvartzman, D. Montes Varela, A. Galvagni. *Hospital del Niño de San Justo, Hospital A. Posadas, Policlinico Bancario, Hospital Zonal de Ezeiza, Buenos Aires, Argentina*

The evolution of the latest 514 children (aged 2 months to 15 years) with ITP (platelet $<100 \times 10^9$/liter; normal or increased megakaryocytes; exclusion of simultaneous systemic disease or infection) was reviewed; 416 of them were available for analysis. Mean age was 55.4±43.8 months, M:F ratio 1:1, and greatest frequency in spring (35.9%). The antecedent of preceding infection (API) - viral illness or attenuated live virus immunization - 6 weeks prior to presentation was detected in 175 children (42.1%), mainly upper respiratory tract infections (69.7%). Petechiae and ecchymosis were present in 99% of patients; epistaxis was the other most frequent hemorrhagic manifestation (42.7%). Clinical severity at diagnosis was classified as mild in 261 children (62.7%), moderate in 139 (33.4%) and severe in 16 (3.8%). Therapies received included: no treatment (220), steroids (179), IV IgG (27), Interferon (7), and/or splenectomy (17).

By 12 months from diagnosis, the recovery rate was 73.6% (306 children); 109 patients (26.2%) had chronic ITP, and 1 child died (0.2%) (intracranial hemorrhage). Age demonstrated to be the main prognostic factor, and 3 groups with significantly different ($p < 0.001$) recovery rates could be established: ***Group A:*** 2 to 12 months = 92.9% (65/70); ***Group B:*** 2 to 8 years = 74.9% (215/287); ***Group C:*** 9 to 15 years = 44.0% (22/50). The API showed prognostic value in Group C (78.6% vs 30.6% recovery rate for API and non-API children, respectively; $p < 0.01$), but not in the other age groups. Sex, severity, platelet count or steroids administration had no incidence on the final outcome of the disease. The time needed to achieve remission (57.5±68.3 days for the whole population) was significantly shorter ($p < 0.05$) in Group A than in the others (A = 50.7±69.3; B = 59.0±67.0; C = 67.1±84.3 days); it was not affected by API or any other factor.

The definition of groups based on age according to our results could be useful to establish differential guidelines for management of children with ITP.

P-1445 IMMUNE THROMBOCYTOPENIC PURPURA AND SPLENECTOMY: EXPERIENCE IN A SERIES OF 540 PATIENTS

Ma.T. Alvarez, M. Canales, M. Dávila[1], J. Sevilla, M. Morado, D. Hernández, M.T. Jiménez, C. Hernández, C. Fernández, J.L. Marijuan[1], A. Villar, M. Magallón, J.A. Rodriguez Montes, F. Hernández-Navarro. *Haemophilia Unit, Department of Haematology; [1]Department of General Surgery, Hospital "La Paz", Madrid, Spain*

Splenectomy for refractory immune thrombocytopenic purpura (ITP) is a surgical procedure associated to 70% of positive responses without increase of the morbidity and mortality. The aim of this study was to, analyze the response and complications (haemorrhagic and infectious) associated with splenectomy in patients with refractory ITP.

A total of 540 patients was diagnosed as ITP in our centre. Eighty-three patients undergone splenectomy between August 1975 and December 1997. The mean age was 36 years. The variables analyzed were: 1) preoperative platelet count; 2) platelet count at discharge (mean interval from surgery 1 week); 3) response to treatment 6 months later; 4); infectious complications over early and late postoperative course; 5) haemorrhagic complications.

The preoperative platelet count was 83×10^9/l and at discharge it was 432×10^9/l (data from the last four years). A positive response ($>30 \times 10^9$/l). was achieved in 81 patients (97.59%); complete response ($>150 \times 10^9$/l) and partial response ($30–150 \times 10^9$/l) was attained in 68 and 13 patients respectively. The platelet count (six months later) was lesser than 30×10^9/l in 2 cases alone. Postoperative infectious complications occurred in 20.4% of patients (10.7% in early and 9.7% in late postoperative course). One case of pneumococcal sepsis was documented nine years later. Haemorrhagic complications during surgery and postoperative course were also recorded in the last four years. A sole case of bruise in the splenic cell was observed among the 18 patients studied. Therefore, we can conclude that splenectomy is an effective treatment for patients with refractory ITP achieving a complete response in as high as 82% in our series. It is a safe procedure without evidence of an increased risk of haemorrhagic or infectious postoperative complications.

P-1446 LYMPHOCYTE IMMNUNOPHENOTYPE IN PERIPHERAL BLOOD AND IN BONE MARROW IN ACUTE AND CHRONIC THROMBOCYTOPENIA

D. De Mattia, B. Martire, P. Giordano, G.C. Del Vecchio, A. De Santis, F. Schettini. *Dipartimento di Biomedicina dell'Eta' Evolutiva, University of Bari, Italy*

An increase in immature B-cell precursors (CD19+, CD10+) has been described in bone marrow of children with non malignant haematologic diseases such as Agranulocytosis or transient Erytroblastopenia. Furthermore, increased numbers of B-cell expressing the pan T-cell marker CD5 in peripheral blood and spleen lymphocytes in Idiopatic Thrombocytopenic Purpura (ITP) patients has been found to be involved in autoantibody production.

In order to characterize lymphocyte subset anormalities and the immunological differences between acute and chronic ITP, we have performed a study of immunophenotype in peripheral blood and in bone marrow of children with acute and chronic ITP.

Materials and Methods: 11 children (6 M, 5 F) with acute ITP at onset, median age 6.5 years (range 2–11) and 13 children (6 M, 7 F) with chronic ITP, median age 8 years (range 2–14), in off therapy since I month, entered this study.

Immunophenotyping was performed by flow-cytometry using monoclonal antibodies on peripheral blood (CD3, CD4, CD8, CD56, CD19), and on bone marrow (CD10, CD19, CD20, CD21).

Results: Our data showed a significant increase in natural-killer (CD8−CD56+) and in cytotoxic lymphocytes (CD8+CD56+) in the two groups of patients compared with normal controls ($p < 0.01$) but no difference between the two groups. The analysis of on bone marrow revealed a significant decrease in immature B-cell precursors (CD19+, CD20+) in patients with chronic ITP compared with acute ITP ($p < 0.01$). We observed an association between the low number of B-cell precursors in bone marrow, the increase of T-cytotoxic lymphocytes (CD8+CD56+) in peripheral blood and chronic ITP. Further studies are necessary to assess if this immunological pattern can be considered a predictive floor of chronic ITP.

Research supported by a grant from MURST (40%).

P-1447 PLATELET KINETIC STUDIES IN PATIENTS DIAGNOSED AS IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

H. Louwes[2], J.Th.M. De Wolf[1], O.A. Zeinali Lathori[1], E. Vellenga[1]. [1]*University-and* [2]*Martini Hospital Groningen, The Netherlands*

We investigated the value of platelet kinetic studies in diagnosis and treatment of 150 patients diagnosed as ITP. Three subgroups could be distinguished. Group A, 11% of the patients, showed a (nearly) normal platelet survival $\geq$6 days (platelet count: median 40, range 11–86), suggesting that the thrombocytopenia is due to an insufficient bone marrow function. Group-B, 58% of the patients showed a normal or increased platelet production. These patients fullfill the conventional picture of ITP: a short platelet survival (1.7 ± 1.6 days), an increased clearance in the spleen in 66% of the patients and a good response to splenectomy: CR/PR in 89% of the cases. In group-C 42% of the patients showed a decreased platelet production. Compared to the patients from group B a significantly longer platelet survival (4 ± 2.4 days, $p < 10^{-6}$) was observed. In addition a significant lower percentage of complete or partial responders was noticed after splenectomy (62%, $p = 0.03$). The results in the last group suggest an ineffective bone marrow production of platelets, rather then an increased peripheral destruction. The response on therapy was not only determined by the platelet kinetic but depended also on sex differences. 56% of the men and 35% of the women showed a favourable response to glucocorticoids ($p = 0.04$). In conclusion, ITP is a heterogenous disease in which subgroups can be identified by platelet kinetic studies. The clinical relevance of this subdivision may become evident from future studies. Furthermore the platelet kinetic studies might be helpful in predicting the effect of therapy and in making therapeutic choices in ITP.

P-1448 IDIOPATHIC THROMBOCYTOPENIC PURPURA IN HODGKIN'S DISEASE

Lj. Tukic[1], D. Stamatovic[1], M. Maleievic[1], L. Ristic[1], Z. Trajkovic[2], S. Berger[2], N. Nunic[1]. [1]*Clinic of Hematology Military Medical Academy, Belgrade;* [2]*Institute of Pathology Military Medical Academy, Belgrade, Yugoslavia*

Immune thrombocytopenia is an uncommon complication of Hodgkin's disease (HD) appearing in patients in remission or with active disease. Response to steroids and/or splenectomy is usually good and prognosis is related to the status of the lymphoma.

We report 2 patients with idiopathic thrombocytopenic purpura (ITP) complicating HD each with a hitherto undescribed feature. In both cases ITP was established after the patients had been successfully treated for HD. The patients were heterogeneus for initial stage of HD, histology and received treatment. The median time from diagnosis of HD to diagnosis of ITP was 68.5 months (range 3 to 134 months). The ITP responded to a single course of oral prednisolone in one patient and he has been in stable remission for more than two years. The second patient, who did not respond to prednisolone, was splenectomised and platelet counts normalised after splenectomy. In the spleen HD was established and this patient was undergoing first line chemotherapy (he is still under treatment).

Thus, the occurrence of ITP in patients with HD in remission, appears to be independent of activity of lymphoma (1st case) and it responded to the therapy as for primary ITP. In the second case ITP preceeded the late relapse of HD and responded to splnectomy and antihodgkin's therapy.

P-1449 IS THE GLUCOCORTICOID THERAPY SUITABLE TO THE INITIAL TREATMENT FOR PATIENTS WITH THE CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA?

T. Kosuge, M. Kato, K. Shikoshi, M. Takata, M. Katoh, M. Umeda. *1st department of Internal medicine, school of Medicine, Toho University, Tokyo, Japan*

Glucocorticoid therapy is recommended to the initial treatment for patients with the chronic idiopathic thrombocytopenic purpura (ITP). But, the glucocorticoid therapy often induces osteoporosis as its most problematic adverse effect. Cepharanthin is a biscoclaurin alkaloid prepared from Stephania cepharantha which is widely used clinically in Japan, and its action includes the inhibition of lipid peroxidation and the stabilization of hematopoietic cells. In addition, this agent is reported to be very useful for the treatment of ITP.

Accordingly, we measured the bone mineral content and some biochemical markers of the bone metabolism in each patient with ITP who has been treated with the glucocorticoid or cepharanthin.

30 patients with ITP were classified into P group of 16 patients who were administered the prednisolone and C group of 14 patients who were administered only cepharanthin. The bone mineral content was measured by the microdensitometry. We determined serum alkaline phosphatase (ALP), ALP isozyme, osteocalcin, intact parathyroid hormone (PTH-intact), urinary pyridinoline (PR), and deoxypyridinoline (DPR) as the biochemical marker of bone metabolism.

Results: 1) The bone density and cortical thickness index in the P group were significantly reduced. On the other side, those in the C grog remained within the normal range.

2) PR and DPR in these markers were remarkably increased in the P group compared with the C group.

Conclusion: In Japan, cepharanthin is usually used to promote the recovery of leukocytes during cancer therapy and to treat ITP. No serious adverse effect has been observed. These results in this study show that cepharanthin may be more suitable to the initial treatment for the moderate to mild cases in the patients with ITP than the glucocorticoid.

P-1450 HUMAN PLATELET ANTIGEN-5 SYSTEM POLYMORPHISM IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

V. Castro, A.F. Origa, G.B. Oliveira, J.M. Annichino-Bizzacchi, F.F. Costa, V.R. Arruda. *Hematology and Hemotherapy Center, State Universty of Campinas, SP, Brazil*

The antibodies involved in the development of Idiopathic Thrombocytopenic Purpura (ITP) have frequently no specificity against platelet specific antigens, although glycoprotein (Gp) IIb/IIIa complex has been described as a target for these antibodies. Recently, Kim *et al* (Thromb Haemost 78: 254, 1997) have studied the frequencies of HPA-1 to 5 system in patients with ITP and have shown an association between HPA-5 system polymorphism and the development of the disease. This system is located in Gpla, which is physicochemically identical to VLA-2 on long-term activated lymphocytes. VLA-2 molecules have a main hole in the regulation of the adhesion and in cytokine production by T activated cells. Thus, distinct HPA-5 system genotypes could be involved in the lymphocyte-mediated platelet cytotoxicity in ITP. In this study we describe the frequencies of HPA-5 system genotypes in a group of 67 patients (19 males, 48 females), median age of 37.6 years (13–80 years) carrying ITP, followed in the Hematology and Hemotherapy Center

of State University of Campinas and in a group of 87 consecutive neonates in the same University Hospital, as a control group. The patients' group was composed by 15 individuals with acute (A-ITP) and 52 individuals with chronic disease (C-ITP). Genotyping was carried out by DNA-based methods. No significant differences were found when we compared the prevalences of homozygous individuals to allele *a* (*aa*) among patients with A-ITP, C-ITP and the control group (67, 75 and 83% respectively). The same was found among the homozygous to allele *b* (*bb*) (6, 0 and 2% respectively) and among the heterozygous (*ab*) individuals (27, 25 and 16% respectively). The difference was not even significant when we compared the genotype frequencies of all ITP patients to the control group (*aa*- 73 vs 83%; *ab*- .25 vs 16%; *bb*- 2 vs 1%). Our data failed to demonstrate a clear association between HPA-5 system polymorphism and the physiopathogenicity of ITP.

P-1451 HEAVY-CHAIN AND LIGHT-CHAIN PHENOTYPE COMPOSITIONS OF GLYCOPROTEIN-SPECIFIC IgG ANTI-BODIES IN CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

D. Stockelberg, M. Hou, H. Wadenvik. *Haematology Section, Department of Medicine, Sahlgrenska university hospital, Göteborg, Sweden*

The purpose of the present study was to define the heavy-chain and light-chain phenotype compositions of the glycoprotein (GP)-specific IgG antibodies in chronic ITP. Sera from 43 patients were analyzed with the MAIPA technique and a panel of monoclonal antibodies (MoAb) capturing each of the five major GPs or GP complexes, namely GPIb/IX, GPIIb/IIIa, GPIa/IIa, GPIV, and GPV. Twenty unequivocal positive reactions were detected among 16 of the 43 patient sera. The heavy-chain phenotypes of the GP-specific antibodies were determined by reassessing the positive patient sera using a subclass-specific MAIPA assays. This approach enabled us to define the heavy-chain phenotypes for 11 (55%) of the 20 GP-specific antibodies. Eight of these 11 (73%) GP-specific "antibodies" displayed a restricted heavy-chain phenotype; the remaining 3 "antibodies" (27/6) expressed mixed heavy-chain phenotypes. The subclass distribution of the GP-specific antibodies were IgG1 (6/11, 55%), IgG3 (6/11, 55%), IgG2 (2/11, 18%), and IgG4 (0/11, 0%). Also, light-chain phenotypes of the GP-specific antibodies were assessed in a kappa- and lambda-specific MAIPA assays. Eighty % (16/20) of the GP-specific antibodies showed a restricted kappa of lambda light-chain phenotype; 20% (4/20) expressed mixed light-chain phenotypes. Moreover, in 6 patients GP-specific antibodies were found to be both light-chain and heavy-chain restricted. These results suggest that GP-specific antibodies are derived from a restricted number of B-cell clones, and that only a limited number of autoantigenic epitopes are present on the platelet surface.

P-1452 HIGH-DOSE INTRAVENOUS IMMUNOGLOBULINS: ADVERSE REACTIONS IN 125 PATIENTS WITH ITP

C. Schiavotto, M. Ruggeri, G. Castaman, F. Rodeghiero. *Haemophilia and Thrombosis Center, Haematology Department, S. Bortolo Hospital, Vicenza, Italy*

Background: High-dose intravenous immunoglobulins (H.D.Ig) are a therapeutical approach used in various haematological and non-haematological disorders. At the moment, there are few available data about the rate and severity of adverse reactions of H.D.Ig, usually considered a safe and well-tolerated treatment.

Patients and Methods: Clinical records of 125 consecutive ITP patients (90 F/35 M, median age 54 y, range 8–84 y), treated at our department between 1985 and 1997 have been reviewed.

Results: 6 patients experienced severe side effects: aseptic meningitis (n = 3), acute renal failure requiring dialysis (n = 1), severe skin reactions (n = 2, erythema multiforme ed diffuse purpuric erythema). I patient with aseptic meningitis developed also transitory alopecia. 7 further patients suffered moderate side-effects: headache (n = 3), fever with chills (n = 1), anaphylactic/oid reactions (n = 3). Thus, 13/125 patients (10%) experienced side effects, severe and unpredictable in 6 of them (5%).

Conclusions: H.D.Ig may cause severe, life-threatening adverse reactions. It is mandatory to carefully evaluate the indications to this expensive treatment.

P-1453 FAMILIAL STUDY IN SUBJECTS WITH THROMBOCYTOPENIA

M. Ferrara, G. Ponte, S. Chiariello, G. Napoli, F. Gangone, A. Coppola, L. Coppola, L. Esposito. *Department of Pediatrics, II University, Naples, Italy*

Thrombocytopenias are a very heterogeneous group of acquired or inherited disease. In many kindreds different hereditary forms of thrombocytopenia has been documented and, between these, the "isolated hereditary thrombocytopenias", inherited either as an X-linked recessive trait or as an autosomal dominant trait. In these cases, in some instances, the affected family members are asymptomatic with a mild reduction of platelet's number, normal platelet function and normal megakaryocytopoiesis, and some time a shortened platelet's survival. In the last years we have observed 350 children with thrombocytopenia, and, in 24 cases, the laboratory data showed a mild reduction of platelet's number (range 80.000–100.000/mmc) in many family members virtually asymptomatic. In 11 of these last cases the patients, all male, aged between 9 months and 3 years, were asymptomatic for bleeding or purpura with range of platelet's number between 70.000 and 100.000/mmc, and normal values of megakariocytes in bone marrow; clinical manifestations of athopic eczema and recurrent mild othitis or/and infections of respiratory tract were present; IgE levels were >100 U.I./ml with a positive Prick-test and Rast for milk cow proteins, IgA levels were increased in 5 cases too. The lymphocyte panel showed in 7 of these cases reduction of CD4 and increase of CD8. In the other 13 patients, 5 male and 8 female, aged between 3 and 9 years, we have observed bleeding and/or purpura with platelet's levels range between 20.000–30.000/mmc and normal values of megakaryocytes. IgE levels were increased >100 U.I./ml with urticaria only in 3 female. Thrombocytopenia in all these last patients followed a viral disease and only in 5 the evolution was chronic. It's possible that many thrombocytopenias are inherited X-linked diseases, with an immunological disorder, like but milder than that observed in WAS (Wiskott-Aldrich syndrome) probably for an involvement of the immunological genes placed on the chromosome X. The hypothesis that there is a greater incidence of ITP in subjects with asymptomatic "isolated hereditary thrombocytopenia" - autosomal or X-linked - after some viral diseases, must to be tested with an accurate anamnesis and the study of the family members, as in aplastic anaemias and heterozygous for Fanconi's anemia.

P-1454 THE GLUCOCORTICOSTEROIDES IN THE TREATMENT OF CHRONIC THROMBOCYTOPENIC PURPURA (ITP)

J. Tasic, M. Bakic, I. Tijanic, L. Macukanovic, G. Marjanovic, J. Antovic, M. Milenovic. *Clinic of Hematology and Clinical Immunology, Clinical Center Nis, Serbia, Yugoslavia*

Background: The glucocorticosteroides act primarily by immediately inhibiting the reticuloendothelial system and for a long run may slightly decrease antibody production.

Methods and Patients: An initial dosage of prednisolone was 1–2 mg per kg per day, in a period of 1–4 weeks. Rarely higher doses or "pulse therapy" were used. The clinical response was consider complete (CR) for platelet increase above 100 and partial (PR) if platelets were between 30 and 100 $\times$ 10^9/l. After a response doses were trapped slowly. Response to steroid therapy and moment of response achievement were compared according to age, sex, long time history of severe thrombocytopenia prior to the therapy. Between December 1988 and January 1998 104 patients were treated (68 women and 36 men), with mean age of 46 years (range 25–78).

Results: From 104 patients (pts) CR was achieved in 48 (46%), PR in 30 (29%). From 48 pts with CR, 43 achieved response in the first two weeks and 5 in next two. From 30 pts with PR it was 12 pts in the first two weeks and 18 in next two. In 34 pts older than 55 years, CR was in 8, PR in 11. Maximal response according to elevation of platelets count was within 4–10 days.

Interpretation: Our data shown that the best response was at the end of first week of treatment, while the worse response was in patients older than 55 years with delayed response to the corticosteroides in patients who had severe thrombocytopenia for a longer period prior to therapy.

P-1455 INTRAVENOUS IMMUNE GLOBULIN AND RESPONSE TO SPLENECTOMY IN CHRONIC IDIOPATHIC PURPURA

M.J. Peñarrubia, A. Jiménez, I. Recio, J. Conde, M.C. Bañuelos, L.J. García-Frade. *Hospital "Del Rio Hortega", Valladolid, Spain*

Recent reports suggest that response to intravenous immune globulin may predict response to splenectomy in patients with chronic idiopathic purpura. We have retrospectively analyzed the relatioship between the outcome of previous course of intravenous immune globulin therapy and that of subsequent splenectomy in a consecutive series of patients with chronic idiopathic purpura treated at our institution over a ten year period.

Results: Fourteen patients (11 females, mean age 37 years, range: 9–73) fulfilled inclusion criteria. The mean interval between diagnosis of chronic idiopathic purpura and splenectomy was 22 months (range: 2–108 months). All but three patients received 0.4 grams of immune globulin per kilogram for five consecutive days. The remaining three patients received 1 gram per kilogram per day for two consecutive days. Response was considered good with a platelet count greater or equal to 50 × 109 per liter, and no response when it was below that level. Mean follow-up after splenectomy was 58 months (range: 8–152).

INTRAVENOUS IMMUNE GLOBULIN	SPLENECTOMY		
	Response	No response	TOTAL
Response	10 (100%)	0	10
No response	2 (50%)	2 (50%)	4
TOTAL	12 (86%)	2 (14%)	14

Therefore, response to intravenous immune globulin predicts response to splenectomy with a sensitivity of 83% and a specificity of 100%. Positive predictive value is 100% and negative predictive value is 50%.

Conclusion: Our results provide evidence that splenectomy has a role even in those patients who did not respond to intravenous immune globulin, in keeping with other previous observations but also in need of further data.

P-1456 HIGH-DOSE DEXAMETHASONE AS A TREATMENT OF IDIOPATHIC THROMBOCYTOPENIC PURPURA

F. Borst, J.J. Keuning, H.A.M. Sinnige, L.H. Van Hulsteijn, G. Vreugdenhil. *Sint Joseph Ziekenhuis Veldhoven, Bosch Medicentrum 's-Hertogenbosch, Sint Joseph Ziekenhuis Veghel, the Netherlands*

Long-term administration of prednisone, currently the first choice treatment of idiopathic thrombocytopenic purpura (ITP), is known to have many side effects. Short regimens of high dose dexamethasone may produce less side effects, thus being a more convenient treatment. The aim of our study is to examine the efficacy and toxicity of pulsed high-dose dexamethasone (HD) as a first and second line treatment of ITP.

Patients and Methods: 25 patients with ITP (defined by a low platelet count, normal bone marrow and the absence of other causes of thrombocytopenia) were analysed prospectively. They were given 40 mg of oral dexamethasone daily for 4 consecutive days. This regimen was repeated on day 29. After the maximal response was obtained, the patients recieved one additional course, with a maximum of 6 courses. Responses were defined as follows: complete response (CR): a thrombocyte count persisting on a normal level (>150 × 10^6/l) after treatment; partial response (PR): a thrombocyte count >50 × 10^6/l; no change (NC) no raise in thrombocyte count or a decline below 50 × ^{6}10/l within 4 weeks after treatment. Study parameters were: response, relapse rate, toxicity and dependency on further or additional treatment after HD treatment was completed.

Results: 11 patients received HD as a first line treatment; 3 had a CR after a follow-up of 6–25 months, 4 patients had a PR after a follow-up of 1–18 months and 4 had NC after a follow-up of 2–26 months. 14 patients received HD as a second line treatment after prednisone or splenectomy; 3 of them had a CR after a follow-up of 6–25 months, 2 patients had a PR after a follow-up of 10–25 months and 9 had NC after a follow-up of 0–13 months. 4 patients with NC after first or second line HD were splenectomised, all successfully with a follow-up of 3–8 months. Side effects were less severe than those experienced during prednisone therapy; reported were fatigue, dizziness and epigastric discomfort; one patient refused further HD because of severe dizziness.

Conclusion: these results suggest that HD provides a well tolerated regimen for both first and second line treatment of ITP. As a second fine treatment however, HD seems to be less effective. Extended studies are required to establish the definite role of HD in the management of patients with newly diagnosed ITP or relapsed ITP.

P-1457 T-LYMPHOPENIA IN IDIOPATHIC THROMBOCYTOPENIC PURPURA IS MAY BE DUE TO DEFECTIVE Fas EXPRESSION

A. Mouzaki, M. Theodoropoulou, E. Mavroides, M. Karakantza, D. Koliperas, N. Beratis, A.K. Maniatis. *Laboratory Hematology and Transfusion Medicine & Dpt. of Pediatrics, Patras University School of Medicine, Patras, Greece*

Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by the production of antibodies against antigens on the membranes of platelets. We and others have observed that ITP patients are lymphopenic (see Table 1). Presently we show data supporting the hypothesis that constitutive expression of the Fas gene is maybe a reason for this lymphopenia. Blood mononuclear cells from 13 normal controls and 10 children suffering from acute ITP were isolated before and 5 days after intravenous immunoglobulin G (IVIG) administration. The cells were counted, phenotyped and assayed for Fas and Bcl-2 gene expression. RNA was isolated directly or after in vitro cell culture with or without mitogens and gene expression studied by semi-quantitative RT-PCR. T-lymphocytes in ITP patients were decreased and the CD4/CD8 ratio inverted regardless treatment (Table 1).

WBC from:	CD3	CD2	CD4	CD8	CD16	CD19	CD4/ CD8
Normal controls (n = 13)	66.5 (±7.7)	43.5 (±5.5)	37.1 (±9)	20 (±3.9)	8 (±4.1)	5.7 (±2.4)	**1.84**
ITP patients (n = 10)	50 (±11.3)	34.5 (±3.5)	13.3 (±3.5)	15.8 (±3.7)	9.5 (±9.2)	8.8 (±5.8)	**0.84**

Our results on Fas and Bcl-2 gene expression are summarised in Table 2:

Genes	WBC from normal controls			WBC from ITP patients before treatment			WBC from ITP patients after IVIG treatment		
	Culture conditions			Culture conditions			Culture conditions		
	ex-vivo	CM	CM + PHA + PMA	ex-vivo	CM	CM + PHA + PMA	ex-vivo	CM	CM + PHA + PMA
β_2-micro	+	+	+	+	+	+	+	+	+
Fas	-	-	+	+	+	++	+	+	+
Bcl-2	+	+	+	+	+	+	+	+	+

In conclusion the data indicate that in ITP patients the Fas gene is constitutively expressed while the expression of Bcl-2 remains unchanged compared to normal controls thus not counteracting the effect of Fas expression. This Fas/Bcl-2 imbalance seems to result in increased levels of apoptosis.

P-1458 PROSPECTIVE STUDY ON THE CLINICAL SIGNIFICANCE OF SPECIFIC ANTI-PLATELET AUTOANTIBODIES IN PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA

F. Fabris, I. Cordiano, R. Sartori, R. Tassan, R. Ramon, A. Girolami. *Istituto Semeiotica Medica, University of Padua, Medical School, Italy*

Autoantibodies against platelet surface glycoproteins (PGP) are assumed to be the cause of peripheral platelet immune-destruction in primary as well as in secondary immune thrombocytopenia (ITP). However, despite sensitive and specific antigen-capture assays, specific anti-platelet autoantibodies are detectable in only about 600%% of patients. Furthermore the clinical significance of these autoantibodies is unknown and the a diagnosis of primary ITP is still made on clinical grounds. We studied 57 patients with a clinical diagnosis of chronic primary ITP without any treatment at time of investigation. In serum of the patients we searched, by monoclonal antibody immobilization of platelet antigens assay (MAIPA), for the prevalence of specific anti-platelet

autoantibodies. Autoantibodies were found in 27 of the patients (47%); in 14 patients the autoantibody reactivity was single (GPIIb-IIIa 15%, GPIb 18%, GPIa-IIa (19%) and in 13 was multiple (48%). Patients were then selected on the basis of MAIPA positivity and the clinical features of the two groups of patients were prospectively compared. The results are the following:

Patients	PAIgG positive	Platelets $\times 10^9$/L	age yrs	sex F/M	months of disease	Hemorrhagic symptoms
MAIPA positive	18/27	45±25	46±17	20/7	44±75	15/27
MAIPA negative	19/30	47±24	46±15	22/8	31±46	20/30

MAIPA positivity was not related with platelet count, PAIgG positivity, time of disease or haemorrhagic symptoms at presentation. We conclude that the presence of specific anti-platelet autoantibodies in the serum, does not select a group of patients with primary ITP with different clinical features at presentation. As far as the clinical course and the response to treatment, the study is still in progress.

P-1459 PREDICTIVE VALUE OF HIGH DOSE IMMUNE GLOBULINS G (IVGG) THERAPY FOR THE SPLENECTOMY RESPONSE IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA (ITP)

F. Fabris, R. Tassan, M.L. Randi, G. Luzzatto, B. Soini, A. Girolami. *University of Padua, Institute of Medical Semeiotics, Italy*

Several factors have been investigated as positive preoperative predictors for splenectomy response. However most of the studies have yielded conflicting results and only the young age of patients seems to be approved as predictive element for a favourable response to splenectomy. Splenectomy and the treatment with IVGG have theoretical similar mechanisms to prolong platelet survival in ITP; they remove and block the reticuloendothelial system. In view of this mechanism, the previous response to IVGG was proposed as predictive for the response to splenectomy. We analysed retrospectively the response to splenectomy in 19 with chronic ITP who were treated, before surgery, with a course of IVGG (0.4 gr/kg/bw during 5 consecutive days). 11 patients were females, 8 were males (mean age of 48±19SD yrs, range 11–73 yrs). The mean time from the diagnosis of ITP to splenectomy was of 9.1±18.3 yrs (range 0.5–60 yrs). The mean time of follow-up after splenectomy was 3.25±2.55 yrs (range 6 months to 7.7 yrs). The patients were considered responsive to IVGG and to splenectomy if platelets increased above 50000/μl. The relationship between IVGG and splenectomy is summarized as follows:

		Splenectomy Responsive	Refractory	Total
IVGG	**Responsive**	10 (91)	1 (9)	5 (58)
	Refractory	5 (62)	3 (38)	8 (42)
(%)	Total	15 (79)	4 (21)	19

The percentage of responders to IVGG and to splenectomy was respectively 58 and 79. The relationship between IVGG and splenectomy response was not statistically significant (chi-square test). However a good response to IVGG may be associated with a subsequent high rate (91%) of positive response to splenectomy whereas the patients who did not respond to IVGG, have yet good chances (62%) to respond to splenectomy.

P-1460 INDIRECT STUDY OF THROMBOPOIESIS (THROMBOPOIETIN, RETICULATED PLATELETS AND GLYCOCALICIN) IN PATIENTS WITH HEREDITARY MACROTHROMBOCYTOPENIA

F. Fabris, A. Steffan[1], I. Cordiano, J.L. Nichol[2], R. Ramon, A. Girolami. *Istituto Semeiotica Medica, University of Padua; [1]Bood Bank CRO Aviano, Italy; [2]Amgen Inc, USA*

Chronic isolated hereditary macro-thrombocytopenia (CHMT) represents the most frequent form of familial thrombocytopenia. This disease is characterized by macro-thrombocytopenia with autosomal-dominant inheritance, mild haemoerrhagic diathesis and absence of other clinical and laboratory abnormalities. We studied 29 patients belonging to 16 unrelated families suffering from CHMT. An indirect evaluation of megakariopoiesis was performed by measuring the percentage of reticulated platelet (RP), the level of serum thrombopoietin (TPO) and plasma glycocalicin (GC). Thiazole Orange was used for labeling RP and fluorescence was analyzed by flow-cytometry. The TPO measurement was kindly performed at Amgen Inc., Usa, by sandwich ELISA using rabbit policlonal antibodies. GC, expressed as plasma GC and as GC index (GC μg/ml $\times$ 250 $\times$ 10^9/L/ individual platelet count), was performed by ELISA using two different monoclonals. 23 patients with chronic immune thrombocytopenic purpura (ITP) and 17 patients with thrombocytopenia due to decreased bone marrow production (Hypoplasia) were also studied. Results are the following:

Patients	Platelet $\times 10^9$/L	RP %	TPO pg/ml	GC μg/ml	GC index
CHMT (29)	66±25	2.2±1.3	205±87 (15)	0.6±0.2	2.0±1.1
ITP (23)	40±21*	8.8±7.9*	360±173 (20)*	0.8±0.9	10.8±18*
Hypop. (17)	30±23*	1.9±1.3	not performed.	0.1±0.04*	0.8±0.1*
Normal (60)	238±67*	0.9±0.5*	104±69 (96)*	0.8±0.16	0.9±0.2*

* statistically significant difference (<0.05) versus CHMT patients.

Patients with CHMT presented a mild but significant increase of RP, TPO and GC index compared to normals; however the levels are considerably lower than those of patients with ITP, being similar to those of patients with hypoplasia. In conclusion the megakariopoiesis in CHMT seems to be both impaired and ineffective.

P-1461 REDUCTION IN LIPOPOLYSACCHARIDE-INDUCED THROMBOCYTOPENIA BY TRIFLAVIN IN A RAT MODEL OF SEPTICEMIA

J.R. Sheu, W.C. Hung. *Graduate Institute of Medical Sciences, Taipei Medical College, Taipei, Taiwan*

Thrombocytopenia frequently occurs early in the course of gram-negative bacterial infection, and is usually identified only when an infection has become systemic. Triflavin, an Arg-Gly-Asp-containing disintegrin purified from snake venom, has been suggested to have an antiplatelet activity by interfering with the interaction of fibrinogen with the glycoprotein (GP) IIb/IIIa complex. The present study was majority to determine whether triflavin could prevent thrombocytopenia in lipopolysaccharide (LPS)-treated rats. In this study, ^{51}Cr-labeled platelets were used to assess blood and tissue platelet accumulation after LPS challenge. The administration of LPS (4 mg/kg, i.v. bolus) for 4 hours induced a reduction in radiolabeled platelets in blood and an obvious accumulation of platelets in liver. Triflavin (500 μg/kg) but not GRGDS (20 mg/kg) significantly prevented the alteration of radiolabeled platelet distribution in blood and liver induced by LPS. Furthermore, triflavin but not GRGDS markedly suppressed the elevation in plasma thromboxane B_2 concentration within the 4 hour period of LPS administration. In LPS-treated rats, 5-hydroxytryptamine level was lower in the blood and higher in the liver as compared with normal saline-treated rats. Pretreatment with triflavin (500 μg/kg) significantly reversed the 5-hydroxytryptamine (5-HT) concentration in blood and liver of LPS-treated rats, however, GRGDS administration showed no significant effect in this reaction. In histological examinations and platelet adhesion assay, triflavin markedly inhibited the adhesion of platelets to subendothelial matrices in vivo and in vitro. These results indicate that triflavin effectively prevents thrombocytopenia during LPS-induced septicemia in rats. This prevention appears to occur through the following two mechanisms: (1) Triflavin markedly inhibits platelet aggregation, resulting in decreased thromboxane A_2 formation. (2) It inhibits the adhesion of platelets to subendothelial matrices, thereby leading to a reversal in the distribution of platelets in blood and liver in LPS-treated rats.

P-1462 POTENTIAL OF CORIPHOSPHINE O FOR THE MEASUREMENT OF RETICULATED PLATELETS

M.S.C. Robinson[1], A. Horton[2], J. Wyatt[2], I.J. Mackie[1], S.J. Machin[1], P. Harrison[1]. [1]*Department of Haematology, UCL, UK;* [2]*Coulter Electronics, Miami, USA*

Several fluorescent nucleic acid dyes have been used for quantification of reticulated platelets by flow cytometry. Although Thiazole Orange (TO) has been widely utilised, the dye has several disadvantages for the purposes of rapid and reliable automation. Not only is there wide variation in methodology, but a prolonged incubation of at least 30 minutes is required and the dye also labels dense granular ADP non-specifically. More recently coriphosphine O (CPO) (Coulter Electronics, Miami) and other dyes have been developed for automated reticulocyte analysis, and may be applicable to reticulated platelet analysis. In this study, the labelling of platelets with CPO was examined in detail to investigate the possibility of reticulated platelets being measured via a simple, rapid, automated method. 5 µl of whole blood or PRP was incubated with 1 ml of CPO solution (8 µg/ml) for different time periods (up to 30 minutes) and the specificity of labelling determined by degranulation experiments and RNAse incubation. Labelling was shown to be rapid (<1 minute) resulting in a Mean Fluorescence Intensity (MnI) at 675 nm of 1.28±0.099 (n = 3). RNA specificity was confirmed by pre-treatment of cells with RNase (50 µg/ml) (0.78 MnI±0.262 2SD, N = 3) following permeabilisation. Degranulation experiments with 80 µM TRAP (verified by CD63 and CD62p) also suggested that the dye is non-specifically labelling dense granules (0.78 MnI±0.365 2SD, N = 3). Combination experiments with RNase and TRAP resulted in near elimination of the total fluorescence to near background levels (0.52 MnI±0.21 2SD, N = 3). Significant uptake of dye into dense granules was shown to be time dependent and could be abrogated by TRAP degranulation. Nevertheless CPO labelling of reticulated platelets peaked prior to (as measured by both % and MnI) platelet recovery in a patient with chronic relapsing TTP, in a manner similar to TO labelling. These results demonstrate the potential of CPO for the rapid measurement of reticulated platelets.

P-1463 LAPAROSCOPIC SPLENECTOMY FOR RELAPSING THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

R. Rahmani, S. Gillis, O. Ben-Tal, A. Szold, A. Eldor. *Tel-Aviv Sourasky Medical Center, Tel-Aviv University, Tel-Aviv and The Hadassah University Hospital, Jerusalem, Israel*

With the advents of plasma therapy, remissions can now be attained in most cases of TTP, however approximately one-half of the patients will relapse. While relapses are usually milder they still carry a significant mortality and preventive therapies are not always effective. In a review of 38 TTP patients (Am. J. Med 83: 437, 1987) we found that patients with relapsing TTP who underwent splenectomy had significantly longer disease free intervals between the first episode and the subsequent relapse. We therefore performed an elective splenectomy during remission in all our patients who presented with relapsing TTP. This report describes 10 patients who were followed after splenectomy for 2 months–10 years. The patients, 4 males 5 females, median age 32 years, had between 1–15 TTP episodes (median 4.2) prior to the operation. Seven patients had an excellent response with no further relapses. One patient who had 15 TTP episodes during one year prior to splenectomy had much fewer and milder episodes which responded to plasma therapy. A second patient had one relapse 8 years after splenectomy. Our last four patients underwent a laparoscopic splenectomy uneventfully and were discharged from the hospital 48–72 hours after the operation in an excellent clinical condition. We found six reports of 17 additional patients who underwent an elective splenectomy for relapsing TTP. None of these cases had a relapse during a follow up of 2–10 years. Based on these results and our experience and despite the fact that the rationale for splenectomy and the relevant pathomechanisms involved are still obscure, this operation seems highly indicated. It should be performed after the first relapse when the patient is in a hematological remission. Laparoscopic splenectomy which offers excellent results with minimal morbidity and very rapid recovery is highly recommended.

P-1464 IS THERE A ROLE FOR DEFIBROTIDE IN THE TREATMENT OF THROMBOTIC THROMBOTICYTOPENIC PURPURA?

L. Levato, F. Iuliano, D. Levato, S. Molica. *Divisione Ematologia, Azienda Ospedaliera "Pugliese-Ciaccio", Catanzaro, Italy*

In the last few years the systematic use of plasma exchange drammatically changed the beahaviour of patients suffering from thrombotic thrombotic-topenic purpura (TTP). Antiplatelet agents (i.e.. dipiridamole, aspirine) although decrease the rate of mortality at 15 days, do not affect response rate. Results dealing with defibrotide, a polydeoxyribonucleotide extracted wich increases the generation of prostacyclin from vascular endothelium, rely on anectodal reports and include a wide range of thrombotic microangiopatic disorders. In this context we would like to report our 7-year experience based on 6 consecutive patients diagnosed as having primitive TTP. All patients were females and median age was 35 years (range, 17–68). At the admission all patients presented severe microangiopatic anemia and thromboticytopenia as well as neurological manifestations. Based on 4 clinical and hematological features proposed byn Rose and Eldor (i.e., neurological impairement, renal impairement, low platelet count and decreased hemoglobin level) patients scored as follows: score 8, one patient, score 6, 3 patients; score 5, 2 patients. In all instances therapy consisted of plasma exchange sessions carried out daily until a clinico-hematological response was achieved. Concomitant steroid therapy (PDN, 2 mg/Kg/day) was given virtually to all patients along with i.v. defibrotide (10–25 mg/Kg/day). Four out of 6 (66.6%) patients were considered early responder on the basis of a platelet count equal or higher than 80 $\times$ 10^9/l and LDH value lower than 400 U/l after 7 days from the starting of diagnosis. The use of defibrotide was not associated with side effects. A patients who received maintainance defibrotide therapy experienced TTP relapse 30 days later its discontinuation. Interestingly, a complete remission was again achieved with i.v. defibrotide administred as a single treatment.

In conclusion, our results although based on a small and not controlled TTP patient series lend support to the use of defibrotide as complementary therapy in such a disease. Randomized, prospective studies are warranted.

P-1465 UTILITY OF SPLENECTOMY IN THE PREVENTION OF RELAPSES IN THROMBOTIC THROMBOCYTOPENIC PURPURA

E. Real, E. Pastor, M. Perella, E. Grau. *Hospital Lluis Alcanyis. Conselleria de Sanitat, Xativa, Spain*

Thrombotic thrombocytopenic purpura (TTP) is a rare, life-threatening disorder of unknown pathophysiology. Treatment with plasma exchange has reduced mortality to 20%. However, between 20 and 40% or surviving patients have relapses. Plasma exchange therapy is usually effective in treating relapses, but this treatment exposes the patient to blood products from numerous donors and does not reduces the relapse rate. We describe a patient with multiple relapses of TTP who was successfully treated with splenectomy. A 27-year-old female had been diagnosed as having TTP in May 1992. Clinical manifestations in the initial episode or TTP were fatigue, headache, hematuria, petechiae and hematomas. The initial episode and two early recurrences through 1992 were successfully treated with fresh plasma transfusions, plasma exchange, corticosteroids and vincristine. The patient remained in remission until 1994. Between March 1994 and February 1996, this patient bad five relapses (incidence of 2.5 relapses/year). The disease-free interval varied from 3 weeks to 9 months. The patient repeatedly responded on therapy plasma infusions and prednisone. Splenectomy was performed 18 days after the last relapse. The patient remains in complete remission without maintenance therapy 2 years after splenectomy. The role of splenectomy in the treatment of TTP is still subject of controversy. Splenectomy has been used primarily in patients in whom plasma exchange failed to improve haematologic values, but it was associated with a high mortality. Elective splenectomy was done in our patient after remission of the seventh relapse, and two years later the patient remains in remission. Our results suggest that splenectomy should be considered in patients with relapsing TTP during haematologic remission.

P-1466 THROMBOPOIETIN, INTERLEUKIN-6 AND P-SELECTIN AT DIAGNOSIS AND AT POST-STEROID RECOVERY PERIOD OF PATIENTS WITH AUTOIMMUNE THROMBOCYTOPENIC PURPURA

İ.C. Haznedaroğlu, Y. Büyükaşık, A. Koşar, Ş. Kirazlı, S. Dündar. *Hacettepe University Medical School, Department of Haematology, Ankara, Turkey*

Plasma levels of the most potent megakaryocytopoietic cytokines thrombopoietin (TPO), and interleukin-6 (IL-6), and the platelet activation marker P-selectin were evaluated in 24 patients with autoimmune thrombocytopenic purpura (ATP) who responded to conventional steroid treatment, at diagnosis and after steroid-induced recovery. TPO (median [interquartile range] = 0 [17.52] pg/ml) was significantly decreased, and IL-6 (38 [19.75] pg/ml) and

P-selectin (485 [393.75] ng/ml) were significantly elevated at diagnosis compared to healthy subjects (100 [68] pg/ml, 8 [7] pg/ml and 166 [69] ng/ml, respectively). After steroid treatment, all values approached to normal, i.e. TPO (20 [18.75] pg/ml) increased and, IL-6 (19.5 [13] pg/ml) and P-selectin (248 [172.5] ng/ml) decreased, significantly. Decrease of TPO in ATP is suggested to occur due to increased megakaryocyte mass and consequently, TPO clearance. The non-lineage-specific cytokine IL-6 may be elevated for compensatory megakaryocytopoiesis/thrombopoiesis. Elevation of P-selectin may reflect compensatory platelet hyperactivation, however this molecule also might be a marker of platelet destruction.

P-1467 ROLE AND SIGNIFICANCE OF THROMBOPOITEIN IN CHRONIC LIVER DISEASES WITH THROMBOCYTOPENIA

G. Shiota, M. Okubo, H. Kawasaki. *Second Department of Internal Medicine, Tottori University School of Medicine, Yonago 683, Japan*

Thrombopoiten (TPO) is the primary regulator of megakaryopoiesis and platelet production. Since liver is the main organ producing TPO, changes of TPO may be associated with thrombocytopenia with liver diseases. To clarify role of TPO, serum TPO and hepatic TPO mRNA were measured in 30 chronic hepatitis (CH), 28 liver cirrhosis (LC), 51 hepatocellular carcinoma (HCC) and 29 normal controls, by using ELISA and reverse transcription polymerase chain reaction, respectively. Hepatic TPO mRNA was assessed as TPO mRNA/β-actin mRNA ratio. Serum TPO in controls, CH, LC and HCC were 0.83±0.36, 2.79±0.40, 1.49±0.20 and 1.97±0.20 fmol/ml, respectively. Serum TPO in CH was significantly higher than in controls, LC and HCC ($p < 0.01$, each). Serum TPO level exhibited a positive correlation with prothrombin time, and a negative correlation with indocyanine green test ($p < 0.01$, $p < 0.05$), whereas no relation between TPO and platelet counts was observed. Serum TPO was increased in CH treated with interferon, in response to thrombocytopenia. Hepatic TPO mRNA was not changed among the diseases. Immunohistochemical analysis also confirmed this result. The results of the present study suggest that impairment of TPO increase in LC and HCC may be one of the factors causing thrombocytopenia accompanying liver diseases.

P-1468 THE DIAGNOSTIC VALUE OF THROMBOPOIETIN LEVEL MEASUREMENTS IN THROMBOCYTOPENIA

C.C. Folman[1,2], L. Porcelijn, M. De Haas, E. Huiskes, B. Bossers, M.A.M. Overbeeke, C.E. Van der Schoot, A.E.G.Kr. Von dem Borne[1,2]. [1]*Central Lab. of the Netherlands Red Cross and the Lab. of Exp. and Clin. Immunol., Univ. of Amsterdam;* [2]*Academic Medical Center, Amsterdam, The Netherlands*

Previously, we and others reported that measurement of blood thrombopoietin (Tpo) levels is useful to discriminate between thrombocytopenia due to increased platelet destruction and decreased platelet production. With our Tpo ELISA and glycocalicin (GC) ELISA we analysed a large group of patients in detail and could confirm and amplify the above notion in detail.

Tpo levels were determined in plasma from 178 clinically and serologically well-defined thrombocytopenic patients: 72 patients with idiopathic autoimmune thrombocytopenia (AITP), 29 patients with secondary AITP, 5 patients with amegakaryocytic thrombocytopenia and 72 patients who suffered from various diseases (46 in whom megakaryocyte deficiency was not, and 26 in whom megakaryocyte deficiency was expected). In addition, the level of GC was measured in these patients as a marker of total body mass of platelets.

In all patients with primary AITP and secondary ITP, Tpo levels were within the normal range or in some (n = 7) cases slightly increased. The level of GC was not significantly different from that of controls (n = 95). The patients with amegakaryocytic thrombocytopenia had strongly elevated Tpo levels and significantly decreased GC levels. Similarly, among the 72 thrombocytopenic patients with various disorders, elevated Tpo levels were only found in patients in whom platelet production was depressed. The mean level of GC in these patients was decreased compared to that in controls and patients with AITP, but was not as low as in patients with amegakaryocytic thrombocytopenia.

In conclusion, all patients with depressed platelet production had elevated levels of circulating Tpo whereas Tpo levels in patients with an immune-mediated thrombocytopenia. were mostly within the normal range. Therefore, measurement of plasma Tpo levels provides valuable diagnostic information for the analysis of thrombocytopenia in general. Moreover, treatment with Tpo may be an option in ITP.

P-1469 PLASMA THROMBOPOIETIN LEVELS IN PATIENTS WITH LIVER CIRRHOSIS, AND IN PATIENTS WITH END-STAGE RENAL FAILURE

A. Olsson, D. Stockelberg, P.O. Andersson, J. Kutti, H. Wadenvik. *Hematology section, Sahlgrenska university hospital, Gothenburg, Sweden*

Recently, c-Mpl ligand (thrombopoietin; TPO) has been cloned by several groups and found to be a primary regulator of thrombopoiesis. Its mRNA expression has been detected in several organs including kindneys, bone marrow stroma cells, muscle, and is very strongly expressed in the liver. To clarify thrombopoiesis and the regulation of TPO in severe liver and renal failure, we analyzed plasma TPO levels in patients with biopsy verified liver cirrhosis (n = 18; mean platelet count 115±52 × 10^9/l), in patients on chronic hemodialysis due to end-stage renal failure (n = 20; mean platelet count 295±94 × 10^9/l), and in healthy individuals (n = 20; mean platelet count 250±46 × 10^9/l). Plasma was prepared from EDTA-anticoagulated whole blood and a commercially available ELISA kit (Quantikine™, Human TPO Immunoassay, R&D systems, USA) was used for the analysis. The mean plasma TPO concentration among the normal individuals was 52±12 pg/ml. In the patients with liver cirrhosis and in patients on hemodialysis the mean TPO levels were 62±19 pg/ml and 46±17 pg/ml, respectively. For both these patient groups no statistically significant differences were seen, as compared to the normals. In conclusion, our results suggest that TPO production is maintained in liver cirrhosis and in renal failure, and that the thrombocytopenia in liver cirrhosis is not due to an impaired TPO production.

P-1470 PLASMA THROMBOPOIETIN LEVELS IN THROMBOCYTOPENIC/THROMBOCYTHEMIC STATES-DIAGNOSTIC VALUE

P.O. Andersson, M. Hou, D. Stockelberg, J. Kutti, H. Wadenvik. *Hematology section, Sahlgrenska University Hospital, Gothenburg, Sweden*

To evaluate the diagnostic value of thrombopoietin (TPO) measurements, we determined the plasma TPO concentration in normal individuals (n = 20; mean platelet count, (plt) 250±46 × 10^9/l), in chronic idiopathic thrombocytopenic purpura (ITP; n = 16; plt 66±54), in severe aplastic anemia (SAA; n = 3, plt 8±5), in chemotherapy-induced bone marrow hypoplasia (n = 10; plt 29±23), in myelodysplastic syndromes (MDS; n = 11; plt 26±20), in essential thrombocythemia (ET; n = 12; plt 435±132), in reactive thrombocytosis (RT; n = 13, plt 657±192). A commercially available ELISA and EDTA-samples were used for the TPO analysis (citrate-anticoagulated whole blood were used for the ET/RT samples); concentration expressed as pg/ml. The mean TPO concentration in normals were 52±12. The corresponding value in patients with SAA and chemotherapy-induced bone marrow hypoplasia were 1514±336 and 1950±1684 respectively. In contrast, the plasma TPO recorded in ITP (64±20) and MDS (68±23) were only slightly higher than normal level. The mean citrate plasma TPO levels in normals, ET and RT were 21±11, 44±45 and 16±9, respectively. Our data support the suggestion that the megakaryocyte mass affect the plasma TPO concentration. In thrombocytopenic states, a substantially increased plasma TPO level suggest a deficient megakaryocyte number. In a thrombocythemic state an elevated TPO level might be used to differentiate ET from RT.

P-1471 BENING FAMILIAL THROMBOCYTOSIS

L. Escoda, M.J. Herranz, L. Florensa[1], A. Ugarriza[2], A. Cartanyà. *Unitat d'Hematologia, Servei de Medicina Interna, Pius Hospital de Valls; [1]Laboratori de citologia, Hospital de l'Aliança, Barcelona; [2]Servei d'Hematologia, Hospital Universitari Joan XXIII, Tarragona, Spain*

Familial presentation of thrombocytosis is extremely rare. We describe a new case, which is the 11th reported in the literature, of a family with four members of three different generations affected with a sustained mild thrombocytosis. These patients were all asymptomatic and had a platelet count in the range of 400 to 700 $\times$ 10^9/l, with otherwise normal hemogram. Routine biochemistry as well as serum ferritine, erythrocyte sedimentation rate, C-reactive protein and vitamin B_{12} were normals. Histological bone marrow examinations showed megakaryocytic hyperplasia in two members and no alterations in another. Karyotypes of bone marrow cells were normal. Platelet aggregation tests disclosed an impaired aggregation to ADP, collagen and epinephrine. Peripheral myeloid progenitor cultures showed spontaneous megakaryocyte colony formation (CFU-Mek) in one subject. With these results reactive thrombocytosis and myeloproliferative disease other than essential thrombocythemia could be discarted in this family.

Conclusions:

- The endogenous colony formation of CFU-Mek clearly discerns between the reactive and the proliferative thrombocytosis, although it is uncertain its meaning in familial thrombocytosis since no previous results have been reported.
- A long term follow-up of this family is required, even though it seems affected by a primary inheritable thrombocytosis of a bening nature.

P-1472 EVALUATION OF RISK FACTORS FOR THROMBOSIS IN PATIENTS WITH ESSENTIAL THROMBOCYTHAEMIA

C. Harrison, S. Donohoe, A. Chitolie, P. Carr, I. Mackie, S. Machin. *Department of Haematology, University College London Hospitals, UK*

Patients with Essential Thrombocythaemia (ET) may present with a thrombosis and are at increased risk of such an event during the course of their disease. The influence of risk factors for thrombosis such as the Factor V Leiden (FVQ^{506}) gene mutation have not previously been investigated. We have studied the incidence of the FVQ^{506} and Prothrombin gene mutations (G20210A) and of anticardiolipin (aCL), anti-β_2 -glycoprotein I (a-β_2GPI), and antiprothrombin (a-Pro) antibodies in a large series of patients with ET. Heterozygotes for the FVQ^{506} and G20210A mutation were identified in 2/49 and 0.49 patients with ET. None of the ET patients heterozygous for either of these mutations had a thrombotic event. By comparison a relatively high proportion of patients with ET had clinically significant levels of aCL or related antibodies detected. Of 53 patients analysed 4 had IgG aCL antibodies (median levels 10.5 range 5.2–15.1) and 19 had IgM aCL (median 19 range 5.7–44.8), one patient had both. Analysis of a-β_2GPI reveals 8 had IgM (median 8.9 range 3.9–24.1), 3 had IgG (levels 3.6, 9, 128) and of a-Pro, 7 had IgM (median 22.5 range 18.3–30.7), and 4 had IgG (Median 8.25 range 4–12.3). Overall 5 patients had 3 positive antibody screens, 5 had 2 and 12 had 1 positive screen. There appeared to be an increased risk of thrombosis in patients with positive aCL 9/22 compared to those without 4/31 (p 0.0264). There was no correlation with antibody specificity or class and thrombosis nor was increasing number of positive screens associated with increased thrombotic risk. In normal controls the reported incidence of aCL is 1.7%, the incidence in this population of ET patients is higher 42% ($p < 0.000001$). We also detected positive screens in 9/27 patients with a reactive thrombocytosis (RT) 3 of which had underlying infections, only 1/3 had positive a-β_2GPI. None of these RT patients with significant antiphospholipid antibodies had a thrombotic event. The apparent increase in occurrence of antiphospholipid antibodies in ET may be as a consequence of chronic exposure to activated and altered platelet membranes acting as an antigenic stimulus. The role of aCL and related factors such as in lupus anticoagulant elevating the risk of thrombosis in patients with ET should be evaluated further. The co-existence of aCL in patients with ET may be an indication for more aggressive cytoreductive and antiplatelet therapy particularly in situations when an increased risk of thrombosis (eg perioperatively) is anticipated.

P-1473 IN-VIVO AND IN-VITRO INHIBITION OF PLATELET FUNCTION OWING TO ANTI GP-Ia/IIa COLLAGEN RECEPTOR EPITOPE

A. Lazzaro, L. Mazzucco, R. Guaschino, C.C. Grangiotti, P. Borzini. *Blood Transfusion Service, Casale Monferrato, Italy*

A 45-years-old woman presented with severe pubic purpura following thrycotomy performed prior minor surgery. A mildly reduced platelet count (58 $\times$ 10e9/L) seemed to be, per se, an inconsistent cause for the development of purpuric lesions. Bleeding time was prolonged (19 min) and spontaneous bleeding was absent. The patient had a chronic liver disease owing to serotype-3 HCV infection. Panreactive anti-HCV antibodies and traces of polyclonal IgG/monoclonal IgM-kappa type II cryoglobulins were detected. The major membrane glycoproteins GP IIb/IIIa, Ib/IX, Ia/IIa were normally expressed; strongly reactive platelet-associated (PA) IgG and IgM were demonstrated and anti-GPIa/IIa antibodies were detected in the serum; cryoglobulin kappa/lamda ratio was similar to PA-Ig kappa/lambda ratio. ADP-induced aggregation resulted depressed, while collagen-induced aggregation resulted completely abrogated as measured by standard platelet aggregometry. In-vitro bleeding time performed by PFA100 (Dade, Miami) disclosed absolute platelet defect owing to impaired platelet adhesion to collagen; a similar defect was inducibile, in a dose-dependent fashion, in normal platelets prior incubation with patient's plasma. These findings suggest an acquired platelet defect due to autoantibodies against the collagen receptor epitope of platelet membrane glycoprotein Ia/IIa. The finding of this antibody and the immunochemical similarities between PA-immunoglobulins and HCV-associated cryoglobulins suggest their mutual relationship and their implication in the pathogenesis of the acquired platelet defect.

P-1474 EVALUATION OF PLATELET FUNCTION IN VON WILLEBRAND DISEASE WITH PFA-100®

M. Cattaneo, A.B. Federici, B. Agati, A. Lecchi, F. Stabile, R. Lombardi, M.T. Canciani, P.M. Mannucci. *Angelo Bianchi Bonomi Hemophilia and Thrombosis Ctr. Dept. of Internal Medicine, IRCCS Ospedale Maggiore, University of Milano, Italy*

Objective: We have evaluated platelet function at high shear with the PFA-100 system in different subtypes of von Willebrand disease (vWD), before and after the intravenous infusions of desmopressin or a factorVIII/vW factor (vWF) concentrate.

Design and Methods: Closure times with the PFA-100 system were determined for both the collagen/ADP and the collagen/epinephrine cartridges in 47 patients with vWD (10 type 1 "platelet normal", 5 type 1 "platelet discordant", 8 type 1 "platelet low", 4 type 2M "1 Vicenza", 6 type 2A, 8 type 2B and 6 type 3) and 30 controls. Measurements were repeated 1 and 4 hours after the i.v. infusion of desmopressin (0.3 μg/Kg) in 25 patients with types 1, type 2M "1 Vicenza" or type 2A vWD, or of a factorVIII/vWF concentrate (Alphanate, 60 U/Kg) in 4 patients with type 3 vWD. At all time points, vWF plasma levels and the bleeding time (Symplate II) were also determined.

Results: Baseline closure times were longer in vWD patients than in controls with both the collagen/ADP and the collagen/epinephrine cartridges. Treatment with desmopressin normalized the closure times in patients with type 1 "platelet normal" or type 2M "1 Vicenza" vWD, shortened but did not normalize the closure times in patients with type 1 "platelet-low" vWD, had no effects in patients with type 1 "platelet-discordant" or type 2A vWD. Infusion of a factorVIII/vWF concentrate in patients with type 3 vWD slightly shortened their prolonged closure times. In general changes in PFA-100® were paralleled by shortenings of the bleeding times and increases in plasma vWF levels.

Conclusions: The PFA-100® test reflects vWF-dependent platelet function under high shear stress and could be useful in the diagnosis and therapeutic monitoring for patients with VWD.

P-1475 ABNORMAL PLATELET FUNCTION RELATED TO ALTERATIONS IN MEMBRANE LIPID CONTENT IN PATIENTS WITH HYPERCHOLESTEROLEMIA

G. Rock, J. Kramer, J. Bormanis, D.S. Ooi. *Ottawa Civic Hospital, Ottawa, Ontario, Canada*

Two young siblings with familial hypercholesterolemia underwent examination for platelet abnormalities because of thrombocytopenia in the less severely heterozygous hypercholesterolemic 20 year old male who had a platelet count of 66 $\times$ 10^9/L, an MPV of 12 fL, and a bleeding time of >20 minutes. His 16 year old homozygous, sister had a normal platelet count, MPV and a bleeding time of 11.5 minutes. There was no history of excessive bleeding or bruising. Platelet aggregation was abnormal with decreased response to ADP and arachidonic acid, absent response to epinephrine and collagen and normal response to ristocetin. There was no ATP release. Electron microscopy showed normal granule content. Flow cytometry analysis showed that GP1b,

IIb/IIIa were present in normal quantities whereas lipid analysis using two different GLC capillary columns showed impaired metabolism of arachidonic acid (20:4n-6) in both siblings. The precursored linoleic acid (18:2n-6), and intermediates were present in normal amounts; three unusual and unknown fatty acids in the region of (20:4n-6) were also present. Linolenic acid elongation and desaturation were decreased. Platelet phosphtidyl-ethanolamine-cholineserine and sphingomyelin content were also abnormal in different degrees in the two. The data suggests the relationship of these lipid alterations with hypercholesterolemia, resulting in altered platelet function, specifically in the conversion of arachidonic acid.

P-1476 CHANGES IN VWF LEVELS IN TTP PATIENTS TREATED WITH CRYOSUPERNATANT VERSUS FRESH FROZEN PLASMA

G. Rock, D.R. Anderson, W.B. Benny, S.N. Caplan, W.F. Clark, J. Freedman, A. Giulivi, M.S. Sternbach, D.M.C. Sutton. *Members of the Canadian Apheresis Group; Canadian Apheresis Group, Ottawa, Ontario, Canada*

We have found that plasma exchange (PE) with cryosupernatant plasma (CSP) results in a 95% survival rate at 1 month compared to the 78% survival seen when fresh frozen plasma (FFP) is used [1]. Examination of the pre and post PE levels of vWF in 10 patients given 1.5-1 plasma volume exchanges on 7 of 9 days shows that the plasma vWF level decreased by 45% after day 5 of treatment when FFP was used for replacement and 66% when CSP was used. None of the patients showed an increase in the ultra high molecular weight vWF multimers at entry. Further, there were no significant alterations during treatment with CSP or FFP with demonstration of high, intermediate and low molecular weight multimers in all patients before and after 3 and 5 days of therapy. Fibrinogen levels were not significantly different in the two groups but FVIII levels were markedly different with much lower levels (0.6 IU) present after CSP PE at day 5 than for FFP exchange (1.19 IU). LDH values were high at entry (mean = 1294 U/L) then decreased over the next 5 days in both groups. We conclude that PE with CSP results in greater reduction in vWF levels compared to PE with FFP but that little change in multimer patterns are seen with either therapy suggesting that multimer pattern is not associated with the basic pathophysiology of this disorder.

(1) [1] Rock et al. Cryosupernatant as replacement fluid for PE in TTP. Br J Haematol 1996; 94:383–6.

P-1477 INCIDENCE OF ANTI-PLATELET ANTIBODIES IN LIVER TRANSPLANT PATIENTS AND REPERCUSSION IN TERMS OF PLATELET REQUIREMENTS

C. Fernández-Lago, E. Romero. *Departement of Hematology Juan Canalejo Hospital, La Coruña, Spain*

Introduction:

Given the special characteristics of orthotopic liver transplants (OLT), these require considerable quantities of blood support. For this reason, it becomes particulary important to identify the existence of antibodies in the patient that may alter the effects of the transfusions administered.

In this study we set out to evaluate the incidence of antiplatelet antibodies in a group of 59 patients that underwent OLT in our hospital in order to determine the repercussions in terms of units of platelet concentrates (PC) required.

Materials and Methods:

The serum of 59 patients was analysed prior to their undergoing OLT, regardless of the initial pathology.

The antibodies were analysed with a capture method in a solid phase (Capture™ - IMMUCOR). Where these tested positive, a study of antibodies followed using a panel of platelets (Capture Ready-Screen - IMMUCOR)

In every case, the amount of units of platelet concentrate used during surgery was quantified.

Results:

The presence of antiplatelet antibodies was detected in three cases corresponding to 5% of the total. In the three cases the antibody was of IgG origin and was directed against antigens from the HLA system.

Patients without the antiplatelet antibody were found to require and average of 19 PC (0–84) while patients with the antibody were found to require an average of 142 PC (102–168)

Conclusion:

The presence of antiplatelet antibodies is relatively frequent in patients who are to undergo OLT and this has significant repercussions in terms of PC requirements, which makes a systematic study, of these antibodies extremely valuable.

Given that the majority of these antibodies act against the HLA system, it would be worthwhile to evaluate the utility of using platelets subject to prior alteration of HLA antigens of the platelet membrane in order to prevent the union of serum antigens in the patient.