

Haematologica

established in 1920

editor-in-chief: Edoardo Ascari

ISSN 0390-6078

Journal of Hematology

volume 84, suppl. to number 9, September 1999

Official Organ of

the Italian Society of Hematology
the Italian Society of Experimental Hematology
the Spanish Association of Hematology and Hemotherapy
the Italian Association of Pediatric Hematology Oncology

37th Congress of the Italian Society of Hematology

September 26-29, 1999
Centro Congressi Lingotto
Turin, Italy

ABSTRACTS



Owned and published by the Ferrata Storti Foundation, Pavia, Italy

Mensile - Sped. Abb. Post. - 45% art. 2, comma 20B, Legge 662/96 - Filiale di Pavia
Il mittente chiede la restituzione dei fascicoli non consegnati impegnandosi a pagare le tasse dovute

Selected Communications

CS01 HAEMATOLOGICAL DATA MODIFICATIONS AFTER ACUTE EXPOSURE TO HIGH ALTITUDE, POSSIBLE IMPLICATION FOR DETECTION OF RECOMBINANT ERYTHROPOIETIN MISUSE

M. BONFICHI, A. BALDUINI^B, A. LORENZI, C. MARSEGLIA,
L. ARCAINI, L. MALCOVATI, L. BERNARDI^O, C. PASSINO^O,
G. SPADACINI^O, P. FEIL^S, C. KEYL^{*}, A. SCHNEIDER^{*},
A. BOIARDI^M, G. BANDINELLI^F, R.E. GREENE^S, C. BERNASCONI

*Inst. of Hematology Lab. of Biotechnology^B,
and Cl. Medica I^O, IRCCS Policlinico S. Matteo,
Pavia, Univ. of Pavia, IRCCS Besta^M, Milan and UO
SM Nuova^F, Florence, Italy; CCU
Univ. Regensburg^{*}, Germany, Highlands Univ.^S Las
Vegas NM. USA*

Erythropoietin is possibly misused by athletes in sports to improve performance. Presently there is no discernible and specific method to identify erythropoietin administration for doping control. Gareau et al. (Nature 380, 113, 1996) recently reported the possible correlation between soluble transferrin receptor/ferritin ratio (sTfr/ftn) and hematocrit (Ht) and hemoglobin (Hb) levels in athlete doping evaluation. The aim of this study is to discuss the modifications of the sTfr/ftn ratio associated to hemoglobin and hematocrit with the acute exposure to high altitude in 24 western subjects, normally living at sea levels. The data were collected during a scientific expedition to the "Pyramid", the CNR laboratory situated in the Kumbu Valley (Nepal) at 5050 m. The blood harvests were performed at standard condition (Katmandu)(A), at the arriving to the Pyramid, after 6 days walking from 2800 m till 5050 m(B) and after 8 days of permanence at the Pyramid (C). The results are reported in the following table

	A (Standard)	B (Arrival at 5050 m)	C (Departure from Pyramid)
Hb g/dl	14.0±1.5	14.7±1.5*	15.±1.5* ^o
Ht %	43.9±1.9	43.4±3.4	45.4±4.3* ^o
STFR/Ftn ratio	1.37±3.4	1.57±2.3	2.48±3.3*
Epo mIU/ml	9.7±3.9	28.7±13*	25.5±15.8*

* p<0.05 vs standard; ^o=<0.05 vs arrival to 5050 m

Significant statistical modifications were observed in Hb values between time A, B and C, in Ht values between time A and C, in Epo data between A vs B and C; sTfr/ftn ratio increased significantly only in C con-

trol. This data are correlated with Gareau's results where non rHuEpo treated subjects showed a low increase of sTfr/ftn ratio, while treated subjects showed an increase of this ratio more than 10 folds. In conclusion we think that the application of the sTfr/ftn ratio may help to discover the possible misuse of rHuEpo especially in those situations where screening data are difficult to evaluate. We have planned to confirm our hypothesis on a higher number of normal subjects, utilizing adults treated with rHuEpo for autologous blood donation as rescue before bone marrow donation.

CS02 DIFFERENTIATION OF ACUTE MYELOID LEUKEMIAS BY THERAPEUTIC TARGETING OF HISTONE DEACETYLASE ACTIVITY

C. NERVI, F.F. FERRARA, F. FAZI, A. BIANCHINI, F. PADULA,
M.C. PETTI, F. MANDELLI, P.G. PELICCI, F. LO COCO

*Dipartimenti di Istologia ed Embriologia Medica e di
Biotecnologie Cellulari ed Ematologia, Università "La
Sapienza", Roma, Istituto Europeo di Oncologia,
Milano*

Differentiation therapeutic approaches targeting a molecular lesion have been successfully employed in acute promyelocytic leukemia (APL). Laboratory and clinical studies have elucidated the mechanisms underlying the maturation arrest of APL blasts and opened new perspectives in differentiation therapy of leukemia. In particular: i) PML/RAR α and PLZF/RAR α fusions resulting from the t(15;17) and t(11;17) translocations, induce alteration of the retinoid signaling pathway by recruitment of histone deacetylase (HDAC) activity, which results in transcriptional repression; ii) Pharmacological doses of retinoic acid (ATRA) release this repressory complex in PML/RAR α -positive APL and recruit the multisubunit activation complex resulting in terminal differentiation of blasts; iii) Treatment with HDAC inhibitors + ATRA restore ATRA sensitivity in PLZF/RAR α APLs and in ATRA-resistant APL blasts *in vitro* and *in vivo*; iv) AML1/ETO, the M2-acute myeloid leukemia (AML) associated oncoprotein, is present in a complex containing HDAC activity. Thus, modification of histone acetylation activities resulting in altered chromatin structure might represent

a general mechanism associated with AML pathogenesis. We tested the *in vitro* effect of ATRA, as differentiating agent in combination with the HDAC inhibitors, trichostatin A and sodium phenylbutyrate in non-M3 AML cell lines and primary cells from patients. We found that such combination enhanced ATRA response of AML blasts, as evaluated by morphological, functional and immunophenotypic studies including NBT dye reduction assay and flow cytometry analysis of differentiation markers and propidium iodide stained cells. Modification of the acetylation status of histones was assessed by immunocytochemistry using anti-acetylated histone H3 antibodies. Finally, we found that in AML blasts, HDAC inhibitors potentiate or restore ATRA signaling on specific ATRA-responsive promoter activities and target gene expression. These findings suggest that therapeutic targeting of transcription may prove effective in non-APL AMLs.

CS03 EPIDEMIOLOGY AND CHARACTERIZATION OF LYMPHOPROLIFERATIVE DISEASES (LPD) VIRUS G (HGV) POSITIVE: CORRELATION WITH HEPATITIS C VIRUS INFECTION

A. DE RENZO, M. PERSICO*, E. PERSICO*, G. FALZARANO,
C. DI GRAZIA, R. NOTARO, R. TORELLA*, B. ROTOLI

*Hematology Unit, Federico II University, Naples.
*Internal Medicine and Hepatology Unit,
II University of Naples, Italy*

A high prevalence of HCV positivity has been shown in Italy in several lymphoproliferative malignancies except Hodgkin Disease (HD). Based on biological similarities between HCV and HGV, we have searched if even HGV shares any epidemiological relation with LPD. We have also evaluated clinical and histological parameters of HGV+ LPD in order to identify possible HGV-associated peculiarities. Comparison with the general population and with HCV+ LPD were also made. **Patients and Methods:** 170 pts. and 134 healthy blood donors. Routine blood examination and anti-HCV determination (ELISA II confirmed by RIBA II) were performed in the two groups. Polymerase chain reaction (PCR) was used for HCV-RNA and HGV-RNA determination.

Histology was reported for all patients according to the WF. **Statistical analysis:** Fisher's exact test and Chi-square. **Results:** Overall, HGV prevalence in the group of patients was significantly higher than in the control group (10.5% vs 1.5%); the same result was found for HCV, but no HGV+ patient was coinfecting with HCV. In addition, while HCV prevalence was elevated in all B-LPDs and was not different from the control group for HD patients, HGV was mainly diffused among both NHL and HD patients. The primary site of disease in HGV+ LPD was in all cases a lymph node.

	NHL	HD	MM	WM	CLL	TOTAL	Controls
n	33	71	48	9	9	170	134
HGV+	4(12%)	11(15%)	3(6%)	0	0	18(10.5%)	2(1.5%)
HCV+	5(15%)	2(2.8%)	4(8%)	4(45%)	2(22%)	17(10%)	3(2.2%)

Conclusions: 1) HGV prevalence seems to be significantly increased in patients with a LPD. 2) HGV prevalence is not associated to the prevalence of HCV. 3) HGV might have a role in lymphomagenesis in NHL and HD.

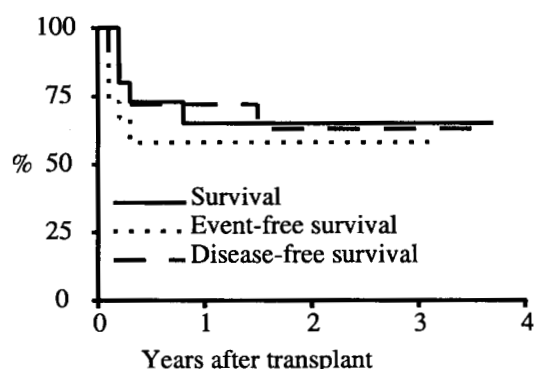
CS04 UMBILICAL CORD BLOOD TRANSPLANT FROM UNRELATED HLA-MISMATCHED DONOR IN CHILDREN WITH HIGH RISK LEUKEMIA

W. ARCESE, C. GUGLIELMI, A.P. IORI, M. SCRENCI,
D. CARMINI, A.M. TESTI, M.L. MOLETTI, A. MENGARELLI,
G. CIMINO, L. ELIA, C. RAPANOTTI, P. PERRONE,
L. LAURENTI, G. GENTILE, A. ROMANO, L. DE FELICE,
F. MANDELLI

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

In the last four years 15 children with high-risk leukemia (12 ALL, 2 AML and 1 CML) underwent cord blood transplantation (CBT) from unrelated HLA mismatched donor at a median of 99 days from the start of search. Nine patients were transplanted in 2nd CR, 1 in accelerated phase, 3 at relapse and 2 patients in first CR. Conditioning regimen (F-TBI, VP-16, CY and ALS) and prophylaxis of GVHD (CSA and 6-methylprednisolone) were identical for all patients. Neutrophils $>0.5 \times 10^9/L$ were reached at a median of 33 days from transplant, but in 4 cases we observed an autologous hematopoietic reconstitution (3

spontaneous, 1 after autologous BM rescue). Acute grade = II and extensive chronic GVHD were observed in 4/15 (27%) and 2/8 (25%) evaluable cases, respectively. Five patients died (3 from transplant related toxicity and 2 from relapse). The degree of HLA disparity, also extended to molecular typing for HLA-C and DQB1 loci, was not significantly correlated either with the rate of engraftment or with the occurrence and severity of GVHD or with transplant-related mortality. The 3 years probabilities of survival, event-free survival and disease-free survival were 65%, 58% and 63%, respectively. CBT from HLA mismatched unrelated donor is a valid alternative to unrelated BM transplant for children with high-risk leukemia.



CS05 G-CSF SELECTIVELY MOBILIZES TH2-INDUCING DENDRITIC CELLS (DC2)

M. ARPINATI, M. LOKEN, C. ANASETTI

*Istituto di Ematologia e Oncologia Medica
"Seragnoli", Università di Bologna, Italy;
Fred Hutchinson Cancer Research Center, Seattle,
WA, USA*

Allogeneic transplantation with G-CSF mobilized peripheral blood stem cells (PBSC) grafts doesn't induce a higher incidence of acute GVHD, although the dose of T cells is 20-fold higher than in marrow grafts. T-lymphocytes from G-CSF treated animals preferentially produce IL4 and IL10, Th2-like cytokines which are associated with diminished GVHD inducing ability. We hypothesized that G-CSF mobilizes antigen presenting cells (APC) which induce T lymphocytes to differentiate to Th2. Dendritic Cells can be classified in DC1 and DC2, according to their ability, respectively, of in-

ducing naive T cells to differentiate to Th1-like and Th2-like effector cells. We used a flow cytometric method to count DC1 and DC2 in peripheral blood of normal donors before or after G-CSF treatment. Both DC1 and DC2 were positive for HLA-DR and negative for lineage markers (lin) (CD3, CD14, CD16, CD19, CD20, CD34, CD56 and IgM). DC1 and DC2 could be identified according to the expression of the adhesion molecule CD11c, which was positive on DC1 and negative on DC2, and of the IL3 receptor α chain (CD123), which was positive on DC2 and negative on DC1. G-CSF administration for 5 days at 16 μ g/kg/die increased peripheral blood DC2 counts from a median of 4.9 $\times 10^6$ /L (n=9) to 24.8 $\times 10^6$ /L (n=13) (p=0.009), while DC1 counts did not change (from 11.2 $\times 10^6$ /L to 10.1 $\times 10^6$ /L) (p=0.52). DC1 purified either from normal or G-CSF treated donors induced allogeneic naive T cells to produce IFN γ , which is typical of Th1 responses, while DC2 induced allogeneic naive T cells to produce IL4 and IL10, which are typical of Th2 responses. Allogeneic PBSC grafts (n=8) contained a higher dose of DC2 than marrow (n=15) (median dose 2.6 $\times 10^6$ /Kg vs 0.5 $\times 10^6$ /Kg) (p=0.006), with a comparable dose of DC1 (0.8 $\times 10^6$ /Kg vs 1.0 $\times 10^6$ /kg) (p=0.4). The presentation of host antigens on donor DC2 might polarise donor T lymphocytes to Th2 cells, thus reducing their ability to attack and damage host tissues, and to cause GVHD.

CS06 ALK⁺ LYMPHOMAS ("ALKomas"): A DISTINCT MOLECULAR, PATHOLOGIC AND CLINICAL ENTITY

B. FALINI

Institute of Hematology, University of Perugia, Italy

The t(2;5)(p23;q35) associated to CD30⁺ anaplastic large cell lymphoma (ALCL) causes the fusion of the nucleophosmin (NPM) and ALK (anaplastic lymphoma kinase) genes leading to the production of a NPM-ALK chimeric oncoprotein. We generated monoclonal antibodies (mAbs) against the cytoplasmic portion of ALK protein (mAb ALKc) and the N- and C-terminus of the NPM molecule (mAbs NPMa and NPMc) and used them to study the expression of these proteins in over 2,000 lymphomas (repre-

sentative of all categories of the REAL classification). The results can be summarized as follows : i) ALK expression is restricted to about 60% of T/Null CD30⁺ ALCL, that we termed ALK⁺ lymphomas or "ALKomas", characterized by a wide morphological spectrum; ii) Anti-ALK antibodies are of great diagnostic value, since allow to distinguish "ALKomas" from other lymphomas and even reactive lesions, and also to detect minimal disease ; iii) Anti-ALK and anti-NPM antibodies differentiate, according to different subcellular distribution patterns of the proteins, "ALKomas" expressing NPM-ALK (about 75%) from the more rare "ALKomas" (about 15%) in which ALK fuses with a partner gene other than NPM; iv) Cloning of the ALK fusion gene partner in one of such a cases is reported; v) Clinically, ALK⁺ lymphomas mostly occur in children and young adults (mean age, 22.01 ± 10.87 years) with a male predominance and usually present as an aggressive, stage III-IV B disease, frequently associated with extranodal involvement (60%). In contrast, ALK⁻ ALCL occur in older individuals (mean age, 43.33 ± 16.15 years) and show a lower M/F ratio (0.9), as well as lower incidence of stage III-IV disease and extranodal involvement at presentation. Overall survival of ALK⁺ lymphomas (both cases bearing NPM-ALK or a variant ALK fusion protein) is far better than that of ALK⁻ ALCL (71% ± 6% vs 15% ± 11%). However, within the good prognostic category of ALK⁺ lymphomas, survival was 94% ± 5% for the low/low intermediate risk group (age-adjusted International Prognostic Index: 0 to 1) and 41% ± 12% for the high/high intermediate risk group (age-adjusted International Prognostic Index : ³2). In conclusion, "ALKomas" represent a distinct molecular, pathological and clinical entity with excellent outcome that should be separated from the bad prognostic category of ALK⁻ ALCL.

CS07

MOLECULAR STUDIES IN HYPERFERRITINEMIA-CATARACT SYNDROME

G. ZECCHINA, M. CICILANO, S. BOSIO, A. ROETTO, V. INFELISE, U. MAZZA, G. LOCKITCH, C. CAMASCHELLA

*Dip. di Scienze Cliniche e Biologiche, Università di Torino, Azienda Ospedaliera S.Luigi, Orbassano; ° Presidio Ospedaliero S.S.Trinità Borgomanero, Italia; *Dept. of Pathology and Laboratory Medicine, Children's and Women's Health Centre of B.C., Vancouver, Canada*

Hereditary Hyperferritinemia-Cataract Syndrome (HHCS) is a dominantly inherited disease characterized by : i) high constitutive L-ferritin synthesis ii) absence of iron overload iii) early onset bilateral cataract. HHCS is caused by heterogeneous mutations in the Iron Responsive Element (IRE) in the 5' untranslated flanking region of the L-ferritin mRNA. The presence of mutations in this nucleotidic sequence abolishes the negative control of ferritin synthesis exerted at translational level by means of the interaction between the IRE structure and two iron-sensitive regulatory proteins named IRP ("Iron Regulating Proteins"). We have identified three italian and one canadian families affected by HHCS. The probands showed serum ferritin levels ranging from 600 µg/L to 2500 µg/L, with normal serum iron and transferrin levels. One subject was affected by a severe iron deficient anemia unrecognized for a long time because of the high serum ferritin levels. PCR amplification and sequence analysis of the IRE element were performed on DNA of affected subjects. Three previously described mutations were found in the italian families: +32 G→A, +39 C→T, +40 A→G. Sequence analysis of the IRE element in the canadian family showed a +51G→C substitution never described before: studies are in progress to evaluate it's functional relevance. By considering the increasing number of observations in the last years HHCS should be taken into account in the differential diagnosis of hyperferritinemic states. Furthermore our data suggest that HHCS may confuse the evaluation of iron balance and therefore complicate the diagnosis of iron deficient anemia.

CS08 G20210A PROTHROMBIN MUTATION AND ASSOCIATED RISK FOR DEEP VEIN THROMBOSIS

V. DE STEFANO, P. CHIUSOLO, K. PACIARONI, I. CASORELLI,
E. ROSSI, A. DI MARIO, G. LEONE

Cattedra di Ematologia, Università Cattolica, Roma

The G20210A prothrombin gene mutation (FII-A) is associated with increased circulating levels of prothrombin and is a risk factor for deep vein thrombosis (DVT). We investigated 334 patients with DVT of the legs objectively documented (M/F 151/183, median age 43 years, range 2-86) and 456 healthy controls (M/F 266/190, median age 44 anni, range 7-93). Heterozygous genotype for the FII-A was found in 32 patients with DVT (9.6%) and in 12 controls (2.6%); other 78 patients with DVT (23.3%) and 11 controls (2.4%) had a different thrombophilic genotype (deficiency of naturally occurring coagulation inhibitors, factor V Leiden). The odds ratio (OR) for DVT associated with FII-A was 3.5 (95% CI 1.7-7.3) after adjustment for other thrombophilic inherited conditions. After stratification according to the circumstances of the first DVT (absence or presence of a circumstantial risk factor), FII-A was associated with an increased risk both for spontaneous DVT (OR 3.8, 95% CI 1.6-9.3) and DVT secondary to circumstantial risk factors (OR 3.3, 95% CI 1.5-7.5); after further stratification according to the age of the first DVT (lower or higher than 45 years) and comparison to controls younger or older than 45 years, FII-A was found associated with an increased risk for secondary DVT in the individuals younger than 45 years (OR 6.6, 95% CI 2.0-21.0) and with an increased risk for spontaneous DVT in the individuals older than 45 years (OR 4.8, 95% CI 1.6-14.2). Among the heterozygous carriers of FII-A the percentage of individuals having been previously exposed to circumstantial risk factors (pregnancy, surgery, hormonal treatment) was 64% (9/14) when the first DVT occurred before 45 years and 25% (2/8) ($p=0.07$) when the first DVT occurred after 45 years. The FII-A genotype was not found associated with an increased risk for recurrent DVT in 202 patients referred for a DVT that occurred in the previous years (in 103 cases as the only thrombotic event; median ob-

servation time after the first thrombosis 4.5 years) (relative risk 1.1, 95% CI 0.4-3.4). In conclusion the heterozygous G20210A prothrombin gene mutation is associated with a moderate increase in thrombotic risk; clinical expression needs a concomitant circumstantial risk factor or a prolonged exposure to the mutated genotype.

CS09 MUTATIONS OF *BCL-6* AND OF IMMUNOGLOBULIN VARIABLE GENES IDENTIFY DISTINCT MOLECULAR SUBSETS OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AND REVEAL HETEROGENEITY IN THE HISTOGENESIS OF THE DISEASE

D. CAPELLO,¹ F. FAIS,² D. VIVENZA,¹ G. MIGLIARETTI,¹
C. ARIATTI,¹ C. VOLTA,¹ N. CHIORAZZI,³ G. GAIDANO,¹
M. FERRARINI²

¹Division of Internal Medicine, Department of Medical Sciences, Amedeo Avogadro University of Eastern Piedmont, Novara; ²Clinical Immunology, IST, University of Genoa, Genova, Italy; ³Department of Medicine, North Shore University Hospital and NYU School of Medicine, Manhasset, NY, USA

B-cell chronic lymphocytic leukemia (B-CLL) is a B-cell tumor involving CD5⁺ small lymphocytes that express CD23 and low levels of surface immunoglobulins (Ig). Within this definition, there is heterogeneity in morphology, genetic lesions and clinical course. Previous studies indicated that leukemic CD5⁺ B cells, like their normal counterpart, use Ig variable (IgV) genes that exhibit minimal, if any, somatic diversity. However, recent reports indicate that a fraction of B-CLL display IgV gene mutations consistent with antigen stimulation and selection. In order to further elucidate the biologic heterogeneity of B-CLL, we have analyzed a panel of 28 B-CLL for the presence of mutations in the 5' non coding-regions of the *BCL-6* proto-oncogene and, for comparison, of IgV genes. Mutations of *BCL-6* are acquired during B-cell transit through the germinal center (GC) and, similar to IgV mutations, represent a histogenetic marker of GC or post-GC derivation of a given B-cell. *BCL-6* mutations were detected in 10/28 cases (36%). The average frequency of *BCL-6* mutation was 2.4×10^{-3} bp (range: $1.3-5.4 \times 10^{-3}$ / bp) and the mutational pattern was similar to that observed

in B-cell disorders known to derive from GC or post-GC cells, namely follicular lymphoma and B-lineage diffuse large cell lymphoma. Mutations of IgV genes occurred in 15/28 (54%) B-CLL and in some instances were consistent with antigen selection. All cases of B-CLL harboring *BCL-6* mutations were found to display also IgV mutations. Conversely, a fraction of B-CLL cases (5/28; 18%) harbored mutations in IgV genes but not in *BCL-6*. Thirteen out of twenty-eight (46%) B-CLL cases displayed unmutated IgV and *BCL-6* genes, consistent with derivation from naive, pre-GC B-cells. The implications of these data are threefold. First, the heterogeneity of *BCL-6* and IgV mutations indicate that B-CLL is molecularly and histogenetically heterogeneous. Second, the presence of *BCL-6* and/or IgV mutations in a proportion of B-CLL corroborates the notion that a subset of cases are derived from a mature, antigen experienced B-cell. Third, *BCL-6* mutations provide a novel molecular marker for disease monitoring. Overall, these results prompt investigations aimed at defining the clinical relevance of the histogenetic heterogeneity of B-CLL as defined in this study.

CS10 INCOMPATIBILITY FOR CD31 AND HUMAN PLATELET ANTIGENS (HPA) AND ACUTE GRAFT-VERSUS-HOST DISEASE AFTER BONE MARROW TRANSPLANTATION

C.L. BALDUINI, F. FRASSONI, P. NORIS, G. GIORGIANI,
M. MORDINI, C. KLERSY, S. BELLETTI, P. SPEDINI,
M. MARTINETTI, R. MACCARIO, A. BACIGALUPO, F. LOCATELLI

*Medicina Interna e Oncologia Medica, Unità
Biometrica, Immunoematologia e Trasfusionale,
IRCCS S. Matteo-Università di Pavia e Ematologia,
Ospedale S. Martino, Genova*

Bone marrow transplantation (BMT) is often complicated by acute graft-versus-host disease (aGVHD). In patients transplanted with an HLA-matched donor, the occurrence of this complication is believed to be favoured by disparities at minor histocompatibility antigens (mHA). However, few of these antigens have been identified. We sought to determine whether donor-recipient incompatibility for HPA-1, HPA-2, HPA-3, HPA-5 or CD31 (codon 125) represent a risk factor for aGVHD and typed these polymorphic molecules in 120 bone marrow do-

nors and their HLA-identical recipients. 70 patients were children: 37 were transplanted from siblings, whereas the remaining 33 underwent BMT from unrelated volunteers. 50 patients were adults and received BMT from siblings. Typing of HPA and CD31 was performed by molecular biology techniques on genomic DNA isolated from donor-recipient pairs before BMT. In the overall patient population, a strong statistical correlation ($p < 0.003$) was observed between donor-recipient CD31-incompatibility and grade II-IV aGVHD (aGVHD in compatible pairs 31.0%, in non-compatible pairs 60.6%). No significant association between aGVHD and incompatibility for any of the HPA's was found, although a possible role in aGVHD for some of the HPA's was suggested by the observation that the higher the number of donor-recipient incompatibilities at CD31/HPA polymorphisms, the stronger the risk of aGVHD. Analysis of pediatric subpopulation confirmed the role of CD31 mismatch in aGVHD and identified a direct correlation ($p < 0.04$) between the number of CD31/HPA incompatibilities and aGVHD. Moreover, HPA-3 incompatibility predicted aGVHD occurrence in HLA-A2 patients ($p < 0.04$), suggesting that HPA-3 mismatch was recognised in an HLA-A2-restricted fashion. In the adult's subpopulation, the frequency of both donor-recipient mismatches for HPA/CD31 and severe aGVHD was low and no statistical correlation was found. In conclusion, our data suggest that allelic variants of CD31 (codon 125) or HPA-3 can serve as mHA in BMT recipients from HLA-identical donors.

CS11 DETECTION OF ABNORMAL PRE- TRANSPLANT CLONES IN PROGENITOR CELLS OF PATIENTS WHO DEVELOPED MYELODYSPLASIA AFTER MYELOABLATIVE THERAPY

E. ABRUZZESE, J.E. RADFORD, J.S. MILLER,
J.J. VREDENBURGH, P.N. RAO, M.J. PETTENATI, A. TENDAS,
D.D. HURD, S. AMADORI

*Cattedra di Ematologia, Università Tor Vergata,
Roma, and Comprehensive Cancer Center, Wake
Forest University, Winston-Salem, NC, USA*

Secondary myelodysplastic syndromes (MDS) have been reported with increasing frequency after autologous transplantation.

It is not clear whether the MDS results from the pre-transplant conventional-dose chemotherapy or from the high dose therapy (HDT) used for the transplant procedure. To address this question we studied pre-transplant marrow or stem cell specimens from 12 patients who had received HDT with autologous marrow or stem cell transplant for the treatment of a lymphoma (7) or solid tumor (5) and have subsequently developed MDS. Post-transplant bone marrow specimens obtained at the time of the MDS diagnosis exhibited one or more MDS-related cytogenetic abnormalities. These abnormalities were used as markers to determine retrospectively, using fluorescence *in-situ* hybridization (FISH) whether the abnormal MDS clone was present pre-transplant. Mean age at time of transplant was 38 years (range 25-65) with 5 males and 7 females. All but one had received chemotherapy and/or radiotherapy prior to HDT. Time between diagnosis and HDT ranged from 1-96 months; time from HDT and MDS diagnosis was 10-60 months. Cryopreserved, pre-transplant bone marrow or peripheral blood stem cell specimens obtained at the time of harvest, or archival bone marrow smears were used. Standard cytogenetic analysis had been performed pre-transplant in 4 patients, showing a normal karyotype. The following cytogenetic abnormalities were examined: del(5)(q31), -5, -7, +8, -11, -21, using FISH probes (Vysis) for interphase analysis. In 9 of 12 cases, the same cytogenetic abnormality/ies observed at the time of MDS diagnosis was detected in asymptomatic pre-HDT specimens, in 20-46% of the cells examined. This finding supports the hypothesis that stem-cell damage leading to post-transplant MDS may result from prior conventional-dose chemotherapy, and may be unrelated to HDT or the transplantation process itself.

CS12

ESSENTIAL THROMBOCYTHAEMIA: PROGNOSTIC FACTORS IN THE ITALIAN SERIES OF TWO THOUSAND PATIENTS

L. GUGLIOTTA, M. LAZZARINO, R. MARCHIOLI, A. AMBROSETTI, S. BARAVELLI, M. BAZZAN, E. CACCIOLA, R. CALORI, A. CIOCCA VASINO, A. DE VIVO, M. FIACCHINI, G. FINAZZI, L. GARGANTINI, A. GROSSI, P.G. IANNACCARO, T. LEVA, U. MAGRINI, M.R. MARFISI, V. MARTINELLI, M.G. MAZZUCCONI, A. MORELLI, A. NOVARINO, F. PALMIERI, E. POGLIANI, F. RADAELLI, L. RANDI, G. REGE CAMBRIN, F. RONCO, M. RUGGERI, S. RUPOLI, D. RUSSO, S. SACCHI, L. VALDRÈ, N. VIANELLI, F. RODEGHIERO, T. BARBUI, S. TURA FOR THE GIMMC (GRUPPO ITALIANO MALATTIE MIELOPROLIFERATIVE CRONICHE)

A series of 2139 patients with diagnosis of Essential Thrombocythaemia (ET), done between 1976 and 1996 in 60 Haematological Centers of GIMMC and verified according to PVSG criteria, have been retrospectively evaluated mainly to define the prognostic factors. The patients, 1315 females and 824 males, with median age 59 years (21% under 40 and 23% over 70 years), at diagnosis had a median platelet count of $910 \times 10^9/L$ (25% <750 and 20% >1200), splenomegaly (21%), hepatomegaly (25%), peripheral granulocyte precursors (8%), functional symptoms (35%), thrombotic risk factors (40%), haemorrhage (5%) and thrombosis (15%). The follow-up was 5.2 ± 3.1 years (median 4.4). The treatment was: antiplatelet drugs 78% (ASA 49%), cytostatic drugs 82%, (alkylating molecules 38%, HU 48%, IFN α 22%, Anagrelide 2%). During the follow-up the platelet count ($10^9/L$) was <400, 400 - 500 and 500 - 600 in 5%, 18% and 26% of cases respectively. The survival curve was the same compared to the general Italian population in the first 10 years of follow-up but later it was appreciably lower mainly because of cancer mortality. The lethal events, censored at 15th year from diagnosis, were 203. Reported as events/100 patient-years deaths were: haemorrhage 0,1%, thrombosis 0,7%, haematological cancer 0,5% and other causes 0,6%. Respect the end-point exitus favourable prognostic factors resulted to be the female sex, (RR -0.6) and the antiplatelet treatment (RR -06), while resulted unfavourable the older age at diagnosis (40 - 60 years RR 3.5; 61-70 years RR. 9.6; >70 years RR 25.2), the thrombosis at onset (RR 1.5), the peripheral granulocyte precursors (RR 1.8), the platelet count during the follow up > $1000 \times 10^9/L$ (RR 2.1). The role of antiplatelet and cytostatic drugs (type, dose, duration) will be furtherly described.