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2. Burgess AW, Begley CG, Johnson GR, et al. Purification and properties of bacterially synthesized human granulocyte-macrophage colony stimulating factor. Blood 1987; 69:43-51.
3. The Royal Marsden Hospital Bone-Marrow Transplantation Team. Failure of syngeneic bone-marrow graft without preconditioning in post-hepatitis marrow aplasia. Lancet 1977; 2:242-4.
4. Anonymous. Red cell aplasia [editorial]. Lancet 1982; 1:546-7.
5. Karlsson S, Humphries RK, Gluzman Y, Nienhuis AW. Transfer of genes into hemopoietic cells using recombinant DNA viruses [abstract]. Blood 1984; 64(Suppl 1):58a.

Books and other monographs (personal authors,[6,7] chapter in a book,[8] published proceeding paper,[9] abstract book,[10] monograph in a series,[11] agency publication[12]):

6. Ferrata A, Storti E. Le malattie del sangue. 2nd ed. Milano: Vallardi; 1958.
7. Hillman RS, Finch CA. Red cell manual. 5th ed. Philadelphia: FA Davis; 1985.
8. Bottomley SS. Sideroblastic anaemia. In: Jacobs A, Worwood M, eds. Iron in biochemistry and medicine, II. London: Academic Press; 1980. p. 363-92.
9. DuPont B. Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the third annual meeting of the International Society for Experimental Hematology. Houston: International Society for Experimental Hematology; 1974. p. 44-6.
10. Bieber MM, Kaplan HS. T-cell inhibitor in the sera of untreated patients with Hodgkin's disease [Abstract]. Paper presented at the International Conference on Malignant Lymphoma Current Status and Prospects, Lugano, 1981:15.
11. Worwood M. Serum ferritin. In: Cook JD, ed. Iron. New York: Churchill Livingstone; 1980. p. 59-89. (Chanarin I, Beutler E, Brown EB, Jacobs A, eds. Methods in hematology; vol 1).
12. Ranofsky AL. Surgical operation in short-stay hospitals: United States-1975. Hyattsville, Maryland: National Center for Health Statistics; 1978. DHEW publication no. (PHS) 78-1785, (Vital and health statistics; series 13; no. 34).

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Haematologica 1998; vol. 83, suppl. to no. 9

Contents

36th Congress of the Italian Society of Hematology

October 2-5, 1997
Catania-Naxos Taormina, Italy

SELECTED ABSTRACTS

36th Congress of the Italian Society of Hematology

October 2-5, 1997
Catania-Naxos Taormina, Italy

SELECTED ABSTRACTS

An introduction

Dear Colleagues,

Almost 600 abstracts were submitted to the Scientific Committee of the meeting. All contributions, without the authors' names and affiliations, were evaluated by three independent reviewers who gave a score based on originality, methodological approach, data explanation and clearness of the message. Based on this evaluation, 564 abstracts were accepted for oral or poster presentation and the 135 of them which were given the highest scores were selected for the present supplement of Haematologica.

The meeting pursued the double task of providing an educational opportunity and facilitating the exchange of both clinical and experimental experience between hematologists from all over the Country. Besides the Italian Society of Hematology (SIE), two other important scientific societies of Italian hematologists (SIES and GITMO) were represented and each of them organized an Institutional Symposium.

The large concourse of people at this congress not only is proof of the interest that research in the field of hematology arouses even in other specialists such as oncologists, basic researchers and internists, but also reaffirms the pivotal role of hematology in biological and clinical studies of neoplastic diseases in general. The high quality of contributions at this meeting confirmed the good shape of the Italian hematological community.

My thanks and gratitude to all the participants for their valuable contributions.

prof. Rosario Giustolisi

General index

Hemopoietic cell proliferation and differentiation

001
INDUCTION OF NITRIC OXIDE SYNTHASE IS INVOLVED IN THE MECHANISM OF FAS-MEDIATED APOPTOSIS IN CD34+ CELLS
Selleri C, Maciejewski JP, Sato T, Raiola A, Risitano AM, Pezzullo L, Rotoli B

002
INHIBITION OF INTERFERON REGULATORY FACTOR-1 EXPRESSION INDUCES INTERFERON-γ TO STIMULATE CD34+ CELLS
Selleri C, Sato T, Young NS, Maciejewski JP

003
FUNCTIONAL INACTIVATION OF p53 BY THE p210 PRODUCT OF THE BCR-ABL REARRANGED GENE OF CHRONIC MYELOID LEUKEMIA
Mianulli AM, Santucci MA, Di Paola MC, Gamberi B, Tura S

004
STEM CELL COMPARTMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): ANALYSIS OF HEMOPOIETIC PRECURSORS
Sala R, Mauro MR, Bellucci R, Lisci A, De Propris MS, Proia A, Cordone I, Foà R, de Fabritiis P

005
G-CSF EXPANDS THE PROGENITOR CELL COMPARTMENT AND MODULATES THE EXPRESSION OF ADHESION MOLECULES ON CD34+ CELLS OF PERIPHERAL BLOOD OF NORMAL DONORS
Bellucci R, De Propris MS, Lisci A, Leone G, Venditti A, de Fabritiis P

006
CLONOGENIC CELL CONTENT OF CHRONIC MYELOGENOUS LEUKEMIA CD34+ CELLS LACKING CD95 EXPRESSION
Regazzi E, Savoldo B, Garau D, Sammarelli G, Mangoni L, Almici C, Tabilio A, Cilloni D, Carlo-Stella C, Rizzoli V

007
GAS6 INHIBITS GRANULOCYTE ADHESION TO ENDOTHELIAL CELLS
Gallicchio M, Gammaitoni L, Bottarel F, Bragardo M, Dianzani U, Dianzani C, Saglio G, Avanzi GC

008
HEMATOPOIETIC PROGENITOR GROWTH IN HEALTHY DONORS BEFORE AND AFTER BONE MARROW HARVESTING
Astori C, Bonfichi M, Alessandrino EP, Balduini A, Bernasconi P, Pagnucco G, Marseglia C, Brera C, Bernasconi C

Molecular biology

009
INTERFERON-α REGULATION OF THE GROWTH-ARREST ASSOCIATED GENE GADD45 IN P210 BCR-ABL EXPRESSING HEMATOPOIETIC PROGENITOR CELLS
Di Paola MC, Santucci MA, Ripalti A, Mianulli AM, Giacca M, Tura S

010
IMMUNOLOCALIZATION OF HHV-6 PROTEIN IN HODGKIN'S DISEASE AND IN ROSAI DORFMAN'S DISEASE
Barozzi P, Luppi M, Maiorana A, Bonacorsi G, Bergonzini S, Morselli M, Cagossi K, Ferrari MG, Marasca R, Artusi T, Torelli G

011
IDENTIFICATION OF NOVEL GENETIC LESIONS IN MATURE B-CELLS NEOPLASMS: ROLE OF A RECURRENT t(4;14)(p16.3;q32) CHROMOSOMAL TRANSLOCATION IN MULTIPLE MYELOMA
Neri A, Ronchetti D, Richelda R, Baldini L, Rocchi M, Lombardi L, Cro L, Maiolo AT

012
QUANTIFICATION OF BCR-ABL TRANSCRIPT IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS BY COMPETITIVE AND QUANTITATIVE RT-PCR AND CAPILLARY ELECTROPHORESIS
Martinelli G, Farabegoli P, Montefusco V, Testoni N, Zaccaria A, Saglio G, Pane F, Petroni D, Amabile M, Vittone A, Terragna C, Tura S

013
MITOCHONDRIAL DNA DELETION IN CHILDREN WITH DE TONI-DEBRE'-FANCONI SYNDROME SECONDARY TO ANTIBLASTIC THERAPY
Di Cataldo A, Palumbo M, Sambataro MP, Schilirò G, Li Volti S

Cytokines

014
EFFECT OF DIFFERENTIAL HEMATOPOIETIC GROWTH FACTORS ON EXPANSION/PROLIFERATION OF ACUTE MYELOID LEUKEMIC CELLS VERSUS PROLIFERATION/DIFFERENTIATION
Gammaitoni L, Severino A, Sanavio F, Aglietta M, Piacibello W

015
COST-EFFECTIVENESS ANALYSIS: A CORRECT ADMINISTRATION OF G-CSF TO TREAT CHEMOTHERAPY-INDUCED NEUTROPENIA IN NON-HODGKIN LYMPHOMAS OF THE ELDERLY
Rupoli S, Capelli D, Cinciripini A, Barulli S, Salvi A, Del Prete S, Olivieri A, Leoni P

016
INTERMEDIATE DOSE OF G-CSF AND MOBILIZATION OF BLOOD STEM CELLS IN 10 HEALTHY DONORS
Bonfichi M, Balduini A, Pagnucco G, Bernasconi P, Alessandrino EP, Astori C, Marseglia C, Brera C, Vanelli L, Bernasconi C

017
FLT3L ENHANCES THE EARLY STEM CELL COMPARTMENT AFTER EX-VIVO AMPLIFICATION OF UMBILICAL CORD BLOOD CD34+ CELLS
Di Pucchio T, Mascolo MG, Breccia M, Agostini F, Guglielmi C, Ricciardi MR, Screnci M, Dominioni F, Tafuri A, Carmini D, Arcese W, De Felice L

018
EFFECT OF THE ADDITION OF TPO IL-3, SCF AND EPO ON THE ERYTHROID AND MEGAKARYOCYTIC DIFFERENTIATION OF CD34+ CELLS FROM HUMAN BONE MARROW AND CORD BLOOD
Grossi A, Pagliai G, Bonsi L, Evangelisti L, Lombardini L, Caporale R, Tonelli R, Strippoli P, Santini V, Bagnara GP

019
THROMBOPOIETIN (TPO) AND TPO-R ARE PRODUCED BY PRIMARY HUMAN MESANGIAL CELLS IN CULTURE
Santini V, Pupilli C, Scappini B, Pagliai L, Grossi A, Lasagni L, Strippoli P, Bagnara G, Rossi Ferrini P

020
HYPERSENSITIVITY TO GM-CSF AND DELAYED APOPTOSIS IN GM-CSF DEPENDENT GF-D8 CELL LINE ENGINEERED TO OVEREXPRESS SHC
Dotti GP, Carlo Stella C, Spinelli O, Savoldo B, Garau D, Regazzi E, Rizzoli V, Barbui T, Pelicci PG, Lanfrancone L, Rambaldi A

021
ALTERED IMMUNOREGULATION BY SELECTIVE EXPRESSION OF Th1-TYPE CYTOKINE mRNAs IN HEMOPHAGOCYTIC SYNDROME PATIENTS
Palumbo GA, Romeo MA, Di Raimondo F, Galvagno F, Milone G

022
HUMORAL-MEDIATED SUPPRESSION OF LYMPHOCYTE BLASTOGENESIS IN HEALTHY DONORS RECEIVING G-CSF
Rutella S, Rumi C, Lucia MB, Sica S, Testa U, Leone G

Chronic myeloproliferative disorders

023
HIGHER EXPRESSION OF FAS RECEPTOR ON CD34$^+$ CELLS OF CHRONIC MYELOGENOUS LEUKEMIA CORRELATES WITH HEMATOLOGIC RESPONSE TO IFN-α
Selleri C, Luciano L, Del Vecchio L, Raiola A, Boccuni PN, Risitano AM, Rotoli B

024
FAS-MEDIATED DOWNMODULATION OF p210 BCR/ABL RESULTS IN APOPTOSIS OF CD34$^+$ CELLS OF CHRONIC MYELOGENOUS LEUKEMIA
Selleri C, Pane F, Maciejewski JP, Luciano L, Raiola A, Mostarda I, Salvatore F, Rotoli B

025
BONE MAST CELLS SARCOMA
Dini D, Savarino M, Bonacorsi G, Artusi T, Torelli G

026
FOLLOW-UP OF THE CYTOGENETIC RESPONSE IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH α-INTERFERON
Zuffa E, Zaccaria A on behalf of the Italian Cooperative Study Group on Chronic Myeloid Leukemia

027
AMIFOSTINE PRETREATMENT ALLOWS THE USE OF HIGHER DOSES OF THE BCR-ABL-SPECIFIC TYROSINE KINASE INHIBITOR AG1112
Cilloni D, Levitzki A, Regazzi E, Garau D, Savoldo B, Sammarelli G, Almici C, Caramatti C, Rizzoli V, Carlo-Stella C

028
CYTOREDUCTIVE THERAPY OF ESSENTIAL THROMBOCYTHEMIA: PROPOSAL OF A SCORE SYSTEM
Spadea A, Peraino M, Bernasconi S, Latagliata R, Petti MC, Mazzucconi MG

029
FOLLOW-UP OF THE PH-POSITIVE CLONE IN CHRONIC MYELOGENOUS LEUKEMIA UNDER INTERFERON THERAPY: COMPARISON OF INTERPHASE CYTOGENETICS AND CONVENTIONAL CHROMOSOME ANALYSIS
Bigoni R, Cuneo A, Roberti MG, Bardi A, Scapoli GL, Emanuelle E, Hagemeijer A, Castoldi GL

Myelodysplastic syndromes

030
MEASUREMENT OF CIRCULATING AND BONE MARROW LONG-TERM CULTURE INITIATING CELLS IN MYELODYSPLASTIC PATIENTS
Selleri C, Sato T, Kim S, Young NS, Maciejewski JP

031
MYELODYSPLASTIC SYNDROMES AND EXPOSURE TO MUTAGENIC AGENTS: CORRELATION BETWEEN PROGNOSIS AND CLINICO-BIOLOGIC AND CYTOGENETIC DATA
Rigolin GM, Cuneo A, Roberti MG, Bardi A, Bigoni R, Minotto C, Castoldi GL

032
IN VIVO MOBILIZATION OF KARYOTYPICALLY NORMAL PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) IN HIGH-RISK MDS, SECONDARY OR THERAPY-RELATED ACUTE MYELOGENOUS LEUKEMIA
Celesti L, Dejana A, Lerma E, Fugazza G, Sessarego M, Podestà M, Frassoni F, Valbonesi M, Carella AM

033
RISK EVALUATION IN MYELODYSPLASTIC SYNDROMES: THE VALIDITY OF THE INTERNATIONAL PROGNOSTIC INDEX AND THE ROLE OF BONE MARROW BIOPSY
Annaloro C, Oriani A, Cortelezzi A, Lambertenghi Deliliers D, Soligo D, Pozzoli E, Somalvico F, Lambertenghi Deliliers G

034
DETECTION OF ABNORMAL MOTILITY PATTERNS OF CIRCULATING NEUTROPHILS FROM PATIENTS SUFFERING FROM MYELODYSPLASTIC SYNDROMES IDENTIFIED BY IMAGE ANALYSIS
Azzarà A, Carulli G, Petrini M

035
HIGHER SPONTANEOUS RATE OF APOPTOSIS CHARACTERIZES MYELODISPLASTIC SYNDROMES AT LOWER RISK OF LEUKEMIC EVOLUTION
Ricciardi MR, Petrucci MT, Ariola C, Gregorj C, Latagliata R, Petti MC, Tafuri A

036
CD34$^+$ MEGAKARYOCYTES OCCUR IN NORMAL BONE MARROW, AND ARE GREATLY INCREASED IN MYELODYSPLASTIC SYNDROMES
Pellegrini W, Marocolo D, Facchetti F, Pelizzari AM, Capucci A, Rossi G

037
EFFECT OF THROMBOPOIETIN ON MEGAKARYOCYTIC AND ERYTHROID PROGENITORS IN MYELODYSPLASTIC SYNDROMES
Sarina N, Cortelezzi A, Cristiani S, Cattaneo C, Silvestris I, Carrabba M, Della Volpe A, Maiolo AT

038
EXPRESSION OF CYTOKINE RECEPTORS IN LONG TERM LIQUID CULTURES OF MYELODYSPLASTIC SYNDROMES
Soligo D, Quirici N, Servida F, Lambertenghi Deliliers G

Acute leukemias

039
COMPARATIVE TOXICITY OF DAUNORUBICIN AND DAUNOXOME ON MDR AND NON-MDR CELL LINES
Michelutti A, Ermacora A, Michieli M, Damiani D, Masolini P, Grimaz S, Candoni A, Bertone A, Rogato A, Marin L, Salmaso F, Geromin A, Russo D, Fanin R, Baccarani M

040
SOLUBLE p55-TNFr SERUM LEVELS CORRELATE WITH PROGNOSIS IN ADULT ACUTE MYELOID LEUKEMIA
Tecchio C, Vinante F, Rigo A, Morosato L, Zanolin E, Locatelli F, Perona G, Pizzolo G

041
PROPOSED MODEL OF CHEMORESISTANCE BASED ON THE INTERACTION BETWEEN P-GLYCOPROTEIN, BCL-2 AND TRANSFERRIN RECEPTOR IN ACUTE MYELOID LEUKEMIA
Del Poeta G,Venditti A, Aronica G, Buccisano F, Tamburini A, Cox MC, Bruno A, Epiceno AM, Postorino M, Masi M, Amadori S

042
P-GLYCOPROTEIN AND TERMINAL TRANSFERASE IDENTIFY PROGNOSTIC SUBSETS WITHIN ACUTE MYELOID LEUKEMIA
Del Poeta G, Venditti A, Aronica G, Buccisano F, Tamburini A, Cox MC, Bruno A, Epiceno AM, Postorino M, Masi M, Amadori S

043
MULTIPLE ADVERSE BIOLOGICAL FEATURES EXPLAIN THE POOR PROGNOSIS OF ACUTE MYELOID LEUKEMIA M0
Del Poeta G, Venditti A, Buccisano F, Aronica G, Tamburini A, Cox MC, Bruno A, Epiceno AM, Masi M, Amadori S

044
THE PATTERN OF CD11b EXPRESSION BY LEUKEMIC CELLS AT BASAL CONDITIONS AND FOLLOWING ATRA IN VITRO DISTINGUISHES M3 FROM NON-M3 BLASTS
Tecchio C, Rigo A, Vinante F, Perona G, Pizzolo G

045
THERAPY OF ACUTE LEUKEMIA: RETROSPECTIVE ANALYSIS OF A RECENT SERIES OF PATIENTS OF ALL AGES TREATED IN ONE CENTER
Cerri R, Clavio M, Pierri I, Isaza A, Beltrami G, Risso M, Rossi E, Sriano M, Canepa L, Miglino M, Van Lint MT, Bacigalupo A, Carella AM, Gobbi M, Damasio E

046
EVALUATION OF THE EXPRESSION OF SEVERAL MDR-RELATED GENES IN ANLL PATIENTS
Petrini M, Galimberti S, Testi R, Da Prato I

047
BONE MARROW METASTATIC INFILTRATION BY ALVEOLAR RHABDOMYOSARCOMA SIMULATING AN ACUTE ERYTHREMIA (DI GUGLIELMO'S SYNDROME)
Volpe S, Marcacci G, Cantore N, Capone F, di Marzo Capozzi F, Santulli B, Volpe E

048
ACUTE MYELOID LEUKEMIA (AML) RELAPSING AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION (AUBMT) IN FIRST REMISSION: ANALYSIS OF 249 CASES
Iacopino P, Vignetti M, Andrizzi C, Barbui T, Carella AM, Alessandrino EP, Benedetti F, Falda M, Sajeva MT, Carlo-Stella C, Lambertenghi G, Sica S, Visani G, Meloni G for GITMO-GIMEMA

049
IN VITRO DRUG-INDUCED CYTOTOXICITY PREDICTS CLINICAL OUTCOME IN ACUTE LEUKEMIA (AL)
Console G, Callea I, Pontari A, Ramirez F, Dattola A, Sculli G, Musolino C, Nobile F, Ronco F, Iacopino P, Morabito F

050
ACUTE MYELOBLASTIC LEUKEMIA IN THE ELDERLY: ANALYSIS OF OUTCOME IN 119 PATIENTS
Audisio E, Marmont F, Allione B, Boccomini C, D'Ardia S, Falda M, Locatelli F, Rus C, Resegotti L

051
ROLE OF FLUORESCENT IN SITU HYBRIDIZATION IN THE DETECTION OF TRISOMY 8 IN ACUTE MYELOID LEUKEMIA (AML)
Cuneo A, Bigoni R, Mancini M, Nanni M, Alimena G, La Starza R, Matteucci C, Mecucci C, Cavigliano P, Bernasconi P, Testoni N, Zaccaria A, Roberti MG, Bardi A, Castoldi GL

052
RESULTS OF A MULTICENTER RANDOMIZED CLINICAL TRIAL ON PLATELET TRANSFUSION THRESHOLD IN ACUTE MYELOID LEUKEMIA (AML)
Barbui T for the GIMEMA group

053
IDARUBICIN IN THE TREATMENT OF AML: LONG-TERM RESULTS OF A SINGLE CENTER'S EXPERIENCE
Oriani A, Annaloro C, Pozzoli E, Della Volpe A, Soligo D, Cortelezzi A, Lambertenghi Deliliers G

054
IN VITRO INDUCTION OF APOPTOSIS BY CHEMOTHERAPEUTIC AGENTS IN HUMAN MYELOID LEUKEMIA CELLS
Mestice A, Coppi MR, Specchia G, De Paolis MR, Ricco A, Attolico I, Mininni D, Contino R, Albano F, Donno A, Vaira A, Liso V

055
EUROPEAN INTERGROUP TRIAL FOR ADULT BCR/ABL$^+$ ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): PRELIMINARY RESULTS OF THE GIMEMA GROUP
Mandelli F, Annino L, Ferrari A, Meloni G, Lo Coco F, Avanzini P, Del Poeta G, Di Raimondo F, Fabbiano F, Falda M, Ladogana S, Levis A, Morra E, Recchia A, Saglio G, Specchia G, Tabilio A, Zagonel V

056
ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): > 5 YEARS LONG-TERM SURVIVORS. A RETROSPECTIVE STUDY
Annino L, Ferrari A, Giona F, Lamanda M, Testi AM, Vegna ML, Mandelli F

057
POOR OUTCOME OF CHILDREN WITH FIRST ISOLATED MEDULLARY RELAPSE OCCURRING FIVE OR MORE YEARS AFTER DIAGNOSIS OF ACUTE LYMPHOBLASTIC LEUKEMIA. AN AIEOP (ASSOCIAZIONE ITALIANA DI EMATOLOGIA ED ONCOLOGIA PEDIATRICA) STUDY
Dibenedetto SP, Rizzari C, Valsecchi MG, Miniero R, Conter V, Aricò M, Messina C, Miano C, Surico GM, Testi AM, Schilirò G, Masera G, on behalf of Associazione Italiana di Ematologia ed Oncologia Pediatrica

058
IMMUNOPHENOTYPIC FINDINGS IN ACUTE MYELOID LEUKEMIA (AML) WITH t(8;21)
Ferrara F, Sebastio L, Di Noto R, Scognamiglio M, Stanziola MC, Lo Pardo C, Boccuni P, Cimino R, Del Vecchio L

059
11q23 REARRANGEMENTS AND ACUTE LEUKEMIAS: CYTOGENETIC AND MOLECULAR ANALYSIS OF 19 CASES
Mancini M, Elia L, Nanni M, Rapanotti MC, Cedrone M, De Cuia MR, Brama M, Celesti F, Alimena G

060
TREATMENT OF RELAPSED ACUTE MYELOID LEUKEMIA (AML): A RETROSPECTVE ANALYSIS OF TEN YEARS EXPERIENCE AT UNIVERSITY "LA SAPIENZA" IN ROME
Luzi G, Meloni G, Montefusco E, Latagliata R, Spadea A, Breccia M, D'Elia GM, Redi R, Betrò P, Petti MC

061
THE ROLE OF A HEMATOLOGICAL EMERGENCY UNIT (HEU) IN THE MANAGEMENT OF PATIENTS WITH ACUTE LEUKEMIA
Tosti S, Morano SG, Battistini R, Coppola L, Girmenia C, Latagliata R, Spadea A, Cimino G, Tafuri A, Alimena G

062
MARROW LEUKEMIC INDEX (MLI) ON THE 14th DAY OF TREATMENT IN ACUTE MYELOID LEUKEMIA
Specchia G, Attolico I, Pastore D, Palumbo G, Carluccio P, Fiore M, Mestice A, Contino R, Lamacchia M, Scorca A, Magno M, Bizzoca R, Liso V

Lymphomas

063
HEPATITIS G VIRUS (HGV) PREVALENCE IN PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS (LPD)
De Renzo A, Persico M, Persico M, Villa MR, Coppola L, Torella R, Rotoli B

064
EPIDEMIOLOGY OF MALIGNANT LYMPHOMAS IN SARDINIA, 1974-1993
Broccia G, Casula P, Monni A, Ferreli A, Onida G.A, Massarelli G, Tanda F, Cocco PL

065
LLO1: A GISL PROTOCOL FOR THE TREATMENT OF LYMPHOCYTIC LYMPHOMA AND B-CELL RELATED LEUKEMIAS
Colombi M, Baldini L, Brugiatelli M, Lombardo M, Molica S, Gobbi P, Avanzini P, La Targia ML, Ginaldi L, Attardo G, Federico M, Silingardi V, Maiolo A.T. for GISL

066
PRELIMINARY DATA OF A RANDOMIZED PLURICENTRIC STUDY (ABVD + RT EF VERSUS IF) IN INTERMEDIATE STAGES HODGKIN'S DISEASE (HD)
Anselmo AP, Proia S, Cavalieri E, Campanella B, Maurizi Enrici R, Cantonetti M, Bellesi G, Biti G, Mandelli F

067
FAILURE OF HDS REGIMEN IN THE MANAGEMENT OF HIGH GRADE NON-HODGKIN'S LYMPHOMA WITH BONE MARROW INVOLVEMENT OR T-CELL OR CD30 IMMUNOPHENOTYPE
Caracciolo D, Tarella C, Gavarotti P, Corradini P, Castellino C, Zallio F, Bianchi A, Cherasco C, Rossi G, Pileri A

068
HDS REGIMEN IN LOW/INTERMEDIATE GRADE NON-HODGKIN'S LYMPHOMA OTHER THAN FOLLICULAR SUBTYPES
Gavarotti P, Caracciolo D, Corradini P, Castellino C, Zallio F, Vitolo U, Bertini M, Novero D, Gallo E, Pileri A, Tarella C

069
VACOP-B vs VACOP-B + AUTOLOGOUS BM TRANSPLANTATION (ABMT) FOR AGGRESSIVE NON-HODGKIN'S LYMPHOMA. A STUDY FROM THE NON-HODGKIN'S LYMPHOMA COOPERATIVE STUDY GROUP (NHLCSG)
Santini G, Congiu M, Salvagno L, Chisesi T, Leoni P, Olivieri A, Centurioni R, Tedeschi L, Porcellini A, Morandi S, Contu A, Quaini R, Majolino I, Palmisano G, Zambaldi G, Indrizzi L, Sertoli M, Rizzoli V for the NHLCSG

070
INTENSIFIED CHEMOTHERAPY WITH DOSE INTENSITY ESCALATION OF CYCLOFOSFAMIDE AND EPIRUBICINE WITH FILGRASTIM SUPPORT (MegaCEOP) FOR POOR PROGNOSIS AGGRESSIVE NON-HODGKIN'S LYMPHOMA
Botto B, Boccomini C, Calvi R, Bertini M, Ciocca Vasino MA, Freilone R, Galliano M, Orsucci L, Levis A, Resegotti L, Vitolo U

071
HUMAN T-CELL LYMPHOTROPIC VIRUS-I TAX IS NEVER DETECTED IN THE SKIN AND PERIPHERAL BLOOD MONONUCLEAR CELLS OF PATIENTS WITH CUTANEOUS T-CELL LYMPHOMA
Multicentric Study of CTCL: Rupoli S, Pauri P, Brugia M, Clementi M, Barulli S, Brandozzi G, Mozzicafreddo G, Offidani AM, Cataldi I, Ricotti G, Simonacci M, Ceschini E, Filosa G, Ciattaglia G, Serresi S,Giangiacomi M, Brancorsini D, Mancini S, Leoni P

072
P-VABEC CHEMOTHERAPY FOR ELDERLY AGGRESSIVE NON HODGKIN'S LYMPHOMA PATIENTS: LONG TERM RESULTS OF 122 PATIENTS TREATED AT SINGLE INSTITUTION
De Sanctis V, Martelli MP, Guglielmi C, Giovannini M, Orgiana L, Palombi F, Martelli M, Mandelli F

073
DIFFUSE LARGE CELL LYMPHOMA (DLCL) WITH BONE MARROW (BM) INVOLVEMENT: INTENSIFIED CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION, COMPARISON WITH STANDARD CHEMOTHERAPY
Freilone R, Vitolo U, Cortellazzo S, Liberati AM, Falda M, Bertini M, Botto B, Cinieri S, Locatelli F, Lovisone E, Marmont F, Pizzuti M, Rossi A, Viero P, Grignani F, Barbui T, Resegotti L

074
REARRANGEMENTS OF BCL6, BCL2, c-MYC AND 6 q DELETION IN B-CELL LYMPHOMA: CLINICAL RELEVANCE IN 71 PATIENTS
Vitolo U, Gaidano G, Botto B, Volpe G, Audisio E, Bertini M, Calvi R, Freilone R, Novero D, Orsucci L, Pastore C, Capello D, Parvis G, Sacco C,Zagonel V, Carbone A, Mazza U, Palestro G, Saglio G, Resegotti L

075
LYMPHOPROLIFERATIVE DISORDERS IN HEART TRANSPLANT RECIPIENTS: DEFINITION OF MOLECULAR APPROACH TO TREATMENT
Dotti GP, Fiocchi R, Ruggeri M, Motta T, Breviario B, Rambaldi A, Barbui T

076
THE "INTERNATIONAL INDEX" IS USEFUL IN THE PROGNOSTIC EVALUATION OF PATIENTS WITH HIV-RELATED NON-HODGKIN'S LYMPHOMA (NHL)
Rossi G, Donisi A, Cattaneo A, Casari S, Stellini R, Cadeo GP, Carosi GP

077
IFOFOSFAMIDE, EPIRUBICIN AND ETOPOSIDE (IEV) PLUS rhG-CSF FOR THE RELAPSED/REFRACTORY LYMPHOMA. A SAFE REGIMEN FOR INDUCTION OF REMISSION AND MOBILIZATION OF PROGENITOR CELLS
De Sanctis V, Proia A, De Propris MS, Bellucci R, Martelli MP,Trasarti S, Bizzoni L, Amaranto P, Martelli M

078
SMALL NON-CLEAVED CELL LYMPHOMA AND MATURE B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA IN ADULTS AND CHILDREN: RESULTS WITH 89-C-41 (NCI) PROTOCOL
Todisco E, Testi AM, Moleti ML, Chiaretti S, Leoni P, Montillo M, Resegotti L, Zagonella V, Mandelli F

079
FDG-PET AND SERUM CA125 ARE RELIABLE, NON-INVASIVE TOOLS FOR STAGING AND MONITORING GASTROINTESTINAL LOCALIZATIONS OF LYMPHOMA
Corazzelli G, Mainolfi C, Abate G, Russo F, Aloia C

080
FRONT-LINE CEVOP-B + rhGM-CSF WITH 96-HOUR INFUSION OF EpiADM AND VP16 IN AGGRESSIVE NON HODGKIN'S LYMPHOMA. A PHASE II STUDY
Abate G, Corazzelli G, De Chiara A, De Rosa V, Frigeri F, Monda VM, Perrone F, Russo F, Scoppa G

081
FLUDARABINE, CYCLOPHOSPHAMIDE, AND DEXAMETHASONE (FLUCYD) FOR THE TREATMENT OF ADVANCED LOW-GRADE NON-HODGKIN'S LYMPHOMA
Lazzarino M, Orlandi E, Montillo M, Tedeschi A, Pagnucco G, Astori C, Crugnola M, Gargantini L, Corso A, Brusamolino E, Morra E, Bernasconi C

082
HEPATITIS C VIRUS (HCV) AND NON-HODGKIN'S LYMPHOMA (NHL): A CASE-CONTROL STUDY COMPARING CLINICAL FEATURES AND RESPONSE TO TREATMENT IN A CONSECUTIVE SERIES FROM A SINGLE INSTITUTION
Zagonel V, Sacco C, Rupolo M, De Vita S, Talamini R, Spazzapan S, Bordonaro R, Boiocchi M, Carbone A

083
CYTOFLUORIMETRIC ANALYSIS OF LYMPH NODE SUSPENSIONS ALLOWS EARLY DIAGNOSIS AND APPLICATION OF THE REAL CLASSIFICATION
Baldini L, Cro L, Pruneri G, Nobili L, Zucal N, Guffanti A, Motta M, Buffa R, Maiolo AT

084
CHARACTERIZATION OF T(11;14) TRANSLOCATION IN MANTLE CELL LYMPHOMA WITH FLUORESCENT IN SITU HYBRIDIZATION
Bardi A, Bigoni R, Cuneo A, Rigolin GM, Roberti MG, Negrini M, Veronese ML, Croce CM, Castoldi GL

085
THE NUCLEOTIDE MUTATIONS IN VARIABLE REGIONS OF HEAVY CHAIN GENES OF IMMUNOGLOBULINS IN HCV-ASSOCIATED IMMUNOCYTOMAS ARE INDICATIVE OF ANTIGEN SELECTION
Efremov DG, Pozzato G, Tulissi P, Fanni Canelles M, Silvestri F, Mazzaro C, Santini GF, Ivanovski M, Burrone O

Chronic lymphoproliferative disorders and myeloma

086
FLUDARABINE (FLU) VERSUS CHLORAMBUCIL AND PREDNISOLONE (CHL+P) IN THE FIRST LINE THERAPY OF B CELL CHRONIC LYMPHOID LEUKEMIA (B-CLL). PRELIMINARY RESULTS OF THE RANDOMIZED MULTICENTRIC STUDY
Spriano M, Cassibba V, Chiurazzi F, Clavio M, Gobbi M, Leoni P, Capalbo S, Mazza P, Molica S, Rossi Ferrini PL, Santini G, Zaccaria A, Damasio E

087
PROPOSED NEW SCORING SYSTEM (MCSS) FOR THE IMMUNOPHENOTYPIC DIAGNOSIS OF MATURE B-CELL CHRONIC LEUKEMIAS
Cro L, Baldini L, Nobili L, Zucal N, Neri A, Maiolo AT

088
EFFICACY OF MAINTENANCE THERAPY WITH HUMAN LYMPHOBLASTOID ALPHA-INTERFERON IN HAIRY CELL LEUKEMIA (HCL)
Pagnucco G, Castello A, Castelli G, Bellio L, Canevari A, Lazzarino M, Bernasconi C

089
SPONTANEOUS AND DRUG-RELATED APOPTOSIS IN EARLY AND ADVANCED CHRONIC LYMPHOCYTIC LEUKEMIA
Impera S, Fiumara P, Stagno F, Consoli U, Formica V, Taibi R, Cacciola E, Guglielmo P, Giustolisi R

090
ATYPICAL B-CLL: A CYTOGENETIC AND INTERPHASE CYTOGENETIC STUDY
Roberti MG, Bigoni R, Cuneo A, Rigolin GM, Bardi A, Negrini M, Bullrich F, Veronese ML, Croce CM, Castoldi GL

091
IMMUNOPHENOTYPIC SUBCLASSIFICATION OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL): CLINICAL AND PROGNOSTIC ANALYSIS
Bellio L,Pagnucco G, Vanelli L, Maiocchi MA, Bo M, Caberlon S, Castelli G, Canevari A, Lazzarino M, Bernasconi C

092
AUTOIMMUNE HEMOLYTIC ANEMIA IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): A RETROSPECTIVE STUDY OF 55 CASES
Mauro FR, Mandelli F, Foà R, Coluzzi S, Sala R, Crescenzi S, Cerretti R, Girelli G

093
COMBINATION OF FLUDARABINE, ARA-C, MITOXANTRONE AND DEXAMETHASONE FOR THE TREATMENT OF ADVANCED CHRONIC LYMPHOPROLIFERATIVE DISORDERS
Mauro FR, Foà R, Martelli M, Lamanda M, La Verde G, Sala R, Rocchi L, Cerretti R, Proia A, Mandelli F

094
INCREASED LEVELS OF SOLUBLE CD27 IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA
Molica S, Vitelli G, Levato D, Dell'Olio M, Mannella A, Levato L, Matera R, Musto P, Gandolfo GM

095
TRIGGERING OF CD40 ANTIGEN INHIBITS FLUDARABINE-INDUCED APOPTOSIS IN B CHRONIC LYMPHOCYTIC LEUKEMIA CELLS
Romano MF, Costantini S, Lamberti A, Turco MC, Alfinito F, Chiurazzi F, Rotoli B, Venuta S

096
AUTOLOGOUS CIRCULATING PROGENITOR CELLS TRANSPLANTATION AS FIRST LINE TREATMENT FOR MULTIPLE MYELOMA
Tribalto M, Adorno G, Andriani A, Bugliosi R, Caravita T, Cudillo L, Del Poeta G, Dentamaro T, Rainaldi A. Avvisati G, La Verde G, Meloni G, Leone G, Sica S, Martelli M, Tabilio A, Fioritoni G, Iacone P, Majolino I, Marcenò R, Amadori S, Mandelli F, Papa G

097
ALLOGENEIC STEM CELL TRANSPLANTS FOR MULTIPLE MYELOMA. BOLOGNA EXPERIENCE
Cavo M, Bandini G, Benni M, Rosti GA, Terragna C, Lemoli RM, Ronconi S, Martinelli G, Gozzetti A, Zamagni E, Motta MR, Rizzi S, Tura S

Autologous bone marrow transplantation

098
HEMATOLOGIC RECONSTITUTION (HR) AFTER PBPC AUTOTRANSPLANTATION: COMPARISON BETWEEN PROGRAMMED CRYOPRESERVATION AND UNCONTROLLED-RATE FREEZING AT –80°C
Olivieri A, Montanari M, Offidani M, Mancini S, Masia MC, Curzi L, Leoni P

099
PBPC MOBILIZATION WITH DHAP REGIMEN FOLLOWED BY AUTOTRANSPLANTATION IN 33 LYMPHOMA PATIENTS AFTER FIRST-LINE CHEMOTHERAPY FAILURE
Olivieri A, Montanari M, Offidani M, Rupoli S, Centurioni R, Cantori I, Leoni P

100
AUTOLOGOUS PBSC (PBSCT) vs AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) AFTER ICE-NOVIA INDUCTION/CONSOLIDATION IN ACUTE MYELOID LEUKEMIA
Visani G, Lemoli RM, Manfroi S, Tosi P, Testoni N, Motta MR, Rizzi S, Mangianti S, Fortuna A, Remiddi C, Pastano R, Pellacani A, Tura S

101
MYELODYSPLASTIC SYNDROMES AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION
Lambertenghi Deliliers G, Annaloro C, Soligo D, Romitti L, Della Volpe A, Tagliaferri E, Bertolli V, Onida F, Oriani A

102
MOBILIZATION AND TRANSPLANTATION OF PHILADELPHIA-(Ph1) NEGATIVE PERIPHERAL-BLOOD PROGENITOR CELLS (PBPC) IN CHRONIC MYELOGENOUS LEUKEMIA
Lerma E, Celesti L, Dejana A, Podestà M, Frassoni F, Sacha T, Carella AM

103
DIFFERENTIAL EXPRESSION LEVEL OF SOME ADHESION MOLECULES ON CD34+ STEADY-STATE BM AND CD34+ MOBILIZED HEMOPOIETIC PROGENITOR CELLS
Stagno F, Consoli U, Fiumara P, Formica V, Privitera A, Santonocito A, Parisi G, Disca S, Guglielmo P, Giustolisi R

104
IMMUNOMAGNETIC SELECTED CD34+ AUTOTRASPLANT FOR TREATMENT OF SEVERE LES
Musso M, Porretto F, Crescimanno A, Bondì F, Polizzi V, Scalone R, Mariani G

105
AUTOLOGOUS PLATELET SUPPORT IN PATIENTS WITH BREAST CANCER RECEIVING HIGH-DOSE CHEMOTHERAPY AND CIRCULATING PROGENITOR CELL TRANSPLANTATION
Da Prada GA, Pedrazzoli P, Perotti C, Battaglia M, Noris P, Torretta L, Pavesi L, Balduini C, Salvaneschi L, Robustelli della Cuna G

106
PRIMARY RESISTANT OR RELAPSED HODGKIN'S DISEASE (HD): RELEVANCE OF HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN A RETROSPECTIVE STUDY
Vitolo U, Freilone R, Botto B, Marmont F, Paolino F, Pantano A, Bertini M, Falda M, Locatelli F, Bertoncelli MC, Orsucci L, Calvi R, Boccomini C, Resegotti L, Levis A

107
MAGNESIUM CHLORIDE (MC) FOR THE PREVENTION AND THE MANAGEMENT OF MUCOSITIS AFTER ALLOGENEIC BMT AND AUTOLOGOUS HEMOPOIETIC STEM CELL TRANSPLANTATION (HSCT)
Irrera G, Rossetti A, Martino M, Messina G, Console G, Pucci G, Azzarà M, Condemi R, Cortese D, Mattia L, Russo L, Morabito F, Iacopino P

108
PCR-DETECTION OF RESIDUAL TUMOR CELLS AFTER HIGH-DOSE CHEMOTHERAPY: COMPARISON BETWEEN PBPC AND BONE MARROW HARVESTS
Corradini P, Tarella C, Astolfi M, Voena C, Cherasco C, Ventriglia A, Cinque F, Ladetto M, Boccadoro M, Pileri A

109
CD34+ SELECTED PBSC AUTOGRAFT IN CLL: PRELIMINARY RESULTS OF A MULTICENTER STUDY
Scimè R, Santoro A, Musso M, Olivieri A, Molica S, Iacopino P, Milone G, Musolino C, Capalbo S, Mariani G, Leoni P, Giustolisi R, Liso V, Narni F, Donelli A, Majolino I

110
MATCHED-PAIR ANALYSYS OF PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (PBSCT) vs AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN HODGKIN'S AND NON-HODGKIN'S LYMPHOMAS: AN UPDATE OF THE EBMT LYMPHOMA REGISTRY STUDY
Majolino I, Pearce R, Taghipour G, Goldstone AH

111
EFFECT OF AG957, A BCR-ABL-SPECIFIC TYROSINE KINASE INHIBITOR, ON CHRONIC MYELOGENOUS LEUKEMIA PROGENITOR CELLS
Garau D, Savoldo B, Regazzi E, Sammarelli G, Cilloni D, Giachetti R, Levitzki A, Rizzoli V, Carlo-Stella C

Allogeneic bone marrow transplantation

112
BONE MARROW LONG-TERM INITIATING CELLS ARE DECREASED AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION
Selleri C, Raiola A, Risitano AM, Boccuni PN, Del Vecchio L, Della Cioppa P, De Rosa G, Rotoli B

113
COMPARABLE OUTCOME OF ALLOGENEIC BONE MARROW AND PERIPHERAL BLOOD CELL TRANSPLANT IN ADULTS WITH HEMATOLOGIC MALIGNANCIES
Bacigalupo A, Van Lint MT, Valbonesi M, Lamparelli T, Gualandi F, Occhini D, Mordini N, Bregante S, Berisso G, Vitale V, Sessarego M, Marmont AM

114
ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR PATIENTS WITH HIGH RISK ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): FAVOURABLE IMPACT OF CHRONIC GRAFT VERSUS HOST DISEASE (cGVHD) ON SURVIVAL AND RELAPSE
Zikos PM, Van Lint MT, Lamparelli T, Gualandi F, Occhini D, Bregante S, Berisso G, Mordini N, Incagliato M, Fugazza G, Sessarego M, Bacigalupo A

115
NEUROLOGIC COMPLICATIONS IN ALLOGENEIC BONE MARROW TRANSPLANTATION
Bavaro P, Di Girolamo G, Di Bartolomeo P, Olioso P, Papalinetti G, Roccaforte S, Marsico S, Torlontano G

116
IN VIVO TREATMENT OF SEVERE REFRACTORY ACUTE GVHD BY ANTIOXIDANT N-ACETYLCTYSTEINE
Colombo A, Alessandrino EP, Bernasconi P, Massa M, Martinelli G, Caldera D, Biaggi G, Bernasconi C

117
SEARCH FOR UNRELATED UMBILICAL CORD BLOOD (UCB) UNIT FOR TRANSPLANTATION OF HIGH RISK LEUKEMIC PATIENTS
Carmini D, Screnci M, Iori A.P, Vulcano F, Guglielmi C, Mengarelli A, Testi AM, Cimino G, Elia L, Perrone MP, Laurenti L, De Felice L, Arcese W

118
SCREENING TESTS FOR PREDICTING THE EXTENSIVE C-GVHD AFTER ALLO-BMT
Alessandrino EP, Bernasconi P, Caldera D, Martinelli G, Colombo A, Biaggi G, Astori C, Malcovati L, Bonfichi M, Vitulo P, Klersy C, Bernasconi C

119
PERIPHERAL BLOOD STEM CELLS (PBSC) ALLOGRAFT. GITMO EXPERIENCE
Falda M, Majolino I, Di Bartolomeo P, Bandini G. Miniero R, Alessandrino EP, Locatelli F, Marmont F, Scimè R, Mordini M, Di Girolamo G, Bonini A, Bacigalupo A for GITMO

120
ROLE OF MIXED LYMPHOCYTE CULTURES IN THE PROGNOSIS OF GVHD
Balduini A, Bonfichi M, Marseglia C, Alessandrino EP, Bernasconi P, Bernasconi C

121
CORD BLOOD STEM CELLS TRANSPLANT IN β THALASSEMIA MAJOR PATIENTS: PRELIMINARY DATA
Vitucci A, Locatelli F, Gigante C, Giorgiani G, Palma A, Tannoia N

122
UMBILICAL CORD BLOOD (UCB) TRANSPLANT FROM UNRELATED MISMATCHED DONOR IN PATIENTS WITH HIGH RISK (HR) LEUKEMIA
Iori AP, Screnci M, Guglielmi C, Mengarelli A, Carmini D, Testi AM, Moleti ML, Cimino G, Perrone P, Laurenti L, Elia L, Boecklin F, Romano A, Vulcano F, De Felice L, Arcese W

Infections in immunocompromized host

123
EARLY DISCHARGE AND HOME CARE MANAGEMENT OF THERAPY-INDUCED NEUTROPENIC AML PATIENTS
Cedrone M, Cartoni C, De Gregoris C, De Sanctis V, Giovannini M, Niscola P, Romani C, Moleti L, Andrizzi C, Tosti S, Girmenia C and Mandelli F

124
REVISITED INDICATIONS FOR BONE MARROW BIOPSY IN HIV-INFECTED PATIENTS
Nosari A, Oreste P.L, Volonterio A, Landonio G, Caroli Costantini M, Moioli MC, Cantoni S, Caggese L, Morra E

125
INVASIVE PULMONARY ASPERGILLOSIS (IPA) IN HEMATOLOGIC NEOPLASMS: CLINICAL MANIFESTATIONS AND TC RADIOGRAPHIC FINDINGS
Nosari A, Stabile F, Oreste PL, Muti G, Cairoli R, Santoleri L, Ribera S, Morra E

126
ENCEPHALITIS IN PATIENTS WITH ACUTE LEUKEMIA. AN UNUSUAL CAUSE OF DEATH IN COMPLETE REMISSION
Pagano L, Vaccario ML, Larocca LM, Masullo C, Antinori A, Equitani F, Accorrà F, Leone G

Thalassemias and hemoglobinopathies

127
HB BRONTE AND HB MADDALONI-CASERTA: TWO NEW α2 GLOBIN GENE ALLELES IN FAMILIES FROM SOUTHERN ITALY
Lacerra G, De Angioletti M, Testa R, Di Girolamo R, Sciorio A, Schilirò G, Carestia C

128
GENOTYPE AND CLINICAL CORRELATION STUDY OF 126 PATIENTS WITH THALASSEMIC SYNDROMES β/β, β/δβ, S/β THALASSEMIA) FROM THE PROVINCE OF REGGIO CALABRIA
D'Ascola DG, Fiorillo MT, Crea AMR, Sorbara M, Trunfio R, Rossi A, Petrou M, Brugiatelli M

129
UNSTABLE VARIANTS HB GUN HILL, HB KOLN AND HB SUN PRAIRIE: VARIABILITY OF CLINICAL HEMOLYTIC DISORDERS
Lacerra G, De Angioletti M, Testa R, Mastrullo L, Di Girolamo R, Sciorio A, Schilirò G, Carestia C

130
TRIPLICATED α GENE AND HETEROZYGOUS β THALASSEMIA: CLINICAL STUDIES
Pietrapertosa A, Campanale D, Ranieri P, Palma A, Modugno E, Tannoia N

131
SURVEY OF SICKLE CELL DISEASE IN ITALY
Russo-Mancuso G, Romeo MA, Guardabasso V, Schilirò G

Thrombosis and hemostasis

132
PREVALENCE OF GENE MUTATION PREDISPOSING TO THROMBOPHILIA IN PATIENTS WITH VENOUS THROMBOEMBOLISM
De Stefano V, Chiusolo P, Paciaroni K, Casorelli I, Rossi E, Leone G

133
RISK OF RECURRENCE IN PATIENTS WITH DEEP VEIN THROMBOSIS AND HETEROZYGOUS MUTATION FOR FACTOR V LEIDEN
De Stefano V, Paciaroni K, Martinelli I, Arcieri P, Chiusolo P, Rossi V, Minotti C, Avvisati G, Mannucci PM, Leone G

Platelets and megakaryocytes

134
THROMBOPOIETIN-STIMULATED EX VIVO EXPANSION OF MEGAKARYOCYTE PROGENITORS OF HUMAN CORD BLOOD
Sanavio F, Garetto L, Severino A, Aglietta M, Piacibello W

135
PLATELETS FROM A PATIENT HETEROZYGOUS FOR THE DEFICIENCY OF 2MeS-ADP BINDING SITES HAVE A SECRETION DEFECT
Cattaneo M, Zighetti ML, Lombardi R, Lecchi A, Gachet C, Cazenave J-P, Mannucci PM

HEMOPOIETIC CELL PROLIFERATION AND DIFFERENTIATION

001
Induction of nitric oxide synthase is involved in the mechanism of Fas-mediated apoptosis in CD34+ cells

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In addition to blocking the cell cycle, inhibitory cytokines exert their effects on the hematopoietic system via induction of apoptosis of progenitor and stem cells. Several intracellular transduction pathways has been described for interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). Recently, induction of nitric oxide synthase (iNOS) with production of the toxic metabolite nitric oxide (NO) have been described as one of the effector mechanisms leading to apoptosis of hematopoietic progenitor cells by IFN-γ and TNF-α. Fas-receptor (Fas-R) expression can also be stimulated by these cytokines and its triggering has synergistic effects on cell cycle inhibition and apoptosis. Transactivation of iNOS promoters and possibly Fas-R by interferon regulatory factor-1 expressed in response to IFN-γ or TNF-α may be a part of the iNOS transduction pathway. The close functional relationship between IFN-γ, TNF-α and Fas-L suggests that the biological effects of these cytokines may be mediated by similar effector mechanisms. We therefore investigated whether the effects of Fas-R triggering in hematopoietic cells are mediated by NO. By Western blotting, we observed that Fas-receptor agonist, the monoclonal antibody (mAb) termed *CH11*, enhanced the expression of iNOS in hematopoietic cells. As shown by reverse transcription polymerase chain reaction, CH11 induced iNOS mRNA expression also in highly purified CD34+ cells. To determine whether NO is involved in Fas-mediated apoptosis, we attempted to inhibit the iNOS-catalyzed production of NO using anti-sense (AS) oligonucleotides directed against iNOS mRNA. After culture of hematopoietic cells in the presence of iNOS AS oligonucleotides, iNOS expression decreased and was no longer enhanced by Fas triggering. This effect was associated with the prevention of Fas-mediated apoptosis, as determined by DNA fragmentation assay and in situ terminal deoxynucleotidyl transferase staining. Functional effects on proliferation were tested in methylcellulose colony assays. While control oligonucleotides showed no effect, iNOS expression was suppressed by specific AS-oligonucleotides, and FAS-mediated inhibition of colony formation by total bone marrow and CD34+ purified progenitor cells was prevented. These data suggest that inhibitory effects of Fas, including induction of apoptosis, are mediated via pathways similar to those described for IFN-γ and TNF-α. These results may also explain the functional synergism between these inhibitory cytokines within the hematopoietic system.

002
Inhibition of interferon regulatory factor-1 expression induces interferon–γ to stimulate CD34+ cells

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Interferon-γ (IFN-γ) is a potent inhibitor of hematopoiesis *in vitro* and has been involved in the pathophysiology of human bone marrow failure syndromes. IFN-γ both inhibits cell cycling and induces expression of the Fas-receptor resulting in apoptosis of hematopoietic progenitor cells. IFN regulatory factor-1 (IRF-1) mediates some of these suppressive effects by activation of downstream inducible genes, such as inducible nitric oxide synthase. However, under certain experimental conditions, IFN-γ has been reported to stimulate proliferation of hematopoietic cells. Using an antisense technique, we inhibited the IRF-1-mediated pathway in KG1a and CD34+ cells before stimulation by IFN-γ. When KG1a cells were transduced with retroviral vectors expressing IRF-1 antisense (IRF-1AS) mRNA, the levels of IRF-1 protein and mRNA were decreased, both constitutively and after stimulation by IFN-γ. In IRF-1AS transduced cells, IFN-γ enhanced proliferation instead of suppressing it. These findings, derived from the study of KG1a cell line, were confirmed in primary hematopoietic cells. Purified human BM CD34+ cells were transfected with retroviral vectors and methylcellulose hematopoietic colony cultures were performed in the presence of a cocktail of growth factors with or without IFN-γ. IFN-γ consistently decreased colony growth of untreated and control virus-transfected CD34+ cells. Colony formation by IRF-1 sense transfected CD34+ cells was significantly decreased compared with that by non-transduced cells and was not affected by the addition of IFN-γ. In contrast, IFN-γ dramatically enhanced colony formation by IRF-1AS transfected

CD34+ cells. Successful transduction of retroviruses into hematopoietic cells was confirmed by PCR analysis of NeoR gene using DNA from pooled colonies. Our results indicate that inhibitory cytokines such as IFN-γ may exhibit diverse biological effects depending on the intracellular balance of transcriptional regulators, in turn influenced by the activation and differentiation status of the target cells.

003
Functional inactivation of p53 by the p210 product of the *bcr-abl* rearranged gene of chronic myeloid leukemia

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The product of oncosuppressor gene p53 is a transcriptional factor which regulates the expression of a number of effector genes. Physiological stimuli (growth and inhibitor factors) and genotoxic damage (ionizing radiation, U.V. and alkylating drugs) activate the transcription rate of p53, whose product binds homologous sequences within promoters of downstream genes. Activated transcription of p53 and p53-dependent genes triggers a cascade of biomolecular events converging in two major pathways: proliferative arrest and apoptotic death. The hypothesis that functional inactivation of p53 is a critical event in the pathogenesis and progression of chronic myeloid leukemia (CML) arises from the evidence that clonal hematopoietic progenitors escape the control of regulated progression through cell cycle phases, particularly that of G1/S transition (G1/S checkpoint), which is required for the correct amount of normal myelopoiesis, and do not die because of apoptosis. To check the hypothesis, avoiding the differences in p53 expression related to the level of hematopoietic differentiation, we compared p53 and p53-dependent gene expression in clonal hematopoietic progenitors, differing only for stable expression of p210 *bcr-abl,* achieved by transfection. In preliminary experiments we assessed p53 gene conformation both in parental and p210 *bcr-abl*-expressing cells, the wild type conformation being mandatory for its functional activation. P210 *bcr-abl* results in abrogation of G0-G1 arrest, following genotoxic damage (low dose ionizing radiations: 400 cGy, U.V. exposure: 5 J/m^2 or alkylating drugs: methylmetansulfonate). P210 *bcr-abl* abrogates G1/S checkpoint by functional inactivation of p53, as proved by transcription and translation rates of p53 and p53-dependent genes: Gadd 45, Gadd 153 and Waf1/Cip1. P53 is the pivotal oncosuppressor gene, being devoted to the control of cell proliferation and genomic stability. Its functional inactivation by p210 bcr-abl thus appears as the critical event in the pathogenesis and progression of CML, since it permits the illegitimate expansion of clonal over normal hematopoiesis and the selection of genomic and/or molecular mutations, favoring the emergence of more *aggressive* subclones, associated with transition from the chronic, indolent phase of the disease to the blast crisis.

004
Stem cell compartment in chronic lymphocytic leukemia (CLL): analysis of hemopoietic precursors

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Fludarabine has increased the rate of response in CLL, thus opening new potential therapeutic strategies including autologous hemopoietic stem cell transplantation. Little is known about the size of the residual hemopoietic compartment in CLL, although a marked increase in circulating progenitor cells has recently been reported. In the present study we evaluated the number and differentiation pattern of CD34+ cells, as well as the CFU-GM, BFU-E and CFU-GEMM from blood (PB) and marrow (BM) of 53 CLL patients. Twenty-four patients were untreated, 29 were studied 2 months after their last course of fludarabine or chlorambucil and 4, studied after fludarabine therapy, were further evaluated after mobilization with cyclophosphamide and G-CSF. Eight PB and 6 BM samples obtained from healthy donors were used as controls. PB of untreated patients had a median number of CD34+ cells, CFU-GM, BFU-E and CFU-GEMM/10^5 seeded cells or per liter of PB similar to those of normal controls. No were any differences found in the number of clonogenic progenitors evaluated per 10^5 cells in patients studied before and after therapy, but significantly fewer BFU-E per liter of PB were found after fludarabine. The numbers of circulating CD34+ per liter of PB were significantly lower in patients treated with fludarabine or chlorambucil than in untreated patients. BM growth was significantly reduced in CLL patients compared to healthy donors, as shown below:

N° of colonies (x10^5 cells)	*Normal BM*	*CLL BM*	*p*
CFU-GM	51.75 (32-70.5)	10 (0-88.5)	< 0.01
BFU-E	21.5 (15.5-32)	7 (0-105)	< 0.05
CFU-GEMM	2.5 (1-3.5)	0 (0-2.5)	< 0.01

Treatment with fludarabine or chlorambucil restored BM progenitors to levels similar to those of normal controls; this effect did not occur for CFU-GM in patients treated with fludarabine. Three-color fluorescence analysis demonstrated a differentiated pattern of $CD34^+$ cells, with a greater expression of CD13 and CD33 after treatment with fludarabine than in untreated patients and normal controls. In 2 of the 4 patients previously treated with fludarabine who underwent cyclophosphamide and G-CSF mobilization therapy, 4×10^6 $CD34^+$ cells/kg were collected. These 2 patients showed a significant increase of $CD34^+$ cells and of clonogenic cells in the PB, but a marked decrease of BM progenitor cells. The 2 patients who failed $CD34^+$ cell mobilization had a reduced growth of CFU-GM both in PB and in BM. These studies indicate that residual hemopoietic progenitors are present in untreated CLL patients and that stem cell mobilization and collection can be carried out following fludarabine treatment.

005
G-CSF expands the progenitor cell compartment and modulates expression of adhesion molecules on $CD34^+$ cells of peripheral blood from normal donors

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CD34 antigen identifies a stem cell population and is present on 1-2% of normal bone marrow (BM) cells and on 0.01-0.1% of peripheral blood (PB) cells. Growth factors are able to mobilize $CD34^+$ cells and to increase their concentration in PB up to 100-1000 fold the baseline level. Mechanisms involved in progenitor cell migration from BM to PB are still poorly understood. In this study we evaluated the immunophenotype and clonogenic potential of $CD34^+$ cells from 20 normal donors who underwent G-CSF mobilization. Samples were taken before and at day 4 and 6 of G-CSF administration, evaluating the differentiation pattern and the expression of adhesion molecules on $CD34^+$ cells, together with the proliferative capacity of myeloid (CFU-GM), erythroid (BFU-E) and multi-lineage (CFU-GEMM) progenitors. The percentage and the absolute number of $CD34^+$ cells significantly increased at days 4 and 6 of G-CSF administration, compared to the steady-state level ($p<0.0001$). Two-color fluorescence analysis showed, at days 4 and 6, a lower proportion of $CD34^+$/c-kit^+ compared to the steady-state level ($p<0.0001$), but a similar expression of CD13, CD33, CD38, HLA-DR and Thy-1 antigens, on $CD34^+$ cells. The expression of adhesion molecules on $CD34^+$ cells revealed a significant reduction of CD18, CD49d and CD62L ($p<0.0001$) at days 4 and 6, compared to the baseline level; CD54 and CD49b expression, in contrast, was not modified by G-CSF administration. The mean fluorescence intensity (MIF) evaluated before and after G-CSF treatment was similar for all the antigens. Three color staining on PB at baseline and at days 4 and 6 of G-CSF administration showed a reduction of the more immature compartment ($34^+/DR^-/13^-$) and an increase of the more differentiated compartment ($34^+/DR^+/13^+$). Evaluation of clonogenic growth showed a significantly greater number of CFU-GM, CFU-GEMM and BFU-E ($p<0.0001$) at day 4 and day 6 compared to the baseline PB level. The immunophenotype and clonogenic characteristics of day 4 G-CSF-stimulated PB were also compared with $CD34^+$ cells from normal bone marrow (BM) and cord blood (CB). A lower proportion of $CD34^+$/c-kit^+ cells was present in PB than in BM or CB, while the proportion of $CD34^+/CD13^+$ or $CD34^+/CD33^+$ in PB was higher than in BM but similar to that in CB. Clonogenic growth of the 3 stem cell sources correlated well with the phenotype, showing a significantly greater number of CFU-GEMM in the BM as compared to day-4 G-CSF PB and a significantly greater number of CFU-GM in PB than in either BM or CB. In conclusion, G-CSF administration can modulate the expression of lineage-specific antigens and of adhesion molecules expressed on $CD34^+$ cells. Although a more differentiated phenotype can be present on the majority of stimulated PB $CD34^+$ cells, the clonogenic potential and the large number of stem cells that can be collected make PB an ideal source of stem cells for transplantation.

006
Clonogenic cell content of chronic myelogenous leukemia $CD34^+$ cells lacking CD95 expression

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Fas antigen (CD95) is a member of the tumor necrosis factor (TNF) receptor superfamily and its triggering by the natural ligand or an anti-Fas antibody induces apoptosis. Chronic myelogenous leukemia (CML) is a clonal disorder of the hematopoietic stem cell characterized by a chimeric BCR/ABL gene. We investigated (i) the expression of CD95 on $CD34^+$ CML marrow cells, (ii) the clonogenic activity and (iii) the molecular status of flow sorted $CD34^+CD95^-$. As compared to normal $CD34^+$, CML-derived $CD34^+$ cells expressed CD95 at higher percentages ($47\pm19\%$ vs $21\pm10\%$, $p = 0.001$). When anti-

Fas antibody (1-5 μg/mL) was added to CML $CD34^+$ cells, CFU-GM colony formation was significantly reduced. In order to analyze the clonogenic cell content of CML-derived $CD34^+CD95^-$ cells, this cell fraction was enriched by flow sorting. Both committed (CFU-Mix, BFU-E, CFU-GM) and primitive (LTC-IC) progenitors could be detected in the $CD34^+CD95^-$ cell fraction. Only 13% (±5%) of committed progenitors were recovered in the $CD34^+CD95^-$ cell fraction, with a 5-fold enrichment. A significantly higher proportion (42±12%) of LTC-IC was recovered in this cell fraction with a 39-fold enrichment. In three newly diagnosed CML patients, individual colonies were analyzed for the presence of BCR/ABL mRNA by RT-PCR. The percentages of BCR/ABL negative CFU-GM generated by $CD34^+$ and $CD34^+CD95^-$ cells were 14±8% and 40±2%, respectively. PCR analysis of individual CFU-GM produced by LTC-IC after 5 weeks in long-term culture revealed 30±10% and 60±7% BCR/ABL negative colonies within the $CD34^+$ and $CD34^+CD95^-$ cell fractions, respectively. In conclusion, our data demonstrate that: (a) CML-derived $CD34^+$ cells have a high ex-pression of Fas antigen which is functionally activate; (b) a significant propor-tion of the primitive LTC-IC is contained in the $CD34^+CD95^-$ cell sub-set; (c) primitive and committed progenitors generated by $CD34^+CD95^-$ cells from CML patients at diagnosis are substantially enriched for non-leukemic clonogenic cells.

007
GAS6 inhibits granulocyte adhesion to endothelial cells

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GAS6 is a ligand for the tyrosine kinase receptors Rse, Axl and Mer, but its function is poorly understood to date. The presence of fibronectin III domains in the extracellular portion of these receptors suggests that the GAS6 system may be involved in cell adhesion. Previous studies reported that both GAS6 and Axl are expressed by vascular endothelial cells (EC), which play a key role in leukocyte extravasation into tissues during inflammation through adhesive interactions with these cells. The aim of this work was to evaluate the GAS6 effect on the adhesive function of EC. Treatment of EC with GAS6 significantly inhibited adhesion of polymorphonuclear cells (PMN) induced by PMA, PAF and thrombin, but not that induced by IL-8. GAS6 did not affect adhesion to resting EC. Titration experiments showed that high concentrations of GAS6 were needed to inhibit PMN adhesion and that inhibition was dose dependent in the 0.1-1 μg/mL concentration range. One possibility was that high concentrations were needed to overwhelm the effect of endogenous GAS6 produced by EC. In line with this possibility, treatment of resting EC with soluble Axl or two anti-GAS6 mAb significantly potentiated PMN adhesion. Analysis of intracellular localization of GAS6 by confocal microscopy showed a rather granular fluorescence distribution pattern, mainly organized in filament-like structures throughout the cytoplasm. In a small but consistent percentage of EC, fluorescence localization was markedly different, clustered in small patches close to the cell surface. In PAF treated cells the fluorescence intensity was markedly lower and exclusively exhibited a diffuse, granular pattern, with no evidence of surface patches. These data suggest that GAS6 may function as a physiologic anti-inflammatory agent that is produced by resting EC and is depleted when pro-inflammatory stimuli turn on the pro-adhesive machinery of EC.

008
Hematopoietic progenitor growth in healty donors before and after bone marrow harvesting

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The harvesting of bone marrow (BM) is now considered an easy procedure and the health and safety of the donor are guaranteed. The aim of this study was to evaluate whether BM harvesting may modify the circulating hematopoietic stem cell number. In 14 healthy voluntary donors who underwent a BM harvest for a BMT to an HLA identical sibling, we evaluated the CFU-GM, BFU-E and CFU-GEMM growth before the harvest, one hour and 24 hours after the procedure and on the seventh day from the harvest. CFU-GM, BFU-E and CFU-GEMM cultures were made in triplicate in methylcellulose (*Methocult Stem Cell*) with recombinant human erythropoietin (2 U/mL) and a standardised human leukocyte conditioned medium (10%) (Hemostim-Stem Cell). In Table 1 we show the results expressed in percentage (mean±SD). The data show a slight reduction of values without statistical significance between standard tests and those performed 1 hour after the BM harvest. The progenitor proliferative activity analyzed after 24 hours was significantly reduced ($p < 0.05$).

The CFU-GM, BFU-E and CFU-GEMM numbers obtained 7 days after the BM harvest were higher than in standard controls but the difference was not statistically significant. In conclusion our data are suggestive of a quick reduction of the hematopoietic prog-

enitors in peripheral blood after a BM harvest. Normalization and often increase of the CFU-GM, BFU-E and CFU-GEMM proliferation is observed within a week.

Table 1.

	Standard	A	B	C
CFU-GM	100	83±64	54±20	150±50
BFU-E	100	89±63	48±30	110±43
CFU-GEMM	100	90±63	58±33	138±78

MOLECULAR BIOLOGY

009
Interferon-α regulation of the growth arrest-associated gene gadd 45 in p210 *bcr-abl* expressing hematopoietic progenitor cells

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Controlled clinical trials have shown that interferon-α (IFN-α) is capable of modifying the natural history of chronic myeloid leukemia (CML): it has, indeed, the potential to control the illegitimate expansion of clonal $Ph1^+$ over normal hematopoiesis and to prevent progression of the disease from the chronic indolent phase to the blast crisis. IFN-α owes its therapeutic efficacy to transcriptional inhibition of the bcr-abl rearranged gene, whose p210 product is relevant in the pathogenesis of CML. Biomolecular mechanisms involved in its activity are still poorly understood. We investigated IFN-α effects on expression of a family of genes, named *growth arrest DNA damage-inducible* (Gadd) genes, devoted to the control of cell proliferation and genomic stability in clonal hematopoietic progenitors where p210 *bcr-abl* has been stably expressed through transfection. To this end, we used a competitive RT-PCR strategy, intended to measure gene expression by co-amplification of unknown amounts of sequences of the target gene and of known amounts of a specific competitor molecule. In preliminary experiments we proved that p210 bcr-abl expression abrogates the induction of Gadd 45 transcription following exposure to genotoxic damage (ionizing and U.V. radiations and alkylating drugs), resulting in the inactivation of one of the biomolecular pathways mandatory for G_0-G_1 recruitment. *In vitro* treatment with IFN-α (500-1,000 U/mL) for 5-7 days, which is effective on cell proliferation and cell cycle distribution, increases Gadd 45 transcription up to 3-5 times the steady-state level. Transcriptional induction of Gadd 45 parallels transcriptional inhibition of p210 bcr-abl. Gadd 45 is the pivotal gene in regulated proliferation and genomic stability because of its ability to control transition from the quiescent (G_0-G_1) to the active synthesis (S) phase of the cell cycle. This dual role involves Gadd 45 in the pathogenesis and progression of CML. In fact, its functional inactivation results in the illegitimate expansion of clonal over normal hematopoiesis and in the genomic instability of *bcr-abl*-rearranged progenitors, which favors the emergence of additional abnormalities and the selection of more aggressive clones, underlying transition of the disease from the chronic indolent phase to the blast crisis. Our results are consistent with the hypothesis that IFN–α is capable of restoring control of both the amount and neoplastic evolution of clonal $Ph1^+$ hematopoiesis.

010
Immunolocalization of HHV-6 protein in Hodgkin's disease and in Rosai-Dorfman's disease

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The aim of this study was to use molecular and immunological techniques to evaluate the presence of human herpesvirus-6 (HHV-6) in a large series of human lymphoid disorders. We used PCR to look for HHV-6 sequences in pathologic lymph nodes from 20 patients with Hodgkin's diseases (HD), 45 with non-Hodgkin's lymphomas (NHL), 12 with angioimmunoblastic lymphadenopathies (AILD) and 70 with reactive lymphadenopathies. We also used immunohistochemical staining with specific antibodies against different viral proteins (p41/38; p101K; gp106; gp116) to look for viral protein expression in the pathologic tissues of the cases positive for HHV-6 DNA by PCR. We identified HHV-6 sequences in 12 out of the 20 HD cases (60%), 7 out of the 45 NHL cases (15%), 7 out of the 12 AILD cases (58%) and in 15 out of the 70 cases of reactive lymphadenopathies (21%), including 2 paracortical lesions, 4 follicular lesions, 5 mixed paracortical and follicular lesions, 1 toxoplasmosis, 1 Kikuchi's disease and 2 Rosai-Dorfman's disease.

Viral protein expression has been documented in a significant number of Reed-Sternberg cells and in plasma cells of lymphomatous tissues from 2 HD cases characterized by a latent infection of HHV-6 with

high levels of viral genome. All 7 NHL cases showed positive monocytes cells for p101K and gp116. Monocytes and plasma cells were also positive for p101K and gp116 in the single 1 AILD case available for immunohistochemical staining. Furthermore, the 15 positive reactive lymphadenopathies, HHV-6 viral protein expression was detected in the only 2 cases of Rosai Dorfman's disease. In particular, we observed expression of p101K and gp116 antigen in dendritic cells and histiocytes, respectively. Our results provide the first evidence of HHV-6 having a putative neoplastic role in HD and Rosai Dorfman's disease. However, assessment of the precise involvement of HHV-6 in HD will require the definition of the exact functions of the viral protein found in Reed-Sternberg cells, as well as identification of which HHV-6 proteins, if any, may have transforming properties.

011
Identification of novel genetic lesions in mature B-cell neoplasms: role of a recurrent t(4;14) (p16.3;q32) chromosomal translocation in multiple myeloma

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Multiple myeloma (MM) is a malignant proliferation of plasma cells characterized by a range of clinical forms that may occur sequentially during the clinical course of the disease (from indolent to aggressive phases) suggesting an involvement of genetic events, such as oncogene activation. Although no specific genetic lesions have been identified in MM, a range of different oncogene and suppressor gene abnormalities including *c-myc*, *ras*, Rb-1 and p53, has been described by us and by others. Chromosomal abnormalities have major biological and prognostic implications in leukemias and lymphomas. Unfortunately, cytogenetic information in MM is limited and difficult to obtain because of the low proliferative rate of malignant plasma cells. However, available data indicate that the 14q+ marker is the most frequent cytogenetic aberration in MM cases (20-40%). This marker reflects rearrangement of the IgH locus at chromosome 14q32, as also suggested by its occurrence in about 40-50% of B-NHL. In one third of positive MM cases the 14q+ results from a t(11;14) (q13;q32), although there is no evidence of rearrangement of the *bcl-1*/cyclin D1 locus. In the other cases, the donor chromosomes supplying the extra material of the 14q+ marker were not characterized by cytogenetic analysis. In an attempt to identify new genes involved in the recombination events with the IgH locus, we carried out a rearrangement analysis by Southern blot using probes specific for the joining (J) and constant (C) regions of the IgH in a panel of 88 MM cases without cytogenetic information. In about 25% of cases our analysis allowed the identification of IgH rearranged alleles as possible candidates for chromosomal translocations. Molecular cloning and fluorescent *in situ* hybridization (FISH) analyses of four cases demonstrated the presence of a t(11;14) (q13;q32) in two (a tumor biopsy and a cell line with no detectable 14q32 translocation) and a new t(4;14)(p16.3;q32) chromosomal translocation in the remaining cases. The breakpoints on 4p16.3 clustered in a genomic region located approximately 2 Mb telomeric to the Huntington's disease gene and 50 kb centromeric to the FGF3R gene. Interestingly, using probes derived from the 4p16.3 region in Southern blot analysis, we detected rearrangements in other tumors. Our FISH analysis revealed the same translocation in a MM cell line where we also found an overexpression of the FGFR-3 gene. We are currently investigating the frequency of this abnormality and the possible role of FGFR-3 gene in the pathogenesis of MM.

012
Quantification of *bcr-abl* transcript in chronic myelogenous leukemia patients by competitive and quantitative rt-PCR and capillary electrophoresis

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In the present study, two types of *bcr-abl* chronic myelogenous leukemia (CML) associated transcripts products were generated by reverse transcription-polymerase chain reaction (RT-PCR) from 5 µg of total RNA extracted from 46 bone marrow samples from 34 CML patients at diagnosis. The PCR products were analyzed by SLAB-gel electrophoresis (SGE) on 2% agarose gels and by capillary electrophoresis (CE) (128 runs; median 3.3 times for each sample). Amplified samples were injected hydrodynamically on CE (40s at 3.45 kPa) and detection was by UV absorbance at 254 nm. After injection, CE separation showed baseline resolution for the two peaks corresponding to the two types of *bcr-abl* junctions: the b2-a2 type (343 base pairs, 10 patients) was revealed at 9.33 min (median 9.32 min; range 8.99-9.40 min; standard deviation [SD] = 0.1) and the b3-a2 type (418 base pair, 24 patients) at 10.03 min (median

9.98; range 9.58-10.70 min; SD = 0.25). Reproducibility of separation on CE product was confirmed by several loadings of the same leukemic samples and of molecular weight markers of same weight and same size fragments at the same conditions. Furthermore, by a competitive RT-PCR approach we assessed a linear correspondence between the amount of PCR product loaded and the area value of its peaks after CE separation. Starting from the sensitivity of our method, which is of 2 nanograms of DNA, we quantified the *bcr-abl* amplified molecules at diagnosis time developing a standard curve and showing a relationship between the amount of the *bcr-abl* product and the peak area detected by CE. We found that the median value of the peak of #10 (30 runs) of b2-a2 PCR product was 0.195 and of #24 (98 runs) of b3-a2 amplified was 0.174. We assessed 94 CML patients at diagnosis time and after 3-6-9-12 months of IFN therapy and found that the amount of *bcr-abl* transcript is lower in patients with cytogenetic conversion and higher in patients in progression of the disease. Our results confirm the greater resolution and enhanced sensitivity observed in CE analysis and the easy detection and quantification of bcr-abl PCR product at diagnosis in CML patients, providing a new method for detecting the evolution of leukemia associated transcript.

013
Mitochondrial DNA deletion in children with De Toni-Debré-Fanconi syndrome secondary to antiblastic therapy

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Children with malignancies who receive chemotherapy are at risk of developing secondary De Toni-Debrè-Fanconi syndrome (DDFs). The aim of this study is to verify whether there are deletions of mitochondrial DNA (mtDNA) and disorders in oxidative phosphorylation complex (OPC), in the pathogenesis of secondary DDFs, as reported in patients with primary DDFs.

We studied 18 pediatric patients with solid tumors, previously treated with chemotherapy, who were off therapy for at least 1 year. All of them had normal renal function at diagnosis. Only four of them received ifosfamide (IFO) and platinum compounds. For all patients we evaluated: 1) renal function; 2) activities of OPC measured on platelets; 3) mtDNA, extracted from platelets, amplified by PCR, using specific primers to detect the common deletions which were further confirmed by the primer shift PCR method.

Only two patients, both treated with IFO and carboplatin, respectively for Wilms tumor and germ cell tumor, developed DDFs, 1 and 3 years after they stopped therapy. They had a decrease in activities of OPC, statistically significant only for NADH-cytochrome-c-reductase and cytochrome-c-reductase. Both children also showed a 650 bp not maternally inherited, specific and unknown deletion of mtDNA.

Our data suggest that treatment with IFO and carboplatin could be responsible for mtDNA deletions, which could cause specific mitochondrial enzyme deficiencies and impairment of transport rates of D-glucose, phosphate and aminoacids. Additional risk factors could be the young age and the reduction of renal tubular surface caused by nephrectomy.

CYTOKINES

014
Effect of differential hematopoietic growth factors on expansion/proliferation of acute myeloid leukemic cells versus proliferation/differentiation

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Human leukemic cells have been shown to express functional receptors for a variety of hematopoietic growth factors (HGFs), including G-CSF, GM-CSF, IL-3, IL-6 and stem cell factor (KL).

Recently, data from our laboratory demonstrated that flt3/flk2 ligand (FL), alone, stimulates both leukemia cell proliferation and clonal growth of the vast majority of AML cases (up to 85%); also, c-mpl ligand (the so-called thrombopoietin, TPO) sustains the proliferation of 40 to 50% fresh AML cells; moreover, it also sustains the clonal growth of more than 50% of AML cells other than M6 and M7.

In addition, we demonstrated that the association of FL and TPO in stroma-free liquid cultures is capable of sustaining a progressive, massive expansion of primitive stem cells from cord blood samples; this phenomenon generated a massive expansion of hematopoietic progenitors for over six months.

The aim of our studies was to investigate whether *primitive* growth factors (KL, FL, TPO, IL-6 and IL-3), alone or in combinations, would sustain leukemia cell growth. So far, 11 AML cases have been studied.

Leukemia cells from either BM or PB were obtained after a simple density cut (1077 or 1070) and, when possible, by $CD34^+$ positive selection. Thymidine incorporation assays, methylcellulose or agar cultures, as well as suspension cultures (either on stromal layers or stroma-free) were performed. In the latter case, at various time-points, cells from the different culture conditions were harvested, counted and the percentages of leukemic cells, of $CD34^+$ and of clonogenic leukemic progenitors were assessed.

Data obtained so far indicate a heterogeneous pattern of response among the AML patients. The expansion of the blast population can be demonstrated, at least in the first few weeks of liquid culture. However, it is transient, as is the expansion of more primitive clonogenic progenitors. The induction of proliferation and self-renewal of primitive leukemic stem cells, by contrast, is not detectable.

015
Cost-effectiveness analysis: a correct administration of G-CSF to treat chemotherapy-induced neutropenia in non-Hodgkin lymphomas of the elderly

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Older age is a poor prognostic factor in non Hodgkin's lymphomas (NHLs), due to dose intensity reduction caused by higher risk of infective complications. Growth factors reduce the duration of neutropenia, febrile episodes and incidence of hospital admission. Our study arose from the necessity to administer an expensive product such as G-CSF consciously and responsibly, evaluating pharmacoeconomic parameters. We planned a randomized prospective clinical study with alternative interventions (arm A: G-CSF administered only if WBC < 1×10^9/L) and prophylactic G-CSF treatment (arm B: G-CSF administered for 10 days, 24 hours after chemotherapy regardless of WBC count) in elderly patients with NHLs, after administration of CHOP regimen.

Thirty-four patients aged between 60 and 82 (average 68 years), affected by high and intermediate grade NHLs and low grade NHLs in advanced stages with active disease, were enrolled from September 1993 to May 1996. The CHOP regimen was given in standard doses at 21 day intervals. Patients with fever higher than 38°C received oral prophylactic ciprofloxacin (500 mg × 2/die) and fluconazol (150 mg/die). We studied the economic impact considering G-CSF, hospitalization, antibiotic and antifungal therapy costs. The treatment groups were compared using a Mann-Witney U-test for continuously measured variables or chi-square test for dichotomous outcomes. There were no statistically significant differences in dose intensity administration (always higher than 97.2% of planned dose). Severe neutropenia was avoided in 88% of B cycles, versus 80.7% of A cycles. Mild neutropenia was absent in 82.8% of B cycles and in 55.4% of A cycles. Clinical parameters such as days of fever or incidence of febrile episodes (23.7% in B cycles vs 24.1% in A cycles), mean and overall duration of febrile episodes (5.6 and 101 days in B cycles versus 5.7 and 115 days in A cycles) were not significantly different between the two arms. Very limited hematologic and extra-hematologic toxicity was observed in both G-CSF therapy groups. We observed 63.6% of CR and 22.7% of PR in 22 cases of high and intermediate grade NHLs. Two patients showed a progressive disease during treatment and one was refractory. On the other hand in 12 low grade NHLs we obtained 41.6% of CR and 58.4% of PR. Median survival was 27 months for high and intermediate grade NHLs while median survival was not achieved for low grade NHLs. The G-CSF comparative cost analysis showed a significantly lower mean cost of the intervention treatment group than the prophylactic one (Italian L. 1,269,036 in cycle A vs Italian L. 2,017,768 in cycle B, p=0.001). Toxicity costs related to chemotherapy (antibiotic therapy and hospitalization) were lower than the cost of growth factors: average cost of antibiotic therapy was Italian L. 114,486 and Italian L. 82,096 in arms A and B respectively; the average hospitalization costs were Italian L. 690,843 in arm A and Italian L. 673,262 in arm B. Considering both G-CSF and average toxicity costs, intervention G-CSF therapy is cheaper than the prophylactic strategy (Italian L. 2,773,127 in arm B vs Italian L. 2,059,077 in arm A, p: 0.0001). Furthermore, the cost-benefit analysis shows that economic advantages of arm A are more evident in the latter cycles (from IV to VI) due to the reduction of G-CSF and hospitalization costs. In conclusion the use of G-CSF as intervention treatment for neutropenic episodes and their sequelae, improves the cure rate in elderly patients with a considerable financial saving.

016
Intermediate dose of G-CSF and mobilization of blood stem cells in 10 healthy donors

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The shortening of the duration of neutropenia duration coupled to a faster engraftment and increased

graft-versus-leukemia are generally observed in bone marrow transplantation with peripheral blood stem cells (PBSC). Moreover, PBSC transplantation following cytokine mobilization avoids the risk of anesthesia and the discomfort associated with bone marrow harvesting in the donor. But the side effects of G-CSF administered to healthy subjects are not well known, and the optimal schedule to collect blood SC in healthy donors remains to be defined. So, with the aim of evaluating the possibility of reducing the duration of G-CSF administration, we report here the growth of progenitor hematopoietic cells and the absolute number of CD34+ cells analyzed after every day of G-CSF therapy in 10 healthy donors. They received G-CSF 5 µg/kg/day subcutaneously for 5 days. The leukaphereses were performed in the morning of the 5th day). The results are reported in Table 1.

Table 1. Hematopoietic progenitor growth and CD34+ cells expressed as mean±standard deviation (SD) in 10 healthy donors treated with G-CSF.

	CFU-GM x10^5 cells	*BFU-E x10^5 cells*	*CFU-GEMM x10^5 cells*	*CD-34+ /µL*
day 0	12.8±4	20±9	1±0.5	4±3.4
day 1	21±10	31±19	1.1±1.4	8.0±2
day 2	33±22	52±38	2.8±2	11.4±4
day 3	45±16	91±31	5±3	39.6±8
day 4	94±27	121±53	14±10	73.1±30
day 5	119±50	134±27	9±6	82±23
leukaphereses	147±49	183±66	15±10	

G-CSF causes a progressive increase of the progenitor and CD34+ cell number after every day of therapy. The highest levels of CD34+ cells and CFU-GM were counted after 4-5 days of G-CSF administration, but high levels of BFU-E, CFU-GM and CFU-GEMM were still observed on the 3rd day of G-CSF therapy. These data support the possibility of reducing the course of this kind of treatment. This fact may have an impact on the duration of G-CSF therapy in healthy subjects.

017
flt3L enhances the early stem cell compartment after *ex vivo* amplification of umbilical cord blood CD34+ cells

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Umbilical cord blood (UCB) cells have been successfully used for transplant in children. The potential of a single UCB unit for transplant in adults remains an open question. *Ex vivo* expansion of hemopoietic stem cells (HSC) has been demonstrated, but the proliferative response of earlier stem cells is uncertain. Short-term stroma-free liquid cultures of immunoselected CD34+ cells from 15 UCB samples were established in the presence of different combinations of the following cytokines: flt3L, SCF, IL-6, IL-3, PIXY-321. The proliferative response was assessed by evaluating: nucleated cells, clonogenic progenitors and immunophenotype. The results show that in cytokine combinations including flt3L, the amplification of both committed and early stem cells was significantly enhanced (Table 1).

Table 1.

	– flt3L	*+ flt3L*	*p*
	(fold amplification)		
NC	32	58	< 0.001
CD34+	6.3	10.2	0.002
CD34+/Thy-1+/CD45RO+	1.5	3.1	0.024
CD34+/CD33+	25.2	33.9	0.017
CFU-GM	4.8	8.5	0.003
BFU-E	10.2	12.7	0.034
CFU-GEMM	4.4	8.7	ns
HPP-CFC	18.2	58.5	0.024

In 8 cases the CD34+ cells were stained at day 0 with PKH26 to monitor cell divisions. After *in vitro* amplification the percentage of PKH26^{bright} cells decreased from 85% at day 0 to 3.7% at day 8. Colonies obtained at day 0, 4 and 8 were replated to determine the secondary plating efficiency (PE2). *In vitro* amplification induced a limited reduction of PE2 from 81% to 54% respectively at day 0 and 8.

In conclusion, our preliminary results show: 1) no exhaustion of UCB proliferative potential; 2) increase of CD34+ cells; 3) persistence of a quiescent cell compartment unresponsive to cytokines (PKH26^{bright}) 4) significant expansion of both committed and early progenitors; 5) significant improvement of hemopoietic progenitor amplification using cytokine associations including flt3L.

018
Effect of the addition of Tpo IL-3, SCF and Epo on the erythroid and megakaryocytic differentiation of CD34+ cells from human bone marrow and cord blood

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$CD34^+$ cells from human bone marrow and cord blood were incubated in semisolid culture in the presence of IL-3, SCF and Epo with or without Tpo. Cells were harvested at different time intervals, and erythroid (GPA^+) and megakaryocytic ($CD41^+$) differentiation analyzed by flow cytometry. Tpo caused an increase in day 7 $CD41^+$ from bone marrow, but not from cord blood cells, compared to Tpo free cultures, and in each instance $CD41^+$ decreased from day 7 to day 14. In the absence of Tpo GPA^+ cells increased from day 7 to 11, and declined to an intermediate value at day 14, either bone marrow or cord blood progenitors were cultured. Addition of Tpo was synergic, increase was maximal at day 7, but values were higher than in Tpo free cultures at each time. As for colony growth, addition of Tpo did not increase the total number of colonies, but augmented the number of pure erythroid colonies at day 7, reducing that of mixed and non erythroid ones. In other experiments Epo, Tpo and SCF used alone or in combination (Tpo+Epo, Tpo+SCF) were tested on $CD34^+$ cells. In this setting the number of either bone marrow or cord blood derived cells collected at days 7 and 11 was inadequate for flow cytometric analysis. At day 14 harvesting was higher from cord blood cultures, but differentiating response was similar in both type of cells. Tpo alone stimulated almost exclusively CD41, but caused a two fold increase of GPA+ cells in combination with Epo, compared to Epo alone, which was unable to induce megakaryocytic differentiation. The percentage of $CD41^+$ cells obtained with Tpo+Epo was comparable to Tpo alone. Only $CD41^+$ cells were present in cultures containing SCF alone, and the mixture SCF+Tpo caused a three fold increase compared to SCF or Tpo alone. The results of this work indicate that the proliferative activity of IL-3, SCF and Epo is not potentiated by Tpo. Megakaryocytic differentiation precedes erythroid differentiation both, either in cord blood and bone marrow cultures, but the two types of cells respond somewhat differently to the addition of Tpo to a combination of early acting cytokines plus Epo. In fact early expression of CD41 in cord blood cells did not require Tpo. In contrast, Tpo was required for full early CD41 expression in cultures from bone marrow progenitors. Tpo was also necessary for full erythroid differentiation. As expected, Tpo and Epo alone possess a selective differentiating activity toward megakaryocytopoiesis and erythropoiesis respectively. Tpo+Epo combination is only effective on erythroid differentiation, in contrast with the synergistic activity toward megakaryocytic differentiation observed with the combination SCF+Tpo. In consideration of the suggested role of GATA-1, GATA-2, NF-E2 in mediating erythroid and megakaryocytic pathways, and the induced expression of GATA-3 in response to Tpo that we have previously described, studies on the expression of transcription factors in $CD34^+$ are in progress.

019
Thrombopoietin (TPO) and Tpo-R are produced by primary human mesangial cells in culture

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Mesangial cells, localized in the centrilobular region of the glomerular tuft, are endowed with both contractile smooth muscle properties and by immunologic, macrophage like functions. Their versatility and their particular embriological derivation, together with the observation that TPO is produced in kidneys, prompted us to verify whether mesangial cells were able to express mRNA for TPO, TPO-R and produce functional proteins.

Mesangial cells were obtained using standard sieving procedures from 3 kidneys excised after informed consent for localized renal tumors and maintained in continuous culture in standard conditions, in RPMI 1640, 17% FCS .Total RNA was extracted from cell cultures and RT-PCR (40 cycles) performed with primers specific for TPO (primer I 5'-TGCCTTGGTCTCCTCCATTCT-3'; primer II 5'-ATAGATTCCTCACCCTTGGC-3'), and TPO-R (primer I 5'- TGGAGATGCAGTGGCACTTG-3'; primer 5'-GAACTGTGTGGGGTCTGTAGT-3'). At the same time, Northern blot analysis was carried out and the presence of mRNA for TPO and TPO-R searched for by the use of specific probes. Moreover, biological assays were carried out to test the activity of the TPO produced by mesangial cells. To do so, M-07e cell line, dependent on IL-3 for proliferation, but growing also upon TPO stimulation, was tested for response to mesangial cell culture supernatant. Supernatants were collected from cultures at day 1, 3 and at cell confluence and were then added 10 and 20% in culture medium of M-07e cells. Thymidine uptake, cell number and cell morphology were analyzed each day, for 7 days of culture. Modulation of platelet aggregation by ADP and epinephrine was also tested after addition of mesangial cell supernatants.

In all cultures of mesangial cells studied it was possible, by RT-PCR, to detect the fragments of 383bp (TPO) and of 226 bp (TPO-R). Northern blot was performed as a further control to check for the quantitative relevance of both transcripts, showing an intense signal for TPO, less pronounced for TPO-R, but clearly expressed by all samples tested. Biological assays indicated a dose dependent stimulation of M-07e cell proliferation in terms of cell number and tritiated thymidine uptake. From our data, mesangial cells are identified as producers of biologically active TPO. Moreover, the contemporary presence of TPO-R on

the surface of these renal interstitial cells is an intriguing finding to shed light on the mechanisms of regulation of TPO and platelet production. Further investigations are ongoing to characterize such aspects.

020
Hypersensitivity to GM-CSF and delayed apoptosis in GM-CSF-dependent GF-D8 cell line engineered to overexpress SHC

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GM-CSF is an essential regulator of acute myelogenous leukemia cell proliferation both *in vitro* and *in vivo*. Alll GM-CSF effects are mediated by a non-tyrosine kinase receptor. The signal transduction pathway of GM-CSF involves a number of transducing molecules including Shc, Grb2, Sos1, Ras, Raf-1. To assess the role Shc may play in leukemic proliferation we engineered the overexpression of Shc in the recently described GM-CSF-dependent GF-D8 cell line by retroviral gene transfer and analyzed subsequent effects on cell proliferation and survival. Early passaged Shc or mock-transfected GF-D8 clones, maintained in RPMI-1640 supplemented with FBS (10%) and GM-CSF (20 ng/mL), were used throughout the study. Western blot analysis confirmed that the transfected clone (GF-D8/Shc) had a significantly higher expression of Shc than the parental clone (GF-D8), or clones retrovirally transduced with the LXSN vector only (GF-D8/SN). To evaluate the clonogenic response of GF-D8/SN and GF-D8/Shc to growth factors, cells were GM-CSF-starved for 24-48 hours and then cultured in methylcellulose with increasing concentrations of different growth factors, including GM-CSF (0.001-50 ng/mL), G-CSF (0.001-50 ng/mL), IL-1 (0.001-50 U/mL), and MGDF (0.1-50 ng/mL). Cell proliferation was analyzed by assaying colonies (40 cells): as compared to GF-D8/Shc cells, GF-D8/SN cells generated significantly lower numbers of colonies upon stimulation with GM-CSF (0.01 to 5 ng/mL). Both GF-D8/Shc or GFD8/SN cells failed to give rise to clonal aggregates in response to G-CSF, IL-1 and MGDF. Cell survival was analyzed by nuclear DNA fragmentation which revealed that GF-D8/SN cells underwent apoptosis 12 hours following GM-CSF deprivation, whereas GF-D8/Shc cells failed to show any evidence of apoptosis up to six days after GM-CSF deprivation. Both in GF-D8/SN and GF-D8/Shc GM-CSF deprivation was associated with a progressive decrease of Bcl-2 and increase of CD95 expression. Our data demonstrate that in this specific model Shc overexpression is devoid of transforming activity but induces hypersensitivity of GF-D8 cells to GM-CSF and prevents apoptosis of these cells following GM-CSF deprivation. The potential relationship in this model between Shc and Ras-MAPK pathways in cell proliferation control and between CD95 and Bcl-2 pathways in apoptosis control remains to be investigated.

021
Altered immunoregulation by selective expression of Th1-type cytokine mRNAs in hemophagocytic syndrome patients

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Hemophagocytic syndromes (HS), also known as hemophagocytic lymphohistiocytosis, are hemopoietic disorders, usually related to viral infections or to hematologic neoplasias (lymphoproliferative disorders), characterised by macrophagic proliferation and activation, with consequent phagocytosis of mature bone marrow cells and peripheral pancytopenia. The pathogenesis is unknown; however, it has been suggested that it might be related to immunoregulation alterations. Recently, a cytokine (CK) and growth factor (GF) overproduction has been reported in HS, which is able to activate monocytes/macrophages (M-CSF, γ-IFN). CKs and GFs are soluble mediators that play a key role in the regulation of immune and hemopoietic systems. Their production is difficult to study with biologic and immunologic assays due to the short range and time of action of these factors. For these reasons in our study we used molecular biology techniques, in particular RT-PCR, to evaluate the messenger RNA (mRNA) production of Th1 and Th2 derived CK/GF in HS patients.

We studied two patients with HS who showed, together with the usual HS biohumoral alterations (high levels of serum triglycerides and LDH), pancytopenia and clear hemophagocytic pictures in bone marrow smears. In the peripheral blood lymphocytes of these subjects we observed a CK/GF mRNA production pattern typical of T helper 1 type (Th1) activation.

	Th1			*Th1-Th2*				*Th2*		
mRNA	IL-2	γ-IFN	TNF-β	IL-3	GM-CSF	TNF-α	LIF	IL-4	IL-5	IL-10
HS	+	+	+	+	–	+	+	+/–	–	–
C	–	–	–	–	–	–	–	–	–	–

We did not observe any CK mRNA in normal control lymphocytes (C). Thus, compared to controls, HS patients undergo a *cytokine storm*, with immune and hemopoietic system involvement. In particular, a

key role in the development of HS-related symptoms may be played by γ-IFN, through macrophage activation and consequent production of TNF-α and LIF (cachexia) and IL-1 (hyperpyrexia). Moreover, TNF-α and γ-IFN may have an inhibiting action on proliferative activity of bone marrow hemopoietic cells.

These results suggest a primary role of the Th1/Th2 lymphocyte subset unbalance in HS pathogenesis, with a prevalent Th1 action not counterbalanced, as in physiological conditions, by Th2 lymphocytes. This hypothesis is further supported in HS patients by the lack of IL-10, a Th2 cytokine with suppressive activity on monocytes and macrophages.

022
Humoral-mediated suppression of lymphocyte blastogenesis in healthy donors receiving G-CSF

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We evaluated lymphocyte blastogenesis to phytohemoagglutinin (PHA) and concanavalin A (Con A) by flow cytometry and propidium iodide (PI) in normal donors receiving rhG-CSF; results were correlated with plasma levels of IL-1 receptor antagonist (IL-1ra), lactoferrin (Lf) and interleukin-10 (IL-10). PB samples were obtained prior to rhG-CSF administration and on days +2, +4, +6 and +30. Heparin-anticoagulated blood was cultured for 72h at 37°C in 5% CO_2 atmosphere in mitogen-containing lyophilized culture medium (PHA 5 µg/mL, Con A 5 µg/mL, PWM 5 µg/mL; Blastest, Ylem). Combined cell surface antigen and DNA staining was performed as indicated by Schmid (*Cytometry 1991; 11:279*). All samples were run through a FACScan flow cytometer (BD). IL-1ra, Lf and IL-10 plasma levels were measured with specific immunoassays (R&D System, UK). The S-phase of PHA-treated cultures decreased from 20% (15-35.5) to 6.7% (1.5-11.9, p=0.0026), 8% (4-12, p=0.0091) and 15% (9-22, p=0.0091) on days +2, +4 and +6 of rhG-CSF treatment. Responsiveness to Con A decreased from 18% (12-20) to 1.8% (0.5-7; p<0.01), 3% (2-8; p<0.01) and 5% (2-11; p=0.009) on days +2, +4 and +6. The S-phase of PHA-stimulated lymphocytes showed an inverse correlation with neutrophil (Rs=-0.75, p=0.0008) and monocyte counts (Rs=-0.64; p=0.007). IL-1ra and Lf levels significantly increased after rhG-CSF as compared with baseline. IL-10 increased from 5 pg/mL to 25 pg/mL on day +2 (p=NS), returned to pre-treatment values thereafter and showed no correlation with S-phase fraction (Rs=-0.22). Interestingly, IL-1ra and Lf inversely correlated with the S-phase of PHA-treated cultures. The present observations indicate an immunoregulatory action mediated by pharmacological doses of rhG-CSF, which could be responsible for the unexpectedly low incidence and severity of acute graft-versus-host disease following allogeneic PB transplantation.

CHRONIC MYELOPROLIFERATIVE DISORDERS

023
Higher expression of FAS receptor on CD34+ cells of chronic myelogenous leukemia correlates with hematologic response to IFN-α

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Fas receptor (Fas-R) is a member of the TNF receptor superfamily and its triggering by the natural ligand (Fas-L) induces apoptosis of target cells. Fas-mediated cell killing plays an important role in the elimination of malignant, virus-infected and allogeneic cells by the immune system. Fas-R is upregulated by IFN-γ and TNF-α on normal $CD34^+$ cells as well as by IFN-a on Daudi cells. Recently, we documented the involvement of Fas-mediated apoptosis in the inhibitory effect of IFN-α in chronic myelogenous leukemia (CML). We investigated whether there was a correlation between Fas-R expression on $CD34^+$ bone marrow (BM) cells and clinical features (such as the Sokal prognostic score, hematologic and cytogenetic response to IFN-α treatment) in a group of patients with CML in the chronic phase. Response to IFN-α treatment was defined as follows: optimal response = complete hematologic recovery after one month of treatment at the maximum tolerated dose (6 or 9 MU); poor response = persisting or increasing leukocytosis (> 80,000) after one month of treatment at the maximum tolerated dose (6 or 9 MU), leading to IFN-α treatment discontinuation. Using two-color flow cytometry with FITC-conjugated anti-CD95 (clone UB2; Amac) and PE-conjugated anti-CD34 (Becton-Dickinson), we found that $CD34^+$ CML cells in the chronic phase show significantly higher expression of Fas-R (mean percentage of $CD34^+CD95^+$ within $CD34^+$ cells: 25.5±22%, n= 40), compared to $CD34^+$ BM normal cells ($CD34^+CD95^+$: 8.4±6%, n=41). Twenty-eight of these CML patients were evaluable for *in vivo* response to IFN-α therapy. Fas-R

expression did not show a significant correlation with the Sokal prognostic score ($CD34^+CD95^+$: 24.5±15 vs 18.2±15 in the group with Sokal < 0.8 or > 0.8, respectively; p=0.28). By contrast, CML patients with optimal response to IFN-α had 25.7±16% (mean±SD) $CD34^+$ cells bearing the CD95 antigen, compared to 12.5±8% in the group of patients showing a poor response ($p<0.05$). We did not observe any correlation between Fas-R and cytogenetic response to IFN-α treatment (p=0.22).

We conclude that $CD34^+$ cells derived from CML patients who have an optimal hematologic response to IFN-α therapy show higher Fas-R expression further suggesting the involvement of the Fas-R/Fas-L system in the immunologic regulation of CML progenitor growth.

024
FAS-mediated down-modulation of p210 bcr/abl results in apoptosis of $CD34^+$ cells of chronic myelogenous leukemia

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Normal progenitor cells do not display significant levels of Fas-receptor (Fas-R). Fas-R expression can be upmodulated upon stimulation by IFN-γ or TNF-α. Following stimulation, the addition of specific agonists acting on Fas-R induces apoptosis of normal $CD34^+$ cells. Recently, we have demonstrated that in CML cells, Fas-R expression can be increased by IFN-α. Subsequent triggering of Fas results in apoptosis of $CD34^+$ cells derived from CML bone marrow (BM). In chronic myelogenous leukemia (CML), the *bcr-abl* gene product has been reported to cause decreased susceptibility to apoptosis, and downregulation of *bcr-abl* expression has been shown to restore the apoptotic potential of cells carrying the Ph-chromosome. We studied 10 patients with CML in the chronic phase to determine whether apoptosis induced by Fas is related to downmodulation of *bcr-abl* and whether there was a correlation between inducibility of apoptosis in BM $CD34^+$ CML cells *in vitro* after triggering of Fas-R and the clinical response to IFN-α. In 6/10 patients tested, addition of the Fas agonist resulted in enhancement of apoptosis as demonstrated by agarose gel electrophoresis of low molecular weight (LMW) DNA extracted or by *in situ* TdT assay from total and $CD34^+$ CML cells. Western blot performed on cell extracts derived from the same cells using identical protein concentrations for each sample, demonstrated that this effect was associated with downmodulation of the *bcr-abl* protein. Bcr-abl downmodulation was enhanced by *in vitro* addition of IFN-α in a dose-dependent fashion in 3 cases, while in 3 patients it occurred only at the highest IFN-α concentration (1000 U/mL). These 6 patients showed a complete hematologic response following IFN-α treatment. In one patient, who showed an optimal response to IFN-α, the effect of Fas triggering *in vitro* was only marginal. By contrast, in 3/10 patients Fas triggering failed to induce apoptosis in IFN-α treated cells, and no change in *bcr-abl* expression was observed; clinically, all these patients had a poor response to IFN-α treatment. In the only case of lymphoid blastic crisis that was studied, we could not document any effect of Fas triggering *in vitro*. Finally, we demonstrated that the decrease in *bcr-abl* protein level caused by Fas triggering is related to a post-transcriptional modulation, since the level of *bcr-abl* mRNA, measured by quantitative RT-PCR, was not affected by Fas triggering in cells susceptible to Fas-mediated apoptosis.

025
Bone mast cells sarcoma

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Systemic mast cell disorders (SMCD) are diseases characterized by proliferation and accumulation of mast cells in tissues. The clinical picture cames from the combined effects of mediator released by these cells and from mechanical consequences of their presence. On a prognostic basis they are classified into indolent SMCD, SMCD with associated hematologic disorder (in which the prognosis depends on the hematologic disorder), aggessive SMCD, mast cell leukemia and mast cell sarcoma (MCS). MCS is a very rare condition, with the major clinical features of solid lesions, principally in the skin and mucosa.

Recently we saw a 56-year-old man who complained of diffuse bone pain, headache, flushing for about 4 months, fever for about 2 weeks, and peripheral paralysis of the XIIth cranial nerve: there were no cutaneous lesions. Laboratory tests revealed anemia, and increased LDH and alcalin phosphatase; skeletal X-ray, abdominal ultrasound and t.b. CT showed diffuse osteolytic lesions, moderate splenomegaly and some lymphoadenomegaly around the left iliac artery. MR and CT of the skull showed a wide osteolytic lesion coming from the occipital region: this lesion was formed by bulky tissue and reached the left hemisphere of the cerebellum. Bone marrow biopsy showed infiltration of strongly $CD68^+$ neoplastic cells: following cytological and cytochemical (weak positivity of CAE) testing of bone marrow aspirate supported the diagnosis of MCS, despite the markedly atypical form.

From December 1996 to February 1997, on the basis of the MCS diagnosis, we gave the patient 3 cycles of chemotherapy with idarubicin and ARA-C. In spite of this, the patient's illness progressed and the patient died.

The clinical course of our patient confirms all that is known regarding the resistance of aggressive SMCD to chemotherapy. Furthermore, to our knowledge, this is second described case of MCS: nevertheless, in our case, there is the peculiarity of bone involvement and lack of cutaneouos or mucous involvement.

026
Follow-up of the cytogenetic response in chronic myeloid leukemia patients treated with α-interferon

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α-interferon (α-IFN) is effective in the treatment of chronic myeloid leukemia (CML), inducing cytogenetic remission and prolonging survival.

From 1986 to 1988 a prospective, randomized trial enrolled 322 patients with CML at diagnosis: 218 were randomized to α-IFN treatment and 104 to treatment with conventional chemotherapy (CHT).

The median survival of the 218 pts who were assigned to α-IFN and of the 104 pts who were assigned to CHT was 76 months and 52 months, respectively (p = 0.002). The proportion of the patients that was projected to be alive after 9 years was 28% in the α-IFN arm and 18% in the CHT arm. The median time from the diagnosis to the progression to accelerated or blastic phase was significantly different between the α-IFN and the CHT arms (74 vs 46 months, p = 0.0005).

A cytogenetic response (Ph-neg >33%) was obtained in 70 of 218 cases treated with α-IFN (32%).

The best cytogenetic response was complete in 23 cases (10%), was major in 23 cases (10%) and was minor in 24 cases (11%). The time to achieve the first response ranged from less than 1 year to 7 years (median 1 year), but the time to achieve the best response was even longer, with a median of 2 years (4 after 1 year, 6 after 2 years, 5 after 3 years and 8 after 5 years or more). However, the majority of the responders got their first response within the first year of treatment and only 7 of 70 cases (10%) did not show any Ph-neg metaphases after the first year of treatment.

Seven out of 70 patients were submitted to allogeneic BMT. Of the remaining 63, 4 died in chronic phase (6%), 36 (57%) are alive in chronic phase and 23 (36%) progressed to accelerated or blastic phase. The proportion of the cases with progression was negatively related to the quality of the cytogenetic response (13% for complete response, 38% for major response and 59% for minor response, p = 0.005). Apart from the patients who were transplanted, α-IFN was discontinued in 11 patients with a major or a minor cytogenetic response because of toxicity or other reasons. Six out of the 11 patients progressed to an accelerated or blastic phase and 5 are alive and in the chronic phase. The median duration of cytogenetic response was 60 months; 17 patients are still in complete or major karyotypic response.

027
Amifostine pretreatment allows the use of higher dose of the *bcr/abl*-specific tyrosine kinase inhibitor AG1112

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Several pieces of evidences demonstrate the existence of residual normal stem/progenitor cells in chronic myelogenous leukemia (CML). The increased protein tyrosine kinase (PTK) activity of the chimeric BCR-ABL gene product (p210BCR/ABL) can be blocked by a number of compounds specifically inhibiting p210BCR-ABL activity, including the tyrphostin AG1112. At high dose, AG1112 might be toxic for residual normal progenitors. Amifostine, a phosphorylated aminothiol, increases the selectivity of specific anticancer drugs for neoplastic cells by protecting normal hematopoietic cells. One potential application of this protector is during stem cell purging to allow the use of higher doses of a given antileukemic compound without damaging residual normal progenitors. We evaluated the effects of amifostine pretreatment on CML-derived marrow and blood progenitor cells exposed to AG1112. Amifostine pretreatment (3 mg/mL, 15 min, 37°C) was followed by AG1112 incubation (50-200 μM, 18 hours, 37 °C). The effect of AG1112 and amifostine was studied on committed (CFU-Mix, BFU-E, CFU-GM) and primitive (LTC-IC) progenitors. Preincubation of CML cells with AG1112 induced a dose-dependent sup-pression of hematopoietic progenitors. AG1112 doses causing 50% inhibition of colony formation (ID50) were 203 μM and 121 μM for CFU-Mix+BFU-E and CFU-GM, respectively. Amifostine pretreatment prior to AG1112 exposure resulted in ID_{50} values which were significantly higher than those detected for AG1112 alone (>300 μM for CFU-Mix+BFU-E, 188 μM for CFU-GM). Analysis of LTC-IC demonstrated 64% and 75% surviving colonies after AG1112 (200μM) and amifostine plus AG1112, respectively.

In conclusion, our data demonstrate that amifostine pretreatment results in a protective effect on primitive and committed progenitors exposed to AG1112. This could encourage us to explore the use of higher AG1112 doses for CML purging in order to improve its antileukemic effects without damaging residual normal stem/progenitor cells.

028
Cytoreductive therapy of essential thrombocythemia: proposal of a score system

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The therapy for essential thrombocythemia (ET) is still a controversial issue, particularly as for asymptomatic patients because of the difficulty in deciding between platelet-lowering agents and a *wait and see* policy using anti-aggregating agents only: the main difficulty derives from our inability to identify patients at risk of developing serious thrombotic or hemorrhagic complications during the course of the disease. In this context, we have produced a scoring system for ET patients asymptomatic at diagnosis, comprising the variables age, platelet value, previous cardiovascular problems, smoking, dysmetabolic diseases. Patients were considered *symptomatic* if at least one of the following signs or symptoms was detected at diagnosis or within the 6 months prior the diagnosis: hemorrhage, bleeding time > 9 min., arterial or venous pathology, disturbances of microcirculation (headache, vertigo, paresthesias, Raynaud-like syndrome). The score was reassessed for each patient every 8 weeks during the follow-up. Cytoreductive therapy was started when score was = 4. Between July 1992 and December 1993, 43 consecutive adult ET patients (pts.) were diagnosed according to the *Polycythemia Vera Study Group* criteria: 17 were males, 26 females, median age was 54 years (range 22-69 years), median platelet level 1,090,000/mmc (range 726,000-2,230,000/mmc). Thirteen out of 43 patients (30%) were symptomatic: all these patients received cytoreductive treatment. Thirty (70%) patients were asymptomatic and therefore were scored according to our criteria: 6 pts. had a score greater than or equal to 4 and received cytoreductive treatment while 24 pts. (80% of the asymptomatic pts.) had a score less than 4 and were treated with anti-aggregating agents only. Median follow-up of all patients was 39 months (range 20-51 months). Nine out of 24 (37.5%) patients started a treatment with platelet-lowering agents, at a median interval from diagnosis of 17 months (range 4-32), because of: development of symptoms (6 pts.), venous thrombosis (1 pt.), prophylaxis of orthopedic surgery (1 pt.) and increase of score (1 pt.). Fourteen out of 24 patients (58.3%) remain without cytoreductive treatment (one patient has been lost to follow-up) after a median follow-up of 38 months (range 31-51). This scoring system appears to be reliable. The study is ongoing in order to enrol a larger series of patients.

029
Follow-up of the pH-positive clone in chronic myelogenous leukemia under interferon therapy: comparison of interphase cytogenetics and conventional chromosome analysis

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In order to analyze the efficiency of dual color fluorescence in situ hybridization (FISH) for the detection and monitoring of Ph+ cells in chronic myelogenous leukemia (CML) under interferon (IFN) treatment, 32 patients were selected for FISH analysis. A commercially available set comprised a BCR probe of approximately 300 kb in size with an ABL probe of 200 kb in size (Vysis Company). With these probes, more than 85% interphase cells showed an efficient hybridization pattern in normal controls, with <1.5% interphase cells having a false positive fusion signal.

All 32 patients were analysed by conventional chromosome analysis CCA (25 metaphase cells) and by dual color FISH (200-300 interphase nuclei) at diagnosis and after 6-32 months of IFN treatment. In addition 30 samples were comparatively analyzed by FISH after direct harvesting and after short-term (24 hours) culture. The difference in the percentage of Ph+ cells as assessed by CCA and by interphase FISH in 98 samples (32 at diagnosis and 66 during IFN therapy) ranged between 0%-9%, median value 4%. Mean differences of Ph+ cells in 32 samples with 0-33% Ph+ metaphases, in 26 samples with 33%-66% Ph+ metaphases, in 40 samples with 66%-100% Ph+ metaphases were as follows: 6.7%, range: 2%-9% in the first group, 7.8%, range 4%-9% in the second group and 4%, range 0-9% in the third group. Results of FISH analyses on direct and short term cultures were similar in all 30 cases studied, with a 2.5% mean difference (range 0%-5%). These data show that: a) overall, interphase FISH and CCA give similar results in assessing the size of the Ph+ clone at diagnosis; b) under IFN therapy, no major differences were detected by either method in cytogenetically-responding patients or in patients showing a minor response or no response; c) the percentage of Ph+ cells does not vary depending on the culture time. Based on these

findings it is suggested that FISH may be safely employed on directly harvested samples for the monitoring of the size of the Ph+ clone in CML. This technique allows the analysis of large numbers of cells, possibly resulting in a more accurate stratification of cytogenetically-responding patients in therapeutic trials using IFN.

MYELODYSPLASTIC SYNDROMES

030
Measurement of circulating and bone marrow long-term culture initiating cells in myelodysplastic patients

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Pancytopenia is a frequent presentation of myelodysplastic syndromes (MDS). The diagnostic distinction between aplastic anemia (AA) and hypocellular MDS may be difficult if chromosome abnormalities are not detected and dysplastic changes are not severe. The autoimmune pathophysiology of acquired AA is well established. Several laboratory findings suggest that similar pathophysiologic mechanisms may also operate in hypoplastic MDS. We studied the number of long-term culture initiating cells (LTC-IC) in the bone marrow (BM) and peripheral blood (PB) of 45 patients with MDS in comparison with those from 17 normal controls and 46 patients with *de novo*, untreated AA. Due to the low numbers of cells available for the analysis, formal limiting dilution analysis could not be performed, and secondary colony-forming cells (sec CFC) after 5 weeks of LTBMC were measured. As sec CFC cells are proportional to the input number of LTC-IC, we used the number of sec CFC cells per 10^6 mononuclear cells (MNC) initiating the culture as a measure of the content of immature stem cells in BM and PB. The MDS group consisted of 34 RA, 3 RARS, 8 RAEB and 2 RAEB-T patients. BM-LTC-IC in normal controls were $147\pm38/10^6$ MNC plated. In MDS patients, the results were as follows: RA and RARS $21\pm7/10^6$ ($p < 0.0001$); RAEB and RAEB-T $39\pm12/10^6$ ($p < 0.0001$). In all groups tested, the decrease in PB sec CFC numbers was consistently less pronounced. In MDS patients with hypocellular BM, secondary CFC were lower but not significantly different in comparison to MDS with hypercellular BM (18±6 vs. 35±11; $p > 0.05$). BM and PB secondary CFC numbers in hypoplastic RA were significantly higher than those in severe AA (19±5 in BM, $p < 0.01$; 7±2 in PB, $p < 0.05$). We conclude that, although the deficiency in the stem cell compartment is less severe in MDS than in AA, similar pathogenetic mechanisms may operate in both diseases leading to the depletion of early hematopoietic progenitor cells.

031
Myelodysplastic syndromes and exposure to mutagenic agents: correlation between prognosis and clinico-biologic and cytogenetic data

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The pathogenetic role of previous radio-chemotherapeutic treatments in the development of secondary acute leukemias and myelodysplastic syndromes (MDS) is well documented. A clear evidence of the role of occupational exposure to mutagenic agents (solvents and pesticides) in the pathogenesis of primary MDS is still lacking. An epidemiological and clinico-biologic study was performed in 195 patients affected by primary MDS in order to evaluate whether exposure to mutagenic agents could define a subset of MDS with particular clinico-biologic features. An adequate occupational history could be collected in nearly 70% of the cases. Overall 43% of the patients had a prolonged exposure to pesticides and solvents in comparison to 20% of a case-controlled group selected on the basis of sex and age. A higher incidence ($p=0.007$) of prolonged exposure was detected among RARS, RAEB and CMMoL versus RA and RAEB-t patients. No differences could be detected as far as principal clinico-biological parameters at presentation were concerned with the exception of age which was relatively higher for exposed patients (67.5 vs 63 yrs, $p=0.03$). Cytogenetic analysis, performed successfully in 130 patients, demonstrated a higher incidence of abnormal karyotypes in exposed subjects with –5/5q–, –7/7q– and 17p– as recurrent abnormalities, a figure usually observed among secondary MDS. Only 3/13 patients affected by classical 5q- syndrome had a previous history of exposure to mutagenic agents while no difference in incidence of exposure was detected for trisomy 8. Exposed patients had a worse prognosis both in univariate and multivariate analysis. Taken together these findings could document that primary MDS in patients with prolonged exposure to pesticides or solvents could identify a subset of myelodysplasia with particular clinico-biological and prognostic features similar to secondary MDS.

032
In vivo mobilization of karyotypically normal peripheral blood progenitor cells (PBPC) in high-risk MDS, secondary or therapy-related acute myelogenous leukemia

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Patients with myelodysplastic syndrome (MDS) with double or complex cytogenetic abnormalities have the worst prognosis. Autografting has been attempted in selected patients but in the majority of them there is no evidence that MDS is cured. The obstacles to more widespread use of autotransplants comprise the problem of providing grafts of progenitor cells which are predominantly, if not completely, disease-free. Our group previously reported that mobilization of Ph1-negative progenitors is possible in a significant number of Ph1-positive ALL (*Br J Haematol 1995; 88:535*) and CML patients (*Bone Marrow Transplant 1993; 12-267*). In this pilot study we employed the same approach in patients with RAEB-t, secondary AML (sAML) and therapy-related AML (t-AML). All patients had double or complex cytogenetic abnormalities in marrow cells before mobilization therapy and none of them had received previous chemotherapy. Nine patients with a median age of 50 years (range, 22 to 68) entered our pilot study. The mobilization protocol consisted of idarubicin 8 mg/m^2/day on days 1-3, arabinosylcytosine 800 mg/m^2 by 2-hour infusion on days 1-3, and etoposide 150 mg/m^2/day by 2 hrs infusion on days 1-3 (mini-ICE protocol). From day +8 G-CSF (5 µ/kg/day) was administered daily until the total neutrophil count was greater than 1.0×10^9/L for three consecutive days. Leukaphereses were performed when the WBC count exceeded 1×10^9/L and the appearance in the PB of CD34$^+$ve cells rose above our threshold of detection (>0.05 of viable MNC). All 9 patients completed the mobilization protocol and no patient died of the mobilizing procedure. Karyotype analysis was possible in all patients on all collections. No cytogenetic abnormalities were found in repeated sampling, in 6 of the 9 patients. Adequate CD34$^+$ cells ($>2\times10^6$/L) and CFU-GM ($>2\times10^4$/kg) were obtained in 7/9 patients. To date, three patients, who had entirely karyotypically normal PBPC collections, have undergone autografting. High-dose therapy consisted of idarubicin, etoposide and single-dose total body irradiation (IVT protocol). G-CSF was given at 5 mg/kg/day from day +8. Evidence of marrow engraftment (ANC $>0.5\times10^9$/L and platelets $>25\times10^9$/L) was attained at 9-17 days and 8-106 days, respectively. One patient is in complete hematologic and cytogenetic remission 3 months after autografting. Two patients showed cytogenetic remission in the marrow at discharge but relapsed within two and six months after transplant; one of them died of refractory leukemia. In conclusion, our preliminary data in these selected high-risk MDS patients suggest that peripheral rmobilization of diploid cells is feasible. Additional patients and time are necessary to establish the role of this procedure.

033
Risk evaluation in myelodysplastic syndromes: the validity of the International Prognostic Index and the role of bone marrow biopsy

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The aim of this study was to apply the International Prognostic Scoring System (IPSS) recently proposed for myelodysplastic syndromes (MDS) to a group of 64 patients (43 males, 21 females; median age: 66 years). Leukemic transformation, overall survival and early death (within 12 months) were chosen as endpoints for the statistical analyses. In addition to the IPSS, we also used univariate and multivariate analysis to evaluate other possible prognostic factors: bone marrow biopsy (BMB), common hematologic and cytogenetic parameters, and FAB classification. As of 30 April 1997, 39 patients had died and 21 presented leukemic transformation. The IPSS and the presence of CD34$^+$ aggregates proved to be the most reliable predictors of leukemic transformation at both univariate ($p<0.01$) and multivariate analysis, whereas BMB was more reliable than the IPSS in predicting early death ($p<0.001$ vs $p<0.01$). Both of the groups with the worst IPSS prognosis ("*Int-2*" and "*High*") had a median overall survival of 20 months; the patients who presented aggregates of immature CD34$^+$ elements at BMB had a median overall survival of 15 months. The overall level of significance deriving from the comparison of the curves obtained using the IPSS was the same as that obtained using BMB histological findings alone ($p<0.0005$). FAB classification and cytogenetic findings proved to be less reliable than the IPSS and histology in relation to the considered prognostic endpoints. The results of this study confirm that the IPSS is a valid method of identifying MDS patients with different prognosis. The two groups with the most unfavorable prognosis accounted for 40% of the study population and also included patients with a relatively long life expectancy, whereas the presence of CD34$^+$ aggregates in BMB samples made it possible to identify patients with a highly unfavorable prognosis who are therefore candidates for a more aggressive therapeutic approach.

034
Detection of abnormal motility patterns of circulating neutrophils from patients suffering from myelodysplastic syndromes identified by image analysis

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Recently, we developed an image processing workstation[1] aimed at evaluating of neutrophil motility in microporous filters, according to the Boyden method. The images are automatically acquired from sequential focal planes through the filter and are subjected to optical-digital conversion by means of specific software which detects and counts the cells. The distance traveled by neutrophils is exactly measured by the algorithm, which calculates the linear interpolation. In addition, the pattern of neutrophil distribution in the filter is evaluated and displayed. Thus, in normal subjects random migration (RMIG) is identified by a Gaussian pattern, and stimulated migration (SMIG) is identified by a typical *peak* of cell concentration far from the initial focal plane,[2] which appears precociously and remains for whole the time of migration.[3] Using this workstation, we evaluated RMIG and SMIG of peripheral blood neutrophils from 18 patients suffering from myelodysplastic syndromes (MDS), who were classified, following the FAB criteria, as having: RA (n=5); RARS (n=4); RAEB (n=4); RAEB-t (n=3); CMMoL (n=2). All patients were evaluated at diagnosis, without any evidence of underlying infections.

RMIG was found to be inhibited in 14 patients, but the normal Gaussian pattern of migration appeared to be preserved in 13 of them. Therefore, the cellular defect responsible for impaired RMIG affected the whole neutrophil population.

SMIG was inhibited in 9 patients, 4 of whom also showed an abnormal distribution of the migration curve (absence of the initial *peak*). In the remaining 9 patients, a similar kinetic defect was observed, despite the fact that normal values of SMIG were registered. As far as the subtype of MDS is concerned, we observed that only neutrophils from patients with RARS did not display any motility defect or abnormality of cell kinetics.

Further studies, carried out on a larger series of patients, could help clarify whether the degree of myelodysplasia could be correlated with the kind of motility defect of the neutrophils.

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035
Higher spontaneous rate of apoptosis characterizes myelodysplastic syndromes at lower risk of leukemic evolution

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Myelodysplastic syndromes (MDS) are characterized by peripheral blood pancytopenia with hypercellular bone marrow and inefficient hematopoiesis. A pathogenetic role of apoptosis (PCD) in this disease has been recently proposed. We therefore evaluated in 18 patients affected by MDS at different risk, the spontaneous rate of apoptosis in leukemic cells $CD34^+$, measuring in addition effects of granulocyte-colony stimulating factor (G-CSF) on PCD. Two different flow cytometric techniques were used to detect PCD: 1) Acridine Orange (AO) that identifies apoptotic cells as a sub-$G_{0/1}$ peak on the DNA frequency histogram; 2) Annexin V, that allows apoptosis to be measured in specific hemopoietic sub-populations. MDS hemopoiesis was evaluated based on DNA-flow cytometric aneuploidy and $CD34^+$. In addition, cell cycle distribution was evaluated in these samples. Results obtained by measuring the overall mononuclear cell population showed differences in the mean percentage of PCD between RAEB (m=14.4%) and RAEB-t (m=5.8%). Detection of the spontaneous rate of apoptosis restricted to the $CD34^+$ compartment, confirmed a significantly (p=0.04) higher spontaneous rate of PCD in RAEB (m=2.2%), than in the sub-type in leukemic transformation (m=0.6%). RAEB were in addition characterized by higher S-phase of the flow cytometric aneuploid fraction compared to RAEB-t (m=23.7% vs. m=12.7%) and by G-CSF protective effects from PCD on $CD34^+$ cells. These findings suggest that mechanisms involved in balance between cell proliferation and survival regulate MDS hemopoiesis in patients at lower risk of leukemic transformation. Cases characterized by a higher rate of apoptosis may lose this growth control mechanism when further additional oncogenetic events lead to leukemic transformation.

036
$CD34^+$ megakaryocytes occur in normal bone marrow, and are greatly increased in myelodysplastic syndromes

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Myelodysplastic syndromes (MDS) are neoplastic proliferations involving all hematopoietic cell lines; morphological abnormalities of megakaryocytes (MGK) are well documented in MDS, but little information is available on associated phenotypical changes. During the characterization of MDS, we identified expression of CD34 antigen (which is normally found on hematopoietic precursor cells, including BFU– and CFU–) on MGK. In this study, we analyzed in detail the occurrence of $CD34^+$ in a large number of MDS patients, and correlated this with other morphological, phenotypical and clinical parameters. Sixty bone marrow biopsies fixed in B5 and embedded in paraffin were obtained from 21 patients with MDS; in addition, 10 biopsies from normal age-matched controls were used. Sections were stained with anti-CD34 monoclonal antibody (Qbend10), and with anti-markers CD61 and factor VIII-RA (FVIIIRA). In normal bone marrow biopsies, mature MGK expressing CD34 were extremely rare and observed in four out of 10 cases. In contrast, in MDS $CD34^+$ MGK were recognizable in 19/21 cases; their number ranged from rare (1 positive cell/high power field), to numerous (> 3 positive cells/h.p.f.); in three cases, the vast majority of MGK strongly expressed CD34. Positivity was predominantly cytoplasmic (diffuse or paranuclear), but cell membrane expression was also recognizable. On morphology, the $CD34^+$ MGK displayed variable features and included mature forms as well as dysplastic and small MGK, typically observed in this disorder. On serially cut sections, it was obvious that the $CD34^+$ MGK also expressed FVIIIRA and CD61. The number of $CD34^+$ MGK was not related to the number of $CD34^+$ small blasts. In addition, no correlation between different forms of MDS, evolution of the disease, cytogenetic abnormalities on the one hand, and the number of MGK expressing CD34 on the other, was found. Finally, since most patients had more than one biopsy during the course of their disease and bone marrow histology greatly varied from one biopsy to another, it was possible to demonstrate that the occurrence of morphologically recognizable MDS changes did correlate positively with the presence of $CD34^+$ MGK.

In vitro studies have demonstrated that CD34 expression on MGK is limited to immature precursors and that it is usually lost during maturation, while CD61 expression is acquired. In normal conditions, the $CD34^+CD61^+$ immature blasts represent a small fraction of MGK precursors. In MDS, the high number of $CD34^+CD61^+$ cells recognizable as dysplastic MGK might reflect high proliferative capacities of these cells (*Blood 1992; 80:3022-35*). In addition, abnormal expression of CD34 on MGK might be responsible for defective/inappropriate signalling by growth factors and cytokines (*Haematologica 1995; 80:367-87*), and therefore result in dysmegakaryopoiesis.

037
Effect of thrombopoietin on megakaryocytic and erythroid progenitors in myelodysplastic syndromes

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Myelodysplastic syndromes (MDS) are clonal disorders often characterized by thrombocytopenia. Many *in vitro* studies have shown that IL-3 and SCF are capable of, at least partially, restoring megakaryocytopoiesis in MDS, but little is known about the effect of thrombopoietin (TPO),* a recently identified cytokine, on bone marrow (BM) precursors. We therefore evaluated its activity on megakaryocytic (MK) and erythroid progenitors by means of short-term cultures of fresh BM samples and after stimulation with TPO alone or in association with IL-3 and SCF.

The BM cells were collected in preservative-free heparin and then separated by means of density gradient centrifugation. The BM mononuclear cells were used partly for short term BFU-MK, partly for liquid cultures. *BFU-MK:* 3×10^5 cells/mL were plated in Plasma Clot containing TPO 50 U/mL alone and with IL-3 100 U/mL, SCF 8 U/mL±TPO. After 18 days of culture the megakaryocytic colonies were scored using anti-gpIIb/IIIa monoclonal antibody. *Liquid cultures:* 1×10^6 cells/mL were resuspended in IMDM and FCS 10% with TPO 50 U/mL alone and with IL-3 100 U/mL, SCF 8 U/mL±TPO 50 U/mL. After 4 days we performed the BFU-MK with IL-3+SCF, and BFU-E in methylcellulose with Epo 2 U/mL, IL-3 and SCF at the above dosages.

TPO alone rarely induced BFU-MK growth in the short-term cultures of normal samples, and never in those of the MDS samples. TPO+IL-3+SCF did not increase the number of colonies in comparison with IL-3+SCF in either the normals or the MDS samples. After liquid culture with TPO alone and in association with IL-3+SCF, the number of MK colonies significantly increased ($p<0.05$) in comparison with the unstimulated control in normal samples. Moreover, we also observed a greater effect of TPO+IL-3+SCF ($p=0.05$) in comparison with the samples pre-stimulated with IL-3 and SCF alone. In contrast, we did not observe any modification in the MDS samples even after pre-incubation. In the normal samples, pre-incubation with TPO led to a significant increase in BFU-E in comparison with the untreated controls. Among the MDS samples 3/7 (42.8%) showed an increase in BFU-E after pre-incubation with TPO±IL-3+SCF with respect to the untreated control. In our *in vitro* system, TPO showed early activity in expanding the normal MK compartment, whereas it did not

seem to have any effect on MK progenitors in MDS. Moreover, TPO seems to be able to expand the erythroid compartment in normal subjects and partially in MDS.

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038
Expression of cytokine receptors in long term liquid cultures of myelodysplastic syndromes

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Abnormalities in response to growth factor (GF) stimulation have been described both in *in vitro* and *in vivo* in myelodysplastic syndromes (MDS). We have recently described a long-term culture system in which bone marrow cells are grown in stroma free, liquid conditions with the frequent addition of multiple cytokines including GM-CSF, IL-6, SCF and IL-3. In this system MDS progenitor cells can either respond to cytokine stimulation with a normal *in vitro* growth, show refractoriness to GFs or rapidly convert to a leukemic growth pattern along the culture. To better understand some mechanisms regulating the *in vitro* growth of these cells we evaluated the pattern of expression of receptors for GM-CSF, c-kit, flt-3 and IL-3 by FACS analysis and immunohistochemistry in 15 patients with different MDS subtypes. MDS patients responding to cytokine stimulation GF receptors were generally up-regulated at the 2nd and 3rd week of culture to decrease sharply at week 4; these findings were similar to those observed in normal bone marrow cultures. In non responding patients GF receptors were either rapidly down-regulated or still present at the 4th week of culture. In patients with a leukemic transformation during the culture they were generally expressed at the onset of the culture and rapidly decreased once the autonomous growth and expansion of the leukemic clone had become apparent. These findings may be explained in several ways including abnormally regulated or structurally defective GF receptors or inappropriate ligand-receptor interactions, this reflecting the heterogeneity of this disease. Nonetheless the combined analysis of the *in vitro* growth characteristics and of GF expression may be useful to dissect MDS further and to predict response to GF treatment *in vivo*.

ACUTE LEUKEMIAS

039
Comparative toxicity of daunorubicin and daunoxome on MDR and non-MDR cell lines

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Anthracyclines are first-line drugs in the treatment of acute leukemia, but sensitivity of leukemic cells to anthracyclines can be downmodulated by multidrug resistance (MDR) transport proteins like the 170 kd glycoprotein, Pgp. In this study we compared the toxicity of daunorubicin (DNR) and daunoxome (DNX), liposomal encapsulated DNR, using the MTT microcultured tetrazolium colorimetric assay. We used the T-cell acute lymphocytic leukemia cell line CCRF CEM and its Pgp overexpressing resistant subline CEM VLB. Results were expressed as ID_{50} (drug dose that inhibited the cell growth to 50% of the control) and resistance index (RI = ID_{50} resistant subline/ID_{50} parental cell line). The use of the cyclosporin derivative SDZ PSC 833 (PSC) increased the toxicity of DNR and DNX on the resistant subline with the consequent reduction of RI. Results are summarized in the Table below.

In conclusion, the toxicity of DNR and DNX was similar in the parental cell line CCRF CEM whereas in the resistant subline DNX was 6 times more toxic than DNR. The addition of PSC increased the toxicity of DNR and DNX and it almost completely neutralized the resistance, leading the RI to 1.6 for DNR and to 1.4 for DNX.

	Daunorubicin		*Daunoxome*	
	ID50	*RI*	*ID50*	*RI*
CCRF CEM	6.5	-	5.4	-
CEM VLB	380.0	58.4	64.0	11.8
CEM VLB+PSC	10.5	1.6	7.8	1.4

040
Soluble p55-TNFr serum levels correlate with prognosis in adult acute myeloid leukemia

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Soluble TNF receptors (sTNFRs) can participate in regulating TNF activities, which have been implicated in acute leukemia cell growth. Thus, pre-treatment serum levels of p55- and p75-sTNFRs were evaluated in 126 adult patients with acute myeloid (AML) and lymphoid (ALL) leukemia (AML 82, ALL 44), using an ELIBA method, and analysed in relation to clinical/hematological features and outcome. RT-PCR, flow cytometry and short-term cultures were used to evaluate expression and release of TNFRs by leukemic cells. Serum levels of both sTNFRs were significantly higher in AML and ALL than in controls ($p<0.01$ for both sTNFRs). AML patients with M4-M5 showed higher figure than those with other subtypes (M0-M3, M6, M7) (p55- and p75-sTNFR, 6.7±5.11 and 9.78±5.6 vs 3.27±1.6 and 4.82±4.3 ng/mL, $p < 0.001$ for both sTNFRs). Leukemic cells expressed both receptors at membrane levels. Following short term cultures, p55- and p75-sTNFR membrane expression was increased and associated with sTNFRs release into SN. At multivariate analysis, AML, but not ALL, with higher p55-sTNFR serum concentration at diagnosis had lower complete remission rate ($p=0.045$), disease free survival ($p=0.027$), and overall survival ($p< 0.001$). p55-sTNFR values analysed as a continuous variable appeared to be a prognostic factor independent from age, sex, number of leukocytes, splenomegaly (present vs absent), FAB sub-type (M4-M5 vs other histotypes), and use of BMT (transplanted vs not transplanted) as consolidation therapy. The independent prognostic significance of p55-sTNFR serum levels seems to be in line with a relevant biological role for this molecule in AML, possibly related to the mechanisms of TNF-mediated leukemic proliferation taking place *in vivo*.

041
Proposed model of chemoresistance based on the interaction between P-glycoprotein, bcl-2 and transferrin receptor in acute myeloid leukemia

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P-glycoprotein (PGP), bcl-2 oncoprotein (bcl2) and transferrin receptor (CD71) are commonly found in normal hemopoietic cells at low intensity levels. Pathologic overexpression analysed through mean fluorescence intensity (MFI) could allow the stratification of AML patients in subgroups with different biological features and clinical outcome.

Here we analyzed 130 consecutive patients with de novo AML, median age 58 yrs. Our results obtained by flow cytometry were based on the MFI ratio (R), determined by dividing the MFI of the positively stained cells by that of cells stained with an isotype control antibody. Bcl2R >10 was significantly related to FAB M0-M1 cases ($p=0.021$), whereas either CD71R >5 or PGPR >6 were associated with M4-M5 groups ($p=0.003$ and = 0.001, respectively). CD34 positivity highly correlated with bcl2R >10 ($p<0.0001$) and CD71R <5 ($p=0.007$); on the other hand, CD14 was often found in PGPR >6 and CD71R >5 cases ($p<0.0001$). Inverse correlations were noted both between bcl2R and CD71R ($P=0.001$) and between bcl2R and PGPR ($p=0.012$). With regard to clinical outcome, PGPR >6 and CD71 >5 were associated with a decreased CR rate (34% v 62%; $p=0.001$; 32% v 68%; $p<0.001$, respectively). Shorter overall survival was found in CD71R >5 ($p=0.022$), in bcl2R >10 ($P=0.011$) and in PGPR >6 ($p=0.003$) cases, while only bcl2R >10 affected CR duration ($p=0.007$). By combining PGPR, bcl2R and CD71R, we were able to distinguish two prognostically different subgroups of patients with regard to CR rate ($p<0.001$), survival ($p=0.00001$) and CR duration ($p=0.007$): 1) best prognosis characterized by [PGPR<6/ bcl2R <10/CD71R <5]; 2) worst prognosis identified by [PGPR >6/bcl2R >10/CD71R >5]. Therefore, our study allowed us to identify two patient groups with opposite biological and clinical features. Multivariate model confirmed the independent prognostic value of CD71R ($p<0.001$), PGPR ($p=0.021$) and bcl2R ($p=0.027$) with regard to CR.

Our model based on additional independent mechanisms of chemoresistance might be exploited for more precise and effective therapeutic approaches.

042
P-glycoprotein and terminal transferase identify prognostic subsets within acute myeloid leukemia

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In acute myeloid leukemia (AML) resistance to cytotoxic therapy could be explained by the imbalance

between P-glycoprotein (PGP) overexpression which confers resistance to agents sharing a mechanism of uptake/efflux and overexpression of bcl-2 which blocks drug induced apoptosis. From 1987 to 1996, we studied 309 consecutive patients with *de novo* AML, median age 58 yrs, all treated by intensive chemotherapy regimens. Terminal transferase (TdT) was expressed in 77/298 (26%) patients, particularly in the immature FAB M0-M1 classes ($p<0.0001$). Close association was found between TdT and CD34 positivity, as 240 of 298 samples had similar patterns of staining ($p<0.0001$). Bcl-2 high fluorescence intensity was closely related to TdT positivity ($p=0.007$) and CD34 expression ($p=0.001$). One hundred and thirty-one out of 234 patients studied (56%) were positive for PGP expression. Strict correlations were noted between PGP negativity and M3/M0 subtypes and between PGP positivity and M5 subtype ($p=0.001$); on the other hand, there was no correlation with CD34 expression. With regard to prognosis, there was a significant difference in CR rates both between PGP^+ and PGP^- cases and between TdT^+ and TdT^- ones (34% v 68%, $P<0.001$ and 35% v 58%, $p=0.001$, respectively). The survival rates were significantly shorter both in PGP^+ ($p<0.0001$) and in TdT^+ patients ($p=0.013$). Moreover, PGP and TdT negative cases showed a trend toward longer remissions ($p=0.002$ and $=0.23$, respectively). By combining PGP and TdT expression, we distinguished two subsets of patients, one with worse prognosis [PGP^+TdT^+] and the other with a better prognosis [PGP^-TdT^-] with regard to CR rate ($p<0.0001$), survival ($p<0.00001$) and CR duration ($p=0.0014$). The two subsets [PGP^+TdT^-] and [PGP^-TdT^+] had an intermediate outcome both for CR rate, survival and CR duration. Multivariate analysis confirmed the independent prognostic weight of PGP ($p<0.001$) and TdT ($p=0.002$) for the achievement of CR. In conclusion, PGP and TdT have a poor prognostic value, implying synergic but distict mechanisms of chemoresistance (increased drug efflux and reduced apoptosis, respectively).

The final implications of our findings are that the use of agents to reverse PGP function in AML may be unsuccessful in the absence of strategies to reduce resistance to apoptosis.

043
Multiple adverse biological features explain the poor prognosis of acute myeloid leukemia M0

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Apart from its biological features, acute myeloid leukemia (AML) M0 is of great clinical interest owing to its frequently reported unfavorable prognosis. We and others recently pointed out that the concurrent expression of factors such as CD7, P-glycoprotein (PGP), complex karyotypes and age older than 60 is a common feature of AML M0. Here, we present an analysis of the biological and clinical findings in 30 AML M0 cases in comparison with 279 AML cases belonging to the other FAB classes. CD34 and TdT were strongly associated with AML M0: 93.3% and 70% of M0 cases were respectively positive ($p < 0.0001$). High intensity of bcl-2 expression was observed in all 12 analyzed M0 cases ($p=0.021$). Lymphoid antigens such as CD2, CD5, CD10 and CD19 were similarly distributed among different FAB classes without any significant correlation. PGP was expressed in 33% of AML M0 cases, the lowest incidence together with M3 among FAB groups; the highest incidence was found in the M5 class ($p=0.001$). Furtherore, AML M0 was associated with a higher incidence of chromosome abnormalities, mainly complex karyotypes, than the other FAB classes ($p=0.005$). With regard to clinical outcome, the CR rate in AML M0 was 36.7%; this compares with an overall CR rate of 51% ($p<0.001$). Significantly shorter overall survival and CR duration were found in AML M0 ($p<0.001$), also after the removal of APL cases. Interestingly, we were able to distinguish subsets with different prognoses within AML M0 using cytogenetic risk classes, PGP and monocytic antigen expression (CD11b and CD15). The following subsets: 1) *poor cytogenetics* (16 cases), 2) PGP^+ (7 cases), 3) $CD15^+$ (7 cases) or $CD11b^+$ (9 cases) had a poorer outcome with regard to CR and overall survival. In conclusion, AML M0 has an extremely unfavorable prognosis since there may be co-expression of different modalities of resistance to cytotoxic drug therapy involving both anti-apoptotic pathways (bcl-2), complex karyotypes, classic multidrug resistance (PGP) and monocytic markers, assembled in a unique fashion with respect to all the other FAB classes.

044
The pattern of CD11b expression by leukemic cells at basal conditions and following ATRA *in vitro* distinguishes M3 from non-M3 blasts

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CD11b is expressed on the surface of normal neutrophils, eosinophils, monocytes, most natural killer cells and a subset of T lymphocytes. It is even reported to be present on acute myeloid leukemia (AML) cells with the exception of M3 blasts, where it is poorly expressed. In the present study we investigated the expression of CD11b on blast cells of 64 AML cases (14 M3, 50 non-M3). The diagnosis of M3 was sup-

ported in all cases by the demonstration of PML/RARα rearrangement. The expression of CD11b (Becton Dickinson) was higher in 50 non-M3 (32.1± 25%; 3 M0: 26.4±30.8%, 4 M1: 34.7±18.5%, 18 M2: 16±12.4%, 18 M4: 43.8±24.2%, 6 M5: 43.8±35.8%, 1 M6: 11%) than in 14 M3 (6.7±6%; p< 0.001). Blast cells from 7 M3 and 5 non-M3 (3 M2, 2 M0) were cultured for 72 hours in 15% FCS RPMI in the presence of 10^{-6} M all-trans retinoic acid (ATRA, Sigma). Following culture, CD11b expression was up-regulated in all individual M3 cases (8±8% at basal condition vs 47.6±21.6% after culture, p< 0.001), whereas it was not in non-M3 (7.9±6.9% both at basal condition and after culture). Our data, although preliminary, suggest that the absent or low expression of CD11b at basal condition and its ATRA-induced up-regulation in culture can be regarded as a typical feature of M3 blasts with PML/RARα rearrangement, possibly representing a immunophenotyping feature to be used for diagnostic purposes.

045
Therapy of acute leukemia: retrospective analysis of a recent series of patients of all ages treated in one center

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The reported good outcome of adult acute leukemia treatment often refers to selected series of patients. We retrospectively analyzed the outcome of all acute leukemia (AL) patients diagnosed and treated in Genoa from 1992 to 1996.

	non M3-ANLL	*M3 - ANLL*	*ALL*
All patients	173	34	48
< 65 ys (%)	103 (59)	29 (85)	38 (79)
Support only (%)	16 (9)	2 (6)	2 (4)
Induction chemotherapy (%)	156 (91)	32 (94)	46 (94)
Early deaths (%)	12 (7)	4 (11)	1 (2)
%CR <65 / >65 yy	66/23	86/80	69/44
Allo 1st CR (%)	15 (9) (15 in <65)	2 (6)	7 (15)
Auto I CR BM (%)	4	2	2
PBSC	(2)	(6)	(4)
	3		1
	(2)		(2)
No BMT < 65 ys			
% 3 ys DFS/Surv	27/26	70/65	27/16
Allogeneic BMT			
% 3 ys DFS/Surv	66/76	–	67/57
ABMT			
% 3 ys DFS/Surv	54/67	–	–

Patients diagnosed by other centers and referred to *Genoa Bone Marrow Transplant Unit* were not analyzed (see Table above). The median interval between diagnosis and BMT was 4 months for both the allogeneic and autologous arms. Our data confirm that allogeneic and autologous BMT represent the best consolidation therapy for acute leukemia patients but point out that few patients may actually benefit from it while in 1st CR.

046
Evaluation of the expression of several MDR-related genes in ANLL patients

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Forty-four acute non-lymphoblastic leukemia patients (35 newly diagnosed) were evaluated by RT-PCR for the expression of several MDR-related genes: *mdr1, mrp, topoisomerase II α, topoisomerase II β, glutathione-S-transferase π, LRP.* Twenty patients were female and twenty-four were male; median age was 62 (range 27 to 81) years. According to the FAB classification 8 were M1, 27 M2, 2 M3, 5 M4 and 2 M5. cDNA obtained from total RNA were amplified by 35 PCR specific cycles. Thirty point seven percent of the evaluated patients were classified as mdr1-mRNA positive, 71% expressed mrp, 45.7% topoisomerase II α, 67.6% topoisomerase II β, 62.9% glutathione-S-transferase π and 36.6% LRP gene. All 44 patients were evaluated for complete remission, overall survival and disease-free survival after a follow-up ranging from 1 to 45 months. Independently from the gene frequency, no significant association was found between each MDR studied gene and the three prognostic parameters, either in responsive or in non-responsive patients. Nevertheless, a significant association between the following MDR genes was found: 1. topoisomerase II β/mrp ($p = 0.01$); 2. topoisomerase II β/glutathione-S-transferase π ($p = 0.01$); 3. mrp/glutathione-S-transferase π ($p = 0.05$). In the group of non-responsive patients, mrp gene expression was significantly associated with a shorter overall survival ($p = 0.04$); besides, frequently mrp/ topoisomerase II β ($p = 0.05$) and mdr1/topoisomerase II β ($p = 0.03$) genes were simultaneously expressed. In conclusion, these results seems to underline the prognostic role of the association of different chemoresistance mechanisms.

047
Bone marrow metastatic infiltration by alveolar rhabdomyosarcoma simulating an acute erythremia (Di Guglielmo syndrome)

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A 65-year-old woman was admitted to our Institute because of fatigue, pallor and cutaneous hemorrhages. Her blood count was: Hb 8.9 g/dL, Plts 9.0×10^9/L, WBC 15.6×10^9/L (blast cells 4 % and erythroblasts 12 %). Biochemical value was: LDH (7331 IU/L), CPK (506 IU/L; isozymes: MM 8.6%, MB 34.1%, BB 57.3%) and ferritin (694 ng/mL). Bone marrow aspiration showed normal cellularity with a massive (> 90%) infiltration by isolated blast cells: these were large cells with round nuclei showing reticular-type nuclear chromatin, one or more large nucleoli, and basophilic cytoplasm without granules but containing many vacuoles; giant bizarre multinucleate cells were frequently observed. They were peroxidase, Sudan black B, specific and non-specific esterase negative and showed strong PAS positivity. Bone marrow immunophenotyping demonstrated negativity for CD: 1a, 2, 3, 4, 5, 7, 8, 10, 11b, 11c, 13, 14, 15, 19, 20, 22, 23, 33, 34, 38, 45, DR and positivity for CD71, CD56 and glycophoyin A. The physical examination showed I grade splenomegaly without hepatomegaly or lymphadenopathy. A diagnosis of acute erythremia was thus suggested. The patient was submitted to bone marrow biopsy, and immunohistochemistry was performed. The final result was a diagnosis of alveolar rhabdomyosarcoma (RMS) because positivity of desmin and vimentin, negativity of cytokeratin, HBA71 and neurofilaments were found. The diagnosis was confirmed by cytogenetics analysis that showed a female karyotype with 99 chromosomes and reciprocal translocation t(2:13)(q35; q14): specific cytogenetic marker of this disease. Some cases of systemic RMS simulating a hematological malignancy have been described in the literature. In this paper we report some features useful for a correct diagnosis: negativity of CD45 (which indicates that it might not be a hematologic malignancy); positivity of desmin and vimentin (which are specific for the diagnosis of RMS). The positivity of glycophoyin A has never been reported and so more studies are necessary. The cytogenetics analysis is helpful, and so to, in our opinion, are the CPK and PAS reactions. As a matter of fact, the PAS positivity that we found is too strong even for an acute erythremia.

048
Acute myeloid leukemia (AML) relapsing after autologous bone marrow transplantation (AUBMT) in first remission: analysis of 249 cases

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While some studies reported good results with second transplants or donor lymphocyte infusions in leukemic patients who relapsed after allogeneic bone marrow transplantation, the benefit of salvage therapy in patients relapsing after autologous transplant remains undefined. This analysis was undertaken to assess the outcome of 259 AML patients who relapsed after AUBMT in first remission. Baseline and follow-up data were obtained from the GITMO and GIMEMA database. One hundred and thirteen patients (43.6%) underwent AUBMT before January 1990; 10.8% of cases was transplanted for secondary AML. The median interval between diagnosis and AUBMT was 6 months (range 3-15). Relapse occurred 5.3 months (range 1-84) after AUBMT. Unfavorable FAB subtypes (M0, M1, M6, M7) generally showed shorter relapse-free survival after AUBMT.

While no significant relationship was found between age, sex, WBC at diagnosis, conditioning regimen and survival, the FAB classification and the duration of first remission (≤ 6 vs. > 6 months) were predictive of survival ($p<0.0048$ and $p<0.0001$, respectively). Multivariate analysis, among the various factors tested, confirmed the favorable role of the M2/M4 FAB subtypes and of a longer first remission, with 23.6% of survival probability for patients having both these features. In conclusion, this retrospective analysis shows that those patients who relapse after AUBMT in first remission can benefit from salvage therapy when they have favorable prognostic factors. Treatment details and response data will be presented.

049
In vitro drug-induced cytotoxicity predicts clinical outcome in acute leukemia (AL)

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In this study we evaluated *in vitro* drug-induced cytotoxicity in 84 cases of AL, mainly at the onset of the disease, classified in 49%, 39% and 12% as acute non lymphoid leukemia (ANLL), acute lymphoblastic leukemia (ALL) and blast crises of chronic myeloid leukemia (BC-CML), respectively. Overall, 66% of cases achieved complete remission (CR), 13% a partial response (PR), while the remaining 21% showed no response to treatment schedules. The *in vitro* cytotoxicity test was assessed by the MTT assay and, after drawing the dose-response curve, the lethal dose $(LD)_{50}$ was calculated for 2-chlorodeoxyadenosine (2-CDA, LD50 39.7±5.7 sem), fludarabine (FAMP, LD_{50} 33.9±4.56 sem), mitoxantrone (Mitox, LD_{50} 1.61±0.15), idarubicin (IDA, LD_{50} 9.49±1.5 sem), daunorubicin (DNR, LD50 11.59±4.26) and aracytin (ARA-C, LD50 29.2±2.97 sem). Among the different AL subgroups, a statistical-

ly significant difference was observed only for the LD_{50} mean values of Mitox (ANLL 1.87±0.2 sem vs LLA 1.14±0.28 vs CB-CML 2.05±0.25, p=0.0028). Also, the LD_{50} of Mitox, FAMP and ARA-C predicted the quality of clinical response to any chemotherapeutic regimen. Finally, longer survival, calculated from the time of the MTT assay study, was associated with the lower LD_{50} of the same drugs. In conclusion, *in vitro* cytotoxicity assay can give overall information on drug resistance regardless of the correlation between *in vivo* and *in vitro* results obtained for each drug.

050
Acute myeloblastic leukemia in the elderly: analysis of outcome in 119 patients

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From January 1985 to December 1996, 119 elderly patients (mean age 70.2 years; range 60-86) with *de novo* AML were studied. FAB classification was: 7 M0, 15 M1, 19 M2, 14 M3, 45 M4, 13 M5 and 5 M6. Thirty-five patients (29.4%) with an older mean age (72.2 vs. 69.3: p=0.01), or with associated chronic diseases, reduced LVEF (41% vs. 66.5%: p=0.006) and impaired PS (Karnofski index ≤ 50: 51.4% vs. 22.6% p=0.004) were treated with palliative chemotherapy (Group 1). The remaining patients (84 = 70.6%) were enrolled into therapeutic protocols: 18 (15.1%) with standard dose Ara-C+DNB (Group 2), 51 (42.9%) with intermediate-dose Ara-C + mitoxantrone (Group 3) and 15 (12.6%) with standard dose Ara-C+IDA+ VP16 (Group 4). A significant difference in the mean doses of the agents delivered during the induction treatment was found, particularly in regard to Ara-C (6.0 gr for Group 3 vs. 0.698 for Group 2 and 0.726 for Group 4: p=0.000). Early mortality (within 10 days of admission) was higher in patients treated with palliative therapy (Group 1) (40% vs. 14.5%: p=0.0001). In this group no patient reached a complete remission (CR). The percentages of CR, resistance (NR), death in aplasia (DA), death during induction (DI) were:

Group (N)	*CR*	*NR*	*DA*	*DI*	
2 (18)	27.8	27.8	38.9	5.6	} (χ^2 6.14: p=0.407)
3 (51)	41.2	17.6	27.5	13.7	
4 (15)	46.7	33.3	20.0	0.0	

The overall survival was 2.5 months with only 6% of the patients surviving at 5 years. A difference in survival was found between patients treated with palliative therapy and those enrolled into protocols (0.6 months vs. 3.2 months : p=0.007). No difference was observed among the three protocol groups (p=0.56). The achievement of CR was a favorable prognostic factor (median survival CR 10.8 months vs. NR 0.6 months: p=0.000) even with a *landmark* placed at 45 days (CR 11.3 months vs. NR 5 months: p=0.01).

Intensive treatment prolongs survival because of the achievement of a significant number of CR. The dose intensification of Ara-C did not provide a significant increase in CR.

051
Role of fluorescent in situ hybridization in the detection of trisomy 8 in acute myeloid leukemia (AML)

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In order to analyze the role of fluorescence *in situ* hybridization (FISH) in the detection and monitoring of numerical chromosome changes in acute leukemia, 176 AML patients were assessed for the presence of +8, which is the most common abnormality in this neoplasia. Trisomy 8 was documented by conventional cytogenetic analysis (CCA) in 33 patients. Seventy-three cases presented complex karyotype without +8, 54 had a normal karyotype, 16 patients had inadequate mitotic yield. FISH confirmed the presence of +8 in all 33 cytogenetically-positive patients, having 10-88% interphase cells cells with 3 signals and detected 14 additional cases with a minor trisomic clone accounting for 4-22% of interphase cells.

Results of FISH analysis in the follow up of patients with +8 showed the following. Two patients with normal karyotype at diagnosis showed a minority of cells with +8 at relapse. In three patients with +8 at diagnosis the reduction of the percentage of trisomic cells was documented in a partial remission phase. In 2 cases in cytologic complete remission (CR), the persistence of 5% cells with +8 was documented, whereas in the remaining patients achieving CR, no residual trisomic cells were detected

We conclude that: a) FISH may be more sensitive than CCA in detecting +8 in AML, especially in those cases having a minority of abnormal cells; b) FISH may disclose cytogenetically-undetected +8 not only in karyotypically normal cases, but in patients with abnormal karyotype as well; c) the finding of persistent +8 in a minority of cells in CR may be important in clinical practice.

052
Results of a multicenter randomized clinical trial on platelet transfusion threshold in acute myeloid leukemia (AML)

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Design. PTTT (*Platelet Transfusion Trigger Trial*) is a prospective, randomized, multicenter clinical trial comparing the safety of a 10,000/mL versus the traditional 20,000/mL threshold for prophylactic platelet transfusion in AML. Two hundred and fifty five patients aged 15-70 with *de novo* AML (FAB M3 excluded) during first remission induction were randomized to be prophylactically transfused at platelet counts <10,000/mL (or 11,000-20,000/mL in case of fever >38°C or before invasive procedures) (group A, 135 cases) versus < 20,000/mL (group B, 120 cases). The two groups were comparable for age, sex, FAB classification, pretreatment blood counts and remission induction regimens. Data on platelet and red cell transfusion, infection and major bleeding (melena, hematemesis, hematuria, any bleeding requiring red cell transfusion, retinal bleeding with visual impairment, cerebral and fatal bleeding) were collected daily during a median observation period of 28 days.

Results. In group A, a median of 6 platelet transfusions per patient was given and 29 patients (21%) developed 36 (26.7%) major bleeding episodes. In group B, 8 median platelet transfusions per patients were administered and 24 patients (20%) had 33 (27.5%) major hemorrhagic events. One fatal cerebral bleeding occurred in group A when platelet count was 32,000/mL. Clinical and laboratory variables associated with major bleeding and laboratory variables associated with major bleeding were evaluated by multivariable stepwise logistic regression analysis. Statistical significance was taken at the p=0.05 level and the most relevant data are summarized in the table.

Variable	*Chi-square*	*p*
Randomization group (A vs. B)	<3.84	not significant
Actual plts count (continuous variable)	42.1	< 0.0001
Hemoglobin (>8 g/dL vs. <8 g/dL)	9. 2	< 0.01
Documented infection (YES vs. NO)	19.0435	< 0.0001
Fever (> 38°C vs. < 38°C)	<3.84	not significant

Conclusions. 1) The rate of major bleeding was similar in patients randomized to be transfused at the 10,000/mL or the conventional 20,000/mL threshold; 2) the more stringent regimen of prophylactic platelet transfusions allowed about 25% reduction of platelet use; 3) main risk factors for major bleeding in AML patients during first remission induction were persistent exposure to a low platelet count (independently from the prophylactic platelet transfusion policy) and the presence of a microbiologically or clinically documented infection.

053
Idarubicin in the treatment of AML: long-term results of a single center experience

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Between 1986 and 1992, 63 patients (37 male, 26 female; median age: 44 years) with acute myeloid leukemia (AML) were treated using a protocol that included idarubicin (IDA) during both induction and consolidation (*Semin Oncol 1993; 20(Suppl 8):27-33*). The patients in complete remission (CR) at the end of the consolidation phase underwent late intensification by means of autologous bone marrow transplantation (ABMT) preceded by myeloablative therapy or, in the case of those who were older than 50 years or who refused transplantation, received a 5-day course of high-dose ARA-C (HDAra-C). CR was achieved by 52 patients (83.87%), 45 (86.53%) after the first induction cycle; 16 of these patients underwent ABMT a median of 11 months after CR. As of the end of April 1997, after a follow-up of 50-127 months (median 107 months), 16 patients were still in CR (10 of whom had received ABMT and six late intensification with HDAra-C), and 29 patients had relapsed a median of 14 months after CR (range 2-75), including ten who relapsed more than two years after CR (two after ABMT and eight treated with HDAra-C). Four patients died of infectious complications after achieving CR. The median disease-free survival (DFS) of the patients as a whole was 25 months; 50-month and 10-year DFS were respectively 41% and 35.06%. No statistically significant differences were observed between the ABMT and HDAra-C treated patients who remained in CR for more than 11 months. Median DFS in the transplanted patients had not been reached after 120 months (actual DFS at 50 months and the probability of DFS at 120 months are both 66.8%). Univariate and multivariate statistical analyses indicate that age is the only variable capable of predicting leukemic relapse.

The long-term results obtained in this unselected patient population confirm the high degree of antileukemic efficacy of IDA which, in addition to allowing rapid CR in a significant percentage of patients, is also capable of favorably influencing the duration of DFS. Furthermore, our data seem to underline the efficacy of late intensification treatment (ABMT or

HDAra-C) at the end of the consolidation phase.The DFS curve of the patients undergoing ABMT is comparable with those published in the literature for allogeneic transplant patients. Furthermore, the DFS obtained in the patients receiving only HDAra-C intensification treatment was not significantly different from that of those who underwent ABMT. Both of these results are of particular interest given the relatively long period of follow-up.

054
In vitro induction of apoptosis by chemotherapeutic agents in human myeloid leukemia cells

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Chemotherapeutic treatment of acute leukemias relies on various alternative underlying mechanisms: cell killing, the induction of differentiation or apoptosis (programmed cell death). A number of anticancer drugs including etoposide (VP-16), cytosine arabinoside (ARA-C), mitoxantrone (MITOX), daunorubicin (DNR) and cisplatin have been shown to induce apoptosis in leukemic cell lines.

We investigated the *in vitro* induction of apoptosis by ARA-C, FLUDA (fludarabine), VP-16, MITOX, DNR and IDA (idarubicin) in the blast cells of 27 adult patients with previously untreated acute myeloid leukemia (3 M0, 2 M1, 12 M2, 3 M4, 4 M5, 2 M6 and 1 M7). Fresh mononuclear cells from the bone marrow and/or peripheral blood were exposed *in vitro* at 37°C for 24h to a wide range of concentrations of ARA-C (1000-0.1 μM), VP-16 (100-0.01 μM), FLUDA (100-0.01 μM), IDA (10-0.001 μM), DNR (10-0.001 μM) and MITOX (10-0.001 μM). Apoptotic activity was evaluated using three different methods: DNA gel electrophoresis, flow-cytometry and light microscopy. The percentage of apoptotic cells was drug concentration-dependent. The drugs most active in inducing internucleosomal fragmentation of DNA were FLUDA (58%), VP-16 (45%) and ARA-C (45%). Only in two of the 27 cases treated with MITOX was apoptosis observed at the highest concentration. DNR and IDA-treated cells did not show DNA fragmentation even at high concentrations. Our results confirm the ability of some drugs to induce apoptosis in vitro in fresh leukemic cells and demonstrate good correlation between the use of DNA electrophoresis, flow cytometry and light microscopy in detecting apoptosis. In 3 out of 13 (23%) evaluable patients a correlation was observed between *in vitro* induction of apoptosis and clinical response to chemotherapy. Further studies in a large number of patients are needed to evaluate the predictive role of *in vitro* drug-induced apoptosis in acute leukemias.

055
European intergroup trial for adult *bcr/abl*$^+$ acute lymphoblastic leukemia (ALL): preliminary results of the GIMEMA group

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In July 1994 the EORTC, French LALA and GIMEMA Group designed a prospective trial on BCR/ABL$^+$ adult ALL. The main aims of this Intergroup study were: 1) to compare duration of CR and DFS in these patients treated early either with allogeneic transplantation (familial or non familial) or autologous transplantation of selected PBSC or non selected PBSC when a HLA compatibile donor was not avalaible; 2) to evaluate the efficacy of IFN-α administered for one year after transplantation.

From August 1994 to March 1997, of the 223 adult ALL enrolled in the GIMEMA ALL0394 and 0496 trials, 42 (19%) pts (23 males, median age 37; range 16-64 yrs) were BCR/ABL rearranged. As of April 1997, 37 pts out of the 42 were evaluable: immunophenotype was pre-pre ALL in 7 cases, pre-B in 3, Common$^+$ in 23, T-ALL in 2 and 2 patients were byphenotypic. Cytogenetics were available in 26 cases: 16 were Ph1+ and 10 Ph1-, 11 were not evaluable. Centralized molecular study revealed 23 p190+, 11 p210+, 1 p210/p190+; in 2 cases the rearrangement was not defined. Out of 37, 27 (77%) achieved hematological CR, 6 were refractory, 2 died during induction and 2 were too early to be evaluated; at this time cytogenetics and molecular analysis were carried out in 15 and 21 cases respectively: only 1 pt. was Ph1+ (FISH), 9 were Ph1-, while PCR molecular CR was achieved in 4 cases, the rearrangement persisted in 16 cases, in one case the analysis is still in progress. Post-CR consolidation was given to 30 patients (28 arm HAM and 2 arm FLANG): 3 pts obtained CR post HAM therapy. Post-consolidation sPBSC harvest was performed in 13 cases: in 5 cases the CD34$^+$ harvests were BCR/ABL$^-$ in 7 BCR/ABL$^+$, one was not defined; the median value of CD34$^+$ harvested was 9.5×10^6/kg (range 1.8-30.6$\times10^6$/kg). At time of this analysis 27 pts were evaluable for post consolidation phase: 11 went off-study [early relapse (6), insufficient harvesting (1), toxicity (3), transplant refusal (1)], 16 underwent transplant [8 alloBMT, 8 autologous (6 sPBSC, 1 sPBSC + ABMT, 1 ABMT)]. Out of 8 alloBMT, 2 relapsed, 6 are in first CCR; of the 8 autotransplanted 2 relapsed, 2 died in CR and 4 are alive in first CCR. The median length of CR has been 8 months (range 1-41 months), overall median survival 13 months (range 1-41 months).

056
Adult acute lymphoblastic leukemia (ALL): > 5-year long-term survivors. A retrospective study

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The possibility that adult ALL patients can be long-term survivors and be considered cured may become a reality. This goal may be realized by more intensified therapeutic strategies based on biological characteristics at diagnosis. However, whether intensification of treatments improve disease outcome is still an open issue. This retrospective study concerns our >5 yr adult ALL long-term survivors, followed at our Department over 19 yrs. From 1972 to December 1991, of 298 consecutive adult (>15 yrs) ALL pts, 105 (35%) survived >5 yrs. At diagnosis median age was 24 (range 15-77), WBC count 8.2×10^9/L (range 1.1-380); 48 had L1 FAB cytotype , 42 L2, 15 were not evaluable.

As concerns immunophenotype, 55 had B-lineage ALL, 13 (12%) T-ALL, 3 hybrid (My^+), 3 biphenotype, 1 B Sig^+,13 null type, and 17 were not classified. Nine (8.3 %) were $Ph1^+$ and 2 were $t(4;11)^+$; none had CNS involvement. From 1972 to 1983 pts were treated with different conventional schedules; from 1983 pts were enrolled in the two consecutive national adult ALL trials, GIMEMA ALL 0183 (31 pts) and ALL 0288 (30 pts). Median follow-up from off-therapy was 4.76 yrs (0.41-19.22). Overall median 1st CR duration was 7.4 yrs (range 0.22-23.15), overall median survival 10.13 yrs (range 5.0-24.22). Forty pts had 1st relapse (35 hematologic) and achieved a 2nd CR, 15 underwent transplant, median 2nd CR duration has been 4.96 yrs (range 0.55-22.19). Twelve and six pts had a 2nd and 3rd relapse, respectively. As of December 1996, 63 (60%) were alive in 1st CR, the median length of which was 9.8 yrs (range 5-19.9); 3 of them were Ph1+ and 2 were $t(4;11)^+$. Three out of 63 underwent transplantation (2 BMT and 1 ABMT). During follow-up 6 females delivered healthy sons. A second neoplasia was recorded in 5 pts: 1 colon and 1 tongue carcinoma (both pts died), 2 ANLL and 1 cutaneous-NHL (pts alive and off-therapy) diagnosed 5, 6, 6, 7 and 14 yrs from ALL diagnosis, respectively.

In conclusion, this retrospective study confirms the favorable impact of traditional prognostic factors (initial median age and WBC count). Since intensification therapy, like transplantation, in 1st CR was applied in very few patients, it is possible that a wider use of these procedures in 1st CR, could increase the future cohort of cured patients.

057
Poor outcome of children with first isolated medullary relapse occurring five or more years after diagnosis of acute lymphoblastic leukemia. An AIEOP study

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The aim of this study was to investigate the clinical features and the treatment outcome of children with very late relapses (VLR) of acute lymphoblastic leukemia (ALL) and to provide some background data for an appropriate choice of a suitable strategy for treatment. Patients included in this study comprise all children who were reported by AIEOP (*Associazione Italiana di Ematologia ed Oncologia Pediatrica*) institutions to have relapsed, for the first time at least 60 months after onset of disease between November 1982 and May 1995. A total of 82 children (50 males, 61%) with a mean age at diagnosis of 5.7 years (SD:3.0; range: 1-14.8 years) who relapsed at least 60 months (range from 61 to 165; mean 78.7±17.6; median 72.3) after diagnosis were registered by AIEOP member institutions in the study period. The frequency of VLR among patients in CCR at 5 years from diagnosis was estimated to be approximately 4.5%. Median follow-up time of the 82 patients was 5.3 years. The 3-year survival (S.E.) and EFS (S.E.) were 70.2% (5.6%) and 51.6% (6.2%), respectively while the same figures at 5 years were 54.1% (6.9%) and 35.4% (6.7%), respectively. The site of first relapse and the WBC count at the time of first diagnosis, but not the duration of the first CCR, were important predictors of the duration of a second CCR in this series. Patients with isolated medullary relapse fared worse [3-yr EFS 34.2 (8.6)] than those with a combined relapse [3-yr EFS 73.3 (10.2)] or with an extramedullary relapse [3-yr EFS 81.2 (9.8); log rank p=0.003]. The overall good outcome for children who relapse after long-term remission might be related to the high incidence of combined or isolated extramedullary relapse for which intensive multiple-drug chemotherapy plus local therapy is an effective treatment. The same therapeutic strategy is not effective for children with isolated medullary relapse, for whom the frequency of EFS is surprisingly low after adequate long-term follow-up.

058
Immunophenotypic findings in acute myeloid leukemia (AML) with t(8;21)

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The t(8;21) identifies a subgroup of AML with distinct, morphologic, molecular and clinic characteristics. In particular, a strong association with FAB subtype M2 and the constant presence of AML1/ETO hybrid gene have been reported. This gene, differently from other hybrid fusion genes, is commonly detectable also in patients in durable complete remission (CR) and virtually cured. So the clinical utility of molecular monitoring in AML with t (8;21) is poor. The aim of this study was to investigate whether, following an extensive immunophenotypic evaluation including conventional surface antigens as well as adhesion molecules (β1 and β2 integrins, cytokine receptors (c-kit/CD117) and surface enzymes (CD45RA/R0), it is possible to identify a surface antigen mosaic specific for AML with t(8;21). Fifty-three patients were investigated, all diagnosed as having AML. Nine showed the t(8;21), 16 cytogenetic abnormalities mostly (12/16) involving chromosomes 8 and 21 but not t(8;21), and 28 had normal karyotypes. Results were evaluated in terms of percentage of positive cells following cytofluorometric analysis. Statistical significance was assessed by the Mann-Whitney test. The group of AML with t(8;21) expressed significantly higher percentage of CD34, CD19 and CD45RA when compared to either patients with normal karyotype or to those with other abnormal cytogenetic findings, while CD56 and CD54 were significantly more expressed only in comparison to the group of patients with normal karyotype. In contrast, the subset of AML with t(8;21) showed a significantly lower expression of CD14, CD11b, CD36 and Cd45R0 as compared to both control groups, while the expression of CD11c and CD33 was significantly lower when compared to the subset with normal karyotype. The analysis of our data demonstrates that AML with t(8;21) displays a distinct immunophenotype characterized by: a) high expression of CD34, CD45RA, CD19. CD56 and CD54; b) constant absence of monocyte-restricted markers such as CD14, CD11b and CD36; c) low expression of CD33 and CD11c. Because molecular analysis in AML with t(8;21) is unreliable in monitoring the minimal residual disease, an immunophenotypic evaluation based on the antigenic mosaic proposed in this study, could be of substantial clinical utility for patients in CR.

059
11q23 rearrangements and acute leukemias: cytogenetic and molecular analysis of 19 cases

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A variety of recurrent chromosomal rearrangements involving band 11q23 has been reported in hematologic malignancies. They are characterized by an extreme heterogeneity, both with regard to the large number of partner chromosomes and in terms of involvement in a variety of acute leukemias (AL). Among the most common 11q23 abnormalities, the t(4;11) is the cytogenetic hallmark of a distinct subset of patients with well defined biological and clinical features and dismal prognosis, while rearrangements such as t(9;11), t(6;11), t(10;11), t(11;19) and del(11)(q23) appear less strongly associated with specific disease entities and their prognostic significance is not yet clear.

Table 1. Biological and clinical features of patients with a given 11q23 rearrangement.

Chromos. aberrations	t(4;11)	t(9;11)	t(10;11)	t(11;19)	del(11)(q23)	others	
No. of patients	3	5	2	1	4	4	
MLL rearrangements	3	5	0	1	1	1	
Add. aberration	no	3	no	no	no	2	
Age	25 (22-34)	16 (20-60)	20 and 25	30	20 (11-66)	34 (2-66)	
Sex	3F	3M, 2F	2F	1F	3M, 1F	3M, 1F	
W.C.C.	270	16.8	78 and 50	9	28	6.4	
($x10^9$L)	(165-290)	(3.5-340)		(3-183)	(4.6-18.6)		
Cytology	3 Pro-B	3M5s 2m4	M5s , Bifen.s	M4	2M4 , 1M2	M2, M4	1 common M7,RAEB-t
Complete remission	3/3	4/5	1/2	1/1	2/4	1/4	
Survival (months)	10, 15, 19	1, 8, 12, 34+, 3+	4, 40+ (TMO)	13 (TMO)	26, 7, 3+, 88+	7, 13, 7, 3	
Deaths	3/3	3/5	1/2	1/1	2/4	4/4	

S=secondary AL.

Between 1990 and 1997 we identified 19 patients with rearrangements involving band 11q23 by using conventional cytogenetics (CC), FISH and molecular analysis of MLL gene. There were 11 females and 8 males, with a diagnosis of AL (13 AML, 4 ALL, 1 biphenotypic) or myelodysplastic syndrome (MDS); median age was 25 years (range 2-66). Patients were subdivided according to the chromosomal abnormality; their clinical and biological characteristics are indicated in Table 1 (previous page).

In our cases the 11q23 rearrangements were predominantly detected in young patients (median age 25 years). The t(4;11) characterized a subgroup of patients with hyperleukocytosis and a pro-B cell immunophenotype, whereas an M4-M5 phenotype was prevalent in both *de novo* and secondary AL carrying the t(9;11), t(10;11) and t(11,19). A high percentage (82%) of MLL rearrangements was detected in all these subgroups of patients (9/11 cases). In the *del(11)* or *other* less common changes the distribution of the cytologic phenotypes appeared to be much more heterogeneous. Moreover, in these latter groups of patients the MLL gene was rearranged only in a low percentage of cases (25%). Finally, 11q23 rearrangement was detected as an isolated abnormality in most patients (14/19); it was associated with an adverse disease outcome irrespectively of the detected karyotypic abnormality, 14 out of 19 (74%) patients had died by a median follow-up time of 10 months.

060
Treatment of relapsed acute myeloid leukemia (AML): a retrospective analysis of ten years experience at "La Sapienza" University, Rome

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From 1982 to 1992, 66 first relapse acute myeloid leukemia patients (median age 36.7 years, M/F 36/30) treated with the standard association DNR+ Ara-C (3+7) as induction therapy, received a second line therapy. Eleven pts. relapsed after autologous transplant (ABMT) in CR1; 2 pts. after allogenic transplant (BMT) in CR1; 53 pts. after consolidation chemotherapy. Median duration of CR1 was 9.9 months (range 1.1÷48.9); in 15 pts. CR1 lasted <6 months. Various reinduction therapies were employed. None out of 9 pts. achieved CR2 with the association Ara-C (1 g/m 2 3 hours i.v. days 1-4 and 8-10) + asparaginase (6000 U/m 2 days 5, 11); 2/2 pts. achieved CR2 with bisantrene (250 mg /m^2 1 hour i.v, days 1-7); 12/16 pts. achieved CR2 with the association Ara-C (1g /m^2 6 hours i.v. days 1-6) + mitoxantrone (6 mg/m^2 days 1-6) either amsacrine (150 mg/m^2 days 4-6) or idarubicin (6 mg/m^2 days 1-5); 23/34 pts. achieved CR2 with the MEC schedule: Ara-C (1 g/m^2 6 hours i.v. days 1-6) + etoposide (80 mg/m^2 days 1-6) + mitoxantrone (6 mg/m^2 days 1-6). Three pts. underwent a double ABMT with bone marrow harvested in CR1 (BAVC conditioning regimen) and in CR2 (CTX + TBI conditioning regimen). Overall 41/66 pts. (62%) achieved CR2, 8/66 pts (12%) died early during reinduction and 17/66 pts (26%) were refractory. Early compared to late relapsing pts. did significantly less well (refractory disease 60% and 15.6%, respectively).

Twenty-two out of 41 pts.(53%) received a bone marrow transplant (21 ABMT and 1 BMT) in post-remission phase; 2 pts died in CR2 during ABMT; 30 pts had a second relapse; 9 pts are still alive in CR2 (1 after BMT, 8 after ABMT). Median survival and DFS were, respectively, 9 and 10 months with 18% pts. still alive at 5 years. In particular pts receiving ABMT in CR2 had a median DFS of 18 months with 39% pts still alive at 5 years. As for reinduction results (62% CR2) our data compare favorably with those in the literature, in particular, the MEC schedule seems to be associated with the best results with moderate toxicity and good tolerability. Finally, taking into account that all pts receiving consolidation chemotherapy relapsed, a bone marrow transplantation procedure represents the only therapeutic option for long lasting CR2.

061
The role of a hematological emergency unit (HEU) in the management of patients with acute leukemia

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At the *Department of Cellular Biotechnologies and Hematology* in Rome a recently rebuilt hematological emergency unit (HEU) is active and operative 24 hour-a-day for emergencies occurring in patients with hematologic diseases. Between March 31, 1996 and March 31, 1997 we saw 402 patients with acute leukemia in various phases of their disease: 236 had acute myeloid leukemia (AML) and 176 had acute lymphoblastic leukemia (ALL). No admission to the ward was needed in 212 (52.7%) cases: in particular, 81 had febrile episodes (35 ALL, 46 AML), 18 had hemorrhagic complications (6 ALL, 12 AML) and 103 had other medical problems. One hundred and ninety (47.3%) patients needed admission to the ward: in detail, 91 for infective complications (25 ALL, 66 AML), 19 for hemorrhagic episodes (5 LAL, 14 AML), 38 for other medical problems (27 ALL, 11 AML); 42 patients were admitted at the onset of

the disease (11 ALL, 31 AML). 174/190 (92%) admitted patients were hospitalized in the HEU ward, 10 in one of the other hematologic wards of the same institute and 6 were referred to other hospitals.

Of the 174 HEU hospitalized patients, 100 were discharged from the HEU ward after a median time of hospitalization of 2.5 days, 65 were transferred to other hematologic wards of the Institute and 9 were transferred to other hospitals. These data demonstrate the important role of a HEU in the management of the various and sometimes life-threatening complications occurring in AL patients which are difficult to sort out in a general emergency unit. The availability of a HEU can shorten the time of hospitalization needed for intensive chemotherapeutic programs (for example, post-remission treatments), thus reducing costs for the National Health System, allowing more patients to be treated and ameliorating their quality of life.

062
Marrow leukemic index (MLI) on the 14th day of treatment for acute myeloid leukemia

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The identification of prognostic factors is gaining increasing importance in the treatment of acute leukemias (AL). In acute myeloid leukemias (AML) these factors include both patient features (age, sex, performance status, presence of fever or infections) and biological aspects (cytotype, cytogenetic and molecular markers, number of blast cells, extramedullary localization of disease). Some authors have recently underlined how speed and value of clearance of blasts in the bone marrow could be predictive of the response to chemotherapy. We aimed to verify the predictivity of the *Marrow Leukemic Index* (MLI=N. of blasts × cellularity on the 14th day/no. of blasts × cellularity at onset × 100)(*Leukemia 1996; 10*) in young patients with AML. One hundred and four pts. aged 16-59 years (median 45 years) with *de novo* AML in the first cycle of treatment were evaluated. Five pts. had M0, 11 had M1, 48 had M2, 18 had M4 and 22 had M5. Sixty-eight pts were treated according to protocol GIMEMA AML8 A/B (DNR 45 mg/m^2 × 3 consecutive days, ARA-C 200 mg/m^2 for 7 consecutive days c.i.); 36 pts. were treated according to protocol GIMEMA-EORTC AML 10 (ARA-C 100 mg/m^2 for 10 consecutive days, VP16 100 mg/m^2 for 5 consecutive days, and a randomized anthracycline: IDA 10 mg/m^2, MITOX 12 mg/m^2 or DNR 50 mg/m^2 for 3 alternate days). Fourteen days after beginning chemotherapy we examined the bone marrow and calculated the MLI. The response to therapy was evaluated on the 28th day or at recovery of peripheral hematologic parameters (WBC, Plts, Hb). Sixty pts (57.6%) achieved complete remission (CR), 14 pts (13.6%) partial remission (PR), whereas 30 pts (28.8%) were non responders (NR). The median value of MLI was 2% (range 0-22) in the group of CR pts, 11% (range 1-36) in PR pts (p=0.0003) and 27% (range 1-132) in NR pts (p=0.0005). Among NR pts, 7 had MLI < 10% on the 14th day but the leukemic cells increased later (within 20 days). These pts were not different from the others as regards age, infection or medullary fibrosis; one had M1, one M2, two M4 and three M5. Among CR pts. only one had MLI >20% on day 14; he achieved CR with a blast clearance on day 28 (OS=14 mths, DFS=13 months); 87% of pts. who had MLI <10% achieved CR. 82% of pts. who had MLI >20% were NR (p=0). The MLI at the 14th day of induction therapy could be used to identify young pts. with AML who may achieve rapid complete remission.

LYMPHOMAS

063
Hepatitis G virus (HGV) prevalence in patients with lymphoproliferative disorders (LPD)

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A correlation between hepatitis C virus (HCV) infection and LPD has recently been reported by our selves and others in Italy (*Am J Hematol 1997; 55:77*). A new hepatitis virus has recently been identified in patients suffering from non A-E hepatitis, termed HGV. This virus has structural and biochemical characteristics similar to those of HCV, including tropism for lymphoid tissues. We have studied HGV prevalence in patients with LPD. HGV-RNA was investigated by nested PCR in the serum of 129 unselected and untreated patients with LPD (47 with NHL, 46 with HD, 10 with Waldenström's macroglobulinemia, 26 with multiple myeloma or MGUS). Serum viral RNA was detected in 6 patients with NHL, in 7 with HD and in 2 with multiple myeloma, showing an overall prevalence of 11.6%. No patient had clinical or laboratory signs of active liver disease. No HGV positive patient had serum HCV-Ab or HCV-RNA. HGV prevalence in patients with LPD is higher than in the general population, which is estimated to be 1-2%, and lower than the prevalence of

HCV in LPD patient from the same area, which we found to be 20-30%. Significantly increased HGV prevalence in LDP patients and absence of co-infection with HCV suggests that even HGV may have a role in lymphomagenesis. Wider studies are needed to confirm these preliminary results.

064
Epidemiology of malignant lymphomas in Sardinia, 1974-1993

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All cases of malignant lymphomas (ML) newly diagnosed in the 20 years from 1974 to 1993 were collected from all pathology institutions of the island of Sardinia. The island's population was 1,594,175 residentb inhabitants according to the 1981 and 1,648,248 according to the 1991 census, with a similar male to female ratio but with a decrease of about 2.5% in age class 0-14 in the 1991 census. In all cases age, sex, residence, histologic diagnosis were taken into consideration; the incidence is given for each of two decades.

Hodgkin's disease (HD): mean incidence per year was 34 cases (range 20-49, median 34) in the period 1974-1983 and 44 cases (range 36-55, median 44) in the period 1984-1993. Age-adjusted incidence rate ($\times 10^5 \times$ year) was 2.6 for males and 1.8 for females in the first ten years of our survey and 2.9 for males and 2.2 for females in the second decade.

Non Hodgkin's lymphomas (NHL): mean incidence per year was 63 cases (range 49-80, median 62) in the period 1974-1983 and 124 cases (range 91-161, median 121) in the period 1984-1993. Age adjusted incidence rate ($\times 10^5 \times$ year) was 5.1 for males and 3.7 for females in the first ten years of our survey and 8.6 for males and 6.4 for females in the second decade.

The increase was evident for age groups over 14 years in females and over 24 years in males and was similar in the other age groups and in both sexes. NHL-HIV+ cases accounted for about 1% in period 1984-1993. The almost stable incidence of HD indicates that effects of potential artifacts (such as improvement in diagnostic evaluation or in population access to medical facilities etc.) are likely to have had a small influence on the observed increase in incidence of NHL.

These data demonstrate that also in the Sardinian population there is an evident increase in the incidence of NHL, as previously observed in the USA and in other countries. The incidence rate in Sardinia still remains lower than that observed in USA, perhaps due to different exposures to risk factors or to different characteristics of the Sardinian population.

065
LL01: a GISL protocol for the treatment of lymphocytic lymphoma and B-cell related leukemias

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In July 1993 GISL proposed a protocol for the study and treatment of low-grade non-Hodgkin lymphomas of extra-follicular origin and related leukemias. This randomized study involves patients with histologic or immunocytomorphological diagnosis (on blood and/or bone marrow) of lymphocytic lymphoma, lymphoplasmocytic lymphoma/immunocytoma, B-monocytoid/marginal zone lymphoma, splenic lymphoma with villous lymphocytes and nodular pattern mantle cell lymphoma. Patients with classic CLL are inelegible. Treatment is given to pts in stage III, IV and II with more than 3 involved sites, and those with *active* leukemia defined as the presence of at least one of the following: B symptoms, bulk, anemia, thrombocytopenia, lymphocyte doubling time count < 12 months or a volumetric increase in at least 3 nodal sites. Group A receives HDChl-P, chlorambucil 15 mg/m^2/day and prednisone 100 mg/day p.o. for 5 days every 28 days; group B receives HDChl-PE, HDChl-P + epirubicin 60 mg/m^2 i.v. on day 1. In the case of CR or PR after 3 cycles, the treatment is continued for a further 5 cycles; in the case of SD or PD, the pts on HDChl-P are switched to CEOP and those on HDChl-PE to FAMP. At the end of 8 cycles, the pts are randomised to aIFN maintenance treatment vs observation. The aims of the study are to evaluate: a) the validity of the proposed criteria for defining indolent disease; b) the effect of epirubicin on therapeutic response; c) the effect of αIFN on response duration; d) the efficacy of FAMP as second-line treatment; e) the behavior of pts with a histologic vs immunocytomorphological diagnosis. As of March 30, 1997, 102 pts (mean age 60.2 yrs, M:F ratio 1,22) had been enrolled: 81 with active and 21 with indolent disease. The diagnosis was histologic in 79 and cytological in 33 pts. More than 90% of the 81 pts with active disease were in stage IV; B symptoms were present in 12 pts and increased serum LDH levels in 32. The median follow up is now 19 months (2-42). Three out of 21 pts with indolent disease have required treatment. In the 58 evaluable pts, induction therapy led to 14 CR (24.1%), 24 PR (41.4%), 16 SD/PD (27.6%), 3 deaths and 1 with-

drawal of consent (6.9%). Four out of 6 pts with PD presented histologic transformation to high-grade NHL. There do not seem to be any differences relating to the type of theraphy (CR+PR in 30 group A vs 28 group B pts: 66.7% vs 64%, with more CR in group A) or the type of diagnosis. No particularly severe toxicity, as designed by WHO, was observed.

066
Preliminary data of a randomized pluricentric study (ABVD + RT EF versus IF) in intermediate stage Hodgkin's disease (HD)

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The patients enrolled in this trial were randomized after 4 courses of ABVD to receive radiotherapy *extended field* (EF) versus *involved field* (IF) (30 Gy + 10 Gy bulky + 36 Gy spleen). The risk factors were: bulky disease, spleen involvement, extranodal disease, 3 or more lymph node regions involved, if A ESR ≥ 50 mm/h or if B ESR ≥ 30 mm/h. With this prospective trial we wanted to evaluate IF and EF irradiation administered at the same dose after an effective but not very toxic ABVD chemotherapy. The trial aims are to evaluate the following: 1) the complete remission rate after 4 courses of ABVD; 2) whether there are significant differences between the two arms; 3) any reduction of toxicity from reducing the dose of RT after effective chemotherapy. The main endpoint is EFS from the starting of RT; other endpoints are RFS and overall survival.

From July 1993 to date 172 HD patients in stages I, II with risk factors and IIIA have been enrolled in this trial. Twenty patients are not evaluable since the follow-up, after treatment discontinuation, is < 12 months; thus 152 patients were evaluable and analyzed. Of these 152 patients 97 were females and 55 males, ages ranged from 15 to 75 years. A complete remission was achieved in all patients except one who is alive with disease. During follow-up, in the EF arm, one patient relapsed 15 months after complete remission; while in IF arm one patient developed LNH 10 months later. Both patients died. One patient died in CR in an accident.

No differences in terms of response duration, hematologic and non hematologic toxicity were recorded. Only two patients in the IF arm, had cardiac failure. Although the follow-up is so far too brief (minimum 1 year), the preliminary results are similar in both arms.

067
Failure of HDS regimen in the management of high grade non-Hodgkin's lymphoma with bone marrow involvement or T-cell or CD30 immunophenotype

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The high-dose sequential (HDS) chemotherapy program has recently been proposed as an alternative to third generation regimens in the treatment of diffuse large cell (DLC) non-Hodgkin's lymphomas (NHL). So far, HDS has been mainly employed in patients with DLC of B cell origin and no bone marrow (BM) involvement; in this subset, a recent randomized study showed HDS superiority compared to MACOP-B in terms of both CR rate and FFS (*Gianni et al., N Engl J Med 1997; 336:1290*). Considering these encouraging results we wanted to verify HDS efficacy in other DLC categories, including patients with bone marrow involvement as well as those with T-cell or CD30 immunophenotype. In the last 3 years 17 patients (median age: 41 yrs., range 26-58) presenting with such characteristics were treated at our Institution with an HDS-approach (study group). There were 8 patients with B-cell DLC and BM invasion, 5 had $CD30^+$ and 4 T-cell NHL. All patients had stage III-IV and/or bulky disease; 16 had disease-related symptoms, 12 had elevated LDH, 10 had poor performance status (WHO 3-4); 3 of the 5 $CD30^+$ patients had both marrow and skin involvement. The original HDS was slightly modified, by postponing PBPC harvest at the end of the high-dose phase and prolonging the initial APO-phase. In the same period, 16 consecutive patients with B-cell DLC and no BM involvement received HDS (control group); their clinical characteristics were well matched with the study group. CR was achieved in 6 patients (35%) of the study group and in 15 (93%) patients of the control group (p=0.08). The low CR rate had an unfavourable impact on the FFS and OS curves, projected at 18 and 28%, respectively. Only 3 patients (1 with BM involvement, 1 high-grade T-cell NHL with cutaneous involvement, 1 with $CD30^+$ NHL and no extranodal involvement) are long-term survivors in CCR. Compared to the study group, the outcome of the control group was significantly better with FFS and OS projected at 73 and 76%, respectively. Results in B-cell DLC without BM involvement were almost identical to those of the recently reported randomized study. We conclude that HDS is highly effective in a specific category of DLC-NHL; its use in subgroups of aggressive lymphoma other than B-

cell+/BM-ve should not be recommended, unless appropriate modifications are introduced both in the high-dose phase and in the autograft procedure.

068
HDS regimen in low/intermediate grade non-Hodgkin's lymphoma other than follicular subtypes

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The high-dose sequential (HDS) chemotherapy regimen has been proposed as a major progress in the treatment of high-grade non-Hodgkin's lymphoma (NHL). Moreover, our experience with follicular lymphomas treated with an HDS-like program gave promising results (*Corradini et al., Blood 1997*). Based on these premises we wanted to verify the applicability of HDS to other subsets of low-intermediate grade NHL. So far, 25 patients (median age 50 yrs., range 26-62) have entered the study protocol. Nine patients had mantle cell and 5 T-AILD histology; the remaining 11 patients had grade A histology, according to Working Formulation: 2 had a monocytoid form while 9 had lymphocytic lymphoma/CLL. All patients presented with advanced stage disease; among grade A patients, 4 had hyperleukocytosis and 4 had a bulky mass. The scheme employed was substantially the same as the one we used in follicular NHL; in particular: i) a prolonged debulking with 2 full-dose APO and 2 DHAP courses was introduced; ii) cytoxan at 7 gr/sqm and PBPC harvest were postponed at the end of the high-dose phase; iii) mitoxantrone (60 mg/sqm) + L-PAM (180 mg/sqm) were employed as conditioning regimen before autograft. There was one toxic death following the initial APO course, due to intracranial hemorrage in a patient who developed DVT and received warfarin; 5 more patients experienced moderate to severe complications (3 pneumonitis, 1 pneumocystis carinii infection, 1 peripheral neuropathy). Among evaluable patients, 7 did not go through the final autograft (2 due to patient refusal, 4 due to marrow residual disease, 1 because aged over 60). Overall, 19 patients (76%) reached CR following HDS; 2 more patients with persistent marrow disease attained CR after allograft from an HLA identical sibling. The OS and EFS curves project 85% and 58% at 7 years. Eight out of 9 patients with mantle cell, 5 out 5 patients with T-AILD and 10 out 11 patients with grade A lymphoma are currently alive at a median follow-up of 2 to 3.2 years. In conclusion, HDS proved to be feasible and highly effective in low/intermediate grade lymphomas well known for their low chemosensitivity. Marrow disease persistence remains the major obstacle to overcome and future improvements should be specifically aimed at eliminating or reducing it at a molecular detection level.

069
VACOP-B vs VACOP-B + autologous BM transplantation (ABMT) for aggressive non-Hodgkin's lymphoma. A study from the *Non-Hodgkin's Lymphoma Cooperative Study Group* (NHLCSG)

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In 1992, the results coming from studies using conventional chemotherapy (CT) and ABMT procedure for ag-gressive NHL, allowed us to draw some conclusions: a) second and third-generation regimens do not improve the per-centage of CR, survival or DFS in aggressive NHL; b) a series of negative prognostic factors present at diagnosis (I.I.) can reduce survival; c) the definite role played by ABMT in the treatment of NHL is still unclear and results of randomized studies are necessary. In consideration of these observations, we divided a series of consecutive patients into 3 groups according to stage, age and negative prognostic factors at diagnosis. From 1992 to 1995, 205 new patients (Groups F-G-H-K/WF), aged 15 to 59 years, entered trials A, B and C, and an analysis re-ferred to study B is now presented. The study included 124 patients in stage II-III plus one or more negative factors at diagnosis and stage IV. Patients were randomized to receive VACOP-B (and possible 2nd line therapy in the case that CR was not attained) (CT arm) or VACOP-B plus ABMT (ABMT arm). The endpoint was to evaluate the effectiveness of high-dose therapy in increasing survival. Sixty-one pts and 63 pts entered the CT arm and ABMT arm, respectively. After VACOP-B the re-sponse was similar in both arms. The addition of radiotherapy or a 2nd-line treatment in the CT arm increased the percentage of CR by 18%. The addition of the ABMT procedure in the ABMT arm increased the percentage of CR by 29%. The conclusive, overall response was similar in both arms. Actuarial survival (69% vs 51%; p=0.9), DFS and progression-free survival curves at three years are similar, showing no advantage in the use of aggressive therapy. The major problem of this study was its feasibility. Seventeen out of 63 pts (27%) of the ABMT arm did not undergo the procedure because of early or late progression, early death, toxicity or refusal. This study failed to prove the definite role played by ABMT in

these patients: therefore a new approach with a different rationale is needed.

070
Intensified chemotherapy with dose intensity escalation of cyclophosphamide and epirubicin with filgrastim support (megaCEOP) for poor prognosis aggressive non-Hodgkin's lymphoma

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The dose intensity (mg/mq/week) (DI) of chemotherapy may correlate with tumor response and outcome in non-Hodgkin's lymphoma. However, data are not conclusive yet. It is possible that only a significant escalation of DI may offer a real advantage. Therefore a phase I trial was designed to determine the maximum tolerated dose intensity (MTD) of cyclophosphamide (CTX) and epirubicin (EPI) in the CEOP regimen (standard doses: CTX 750 mg/sqm + EPI 65 mg/sqm + VCR 1.4 mg/sqm + PDN 40 mg/mq every 21 days) as outpatient regimen with filgrastim support per 6-8 courses. The MTD was defined as the DI that determines a grade 4 (WHO) hematologic toxicity in 25% of the courses. Patients were treated according to three DI escalation steps mantaining standard VCR and PDN doses: first level CTX 1000 mg/sqm + EPI 100 mg/smq + filgrastim 5 ug/kg (10 days) every 15 days (DI CTX 200%, DI EPI 230%); second level CTX 1100 mg/sqm + EPI 100 mg/sqm + filgrastim (10 days) (DI CTX 220%, DI EPI 230%); third level CTX 1200 mg/smq + EPI 110 mg/sqm + filgrastim (10 days) (DI CTX 240%, DI EPI 250%). Eighteen patients entered the study: median age was 50 yrs (range 22-63), 7 were males and 11 females; 15 with diffuse large cell and 3 with large cell follicular lymphoma; 4 pts with stage II, 3 with stage III, 11 stage IV; 7 pts with >1 extranodal sites and 5 with bone marrow involvement at diagnosis. Thirtynine percent were at intermediate risk and 65% at intermediate-high or high risk according to the *International Prognostic Index* criteria. Three pts were treated at the first level (18 cycles) with grade 4 neutropenia in 5% of courses, 3 at the second (18 cycles) with severe neutropenia in 5% and 12 pts at third (80 cycles) with 25% WHO grade 4 neutropenia. Red blood cell transfusions were used in 11% of courses at the first level, 0% at the second and 16% at the third; no platelet transfusions were required. WHO grade 1-2 infections were registered in only 3 patients, grade 1 mucositis in 7 and gastrointestinal grade 1-2 toxicity in 7. No severe extrahematological toxicity occurred. Fourteen patients (78%) obtained a complete remission; four relapsed with a median follow-up of 29 months. In conclusion MegaCEOP regimen allows safe escalation of the DI of CTX (240%) and EPI (250%) with filgrastim support. Third level dose is feasible in an outpatient setting and induces a high CR rate, without significant increase in toxicity. Its efficacy needs to be tested in larger series.

071
Human T-cell lymphotropic virus-I TAX is never detected in the skin and peripheral blood mononuclear cells of patients with cutaneous T-cell lymphoma

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Most investigators suggest that cutaneous T cell lymphomas (CTCLs), mycosis fungoides (MF) and its leukemic variant called Sézary syndrome (SS) are caused by the human T lymphotropic viruses (HTLV-I/II); however this association has been the matter of several contradictory reports. For example, Pancake *et al.*[1] conclude that MF/SS is an HTLV-associated disease, while Bazarbachi *et al.*[2] never reported the presence of HTLV-I proviral sequences. Like other retroviruses, HTLV-I has *gag*, *pol*, *env* and long terminal repeat sequences (LTR). In a unique region between env and 3'-LTR sequences, referred to as the Px region two genes have now been identified: the HTLV-I/II tax gene, which encodes proteins of 40 and 37 Kd, and the rex gene which encodes proteins of 27/21 Kd for HTLV-I and 26/24 for HTLV-II. In particular tax can transactivate the transcription of various viral and cellular genes and may be implicated in leukemogenesis and tumor progression.

Our group studied 34 patients aged from 34 to 81 years with CTCL (33 MF, 1 SS) in various stages: 24 were classified as stage I (10 IA and 14 IB), 8 as stage II (5 IIA and 3 IIB), one patient as stage IIIA; one patient had a SS. None of the patients had typical risk factors or came from areas with high prevalence of HTLV-I infections and only one patient had received blood products prior to the diagnosis of MF. Plasma samples from all our patients are routinally tested for HTLV-I/II antibodies using ELISA and Western blot assays obtained from Medical System and Genelabs

Diagnostics. The prototypic HTLV-I cell line MT-2 was used as positive control for PCR analyses. PBMC from 15 randomly chosen healthy adult volunteers and from 10 patients with lymphomas of T and B phenotypes were collected and processed by the same procedures and in parallel with specimens of MF patients. DNA extracted from 1×10^5 cells (PBMNCs and bone marrow) was used as a template to amplify with PCR, 233 pb between sites 7324 and 7556 in the pX region. Analysis of serum antibodies by ELISA was negative. A small percentage of patients (4/34, 11.7%) showed reactivity to gp21 proteins by WB. PCR amplification was negative for all DNA samples. In order to exclude that this negative result could be correlated with low levels of virus due to the low number of circulating atypical cells, we tested the HTLV-I/II tax gene in DNA extracted directly from skin biopsy specimens. Our study revealed that none of MF/SS patients harbor HTLV-I/II tax in DNA derived from paraffin-embedded or frozen skin sections. In conclusion, we tried to reveal proviral sequences in early stages of MF but on the basis of our negative results it seems reasonable to exclude a causal role of HTLV-I infection in CTCL. The detection of proviral DNA sequences in more advanced MF might represent a secondary phenomenon due to CTCL-associated immunosoppression or might be related to concomitant risk factors for HTLV-I infections.

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072
P-VABEC chemotherapy for elderly aggressive non Hodgkin's lymphoma patients: long term results of 122 patients treated at a single institution

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Purpose. To evaluate the long-term results of the P-VABEC regimen for elderly patients (pts) with aggressive non Hodgkin's lymphoma (NHL).

Patients and Methods. From October 1988 to April 1995, 122 previously untreated aggressive NHL (F-G-H-J according to Working Formulation, stage II-IV) pts older than 60 years of age were treated at our institution with the standard (92 pts) or increased (30 pts) P-VABEC regimen. The schedule consisted of doxorubicin (30 or 40 mg/m^2), etoposide (100 or 150 mg/m^2) and cyclophosphamide (350 mg/m^2) alternated weekly with vincristine (1.2 mg/m^2) and bleomycin (5 U/m^2 or 15 U t.d.) for a total of 8 courses. Oral prednisone was administered daily during the entire treatment period. From October 1988 to December 1990 60 pts were treated with the standard dose schedule. Subsequently, from February 1991 to October 1992, 30 pts were treated with the increased dose schedule. From January 1994 to April 1995 another 32 consecutive pts were again treated with the standard dose schedule. All pts were treated on an outpatient basis without the use of growth factors.

Results. The results are depicted in Table 1 (below).

Table 1.

Regimen	*n° pts*	*% CR*	*OS (5yrs)*	*EFS (5 yrs)*	*DFS (5yrs)*	*toxic deaths (%)#*	*un related deaths (%)°*
P-VABEC (1)	60	75	48	44	53	3.3	11.6
P-VABEC (2)	30	56	32	19	27	16.6	0
P-VABEC (1)	32	69	53*	44*	46*	0	0
Total	122	67	34	34	45	5.7	5.7

*1=standard dose; 2=increased dose; * calculated at 2 yrs; #all pts who died during treatment and within three months of completion of treatment; °all pts in CR who died later of causes unrelated to lymphoma or its treatment.*

Conclusions. The P-VABEC is an active and well-tolerated chemotherapy regimen when used at standard dose, whereas the increased dosage mainly increases the toxic death rate without an improvement of the efficacy. Since October 1995 an Italian multicenter prospective randomized study has been in progress. The aim of this study is to evaluate the efficacy of consolidation chemotherapy after a standard dose P-VABEC regimen in reducing the relapse-rate in elderly pts aggressive NHL.

073
Diffuse large cell lymphoma (DLCL) with bone marrow (BM) involvement: intensified chemotherapy with autologous stem cell transplantation, comparison with standard chemotherapy

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A subset of DLCL patietns have bone marrow involvement (10-15%) and they usually have a poor outcome if treated with standard chemotherapy. From January 1992 through to August 1994, 19 patients with DLCL and BM involvement were treated with a new intensified scheme with autologous stem cell transplantation (ASCT). This scheme included 3 phases: induction with 8 weeks of MACOPB; intensification with two courses of Mitoxantrone 8 mg/m^2 + HDARAC 2 g/m^2/12 h + dexamethasone 4 mg/m^2/12h for 3 days (MAD) followed by GCSF 5 µg/kg dd 4-17 with peripheral blood progenitor cells (PBPC) harvest; consolidation with BEAM + ASCT with PBPC or marrow or both. Median age was 49 years (29-57), 11 were male, 8 female; 16 pts had high tumor burden, the LDH was elevated in 14, 11 with performace status >1 and 9 had >1 extranodal sites. Leukapheresis yielded a median of 23×10^6/kg CD34$^+$ cells and 75×10^4/kg CFU-GM. Thirteen patients were autografted: 11 with PBPC alone, 1 with marrow and 2 with both. Five patients were not transplanted: 4 because of progressive disease and 1 due to toxic death (mucormycosis infection). Hematologic engraftment after BEAM was fast and sustained: neutrophils > 500 in 11 dd (7-17), platelets > 50,000 in 11 dd (8-60). At the end of therapy 11 (58%) were in CR, 7 NR and 1 died of toxicity. With a median follow-up of 32 months DFS rate is 80%, FFS 45% and OS 53%. These results compare favourably with those achieved in 21 patients with DLCL and BM involvement with similar clinical characteristics, previously treated (1986-1990) with MACOPB alone for 12 weeks: CR 33%, DFS 29%, FFS 10%, OS 9% (median follow-up 7 years). The new scheme of intensified chemotherapy and ASCT allows a good yield of PBPC also in patients with BM involvement. This aggressive approach improves the outcome of this poor prognosis subset of DLCL patients.

074
Rearrangements of BCL6, BCL2, c-MYC and 6q deletion in B-Cell lymphoma: clinical relevance in 71 patients

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B-diffuse large cell lymphoma (DLCL) has been associated with some molecular lesions, but the role of these lesions as prognostic marker is still controversial. The frequency and clinical correlations of BCL6, BCL2, c-MYC rearrangements and 6(q)deletion in B-DLCL was investigated in this study. The presence of these genetic lesions was analysed in samples of lymph nodes or bone marrow collected at diagnosis from 71 patients with B-DLCL, all treated with an antracycline-containing chemotherapy regimen. Rearrangement of BCL6 was found in 11 patients (15%), rearranged BCL2 in 12 (17%), 6(q) deletions in 10 patients (14%) and 4 patients (6%) showed c-MYC rearrangement. Patients with rearranged BCL6 tended to have more aggressive disease than patients with germ-line BCL6 (intermediate-high/high risk according to IPI criteria: 73% vs 43%), 3-yr survival rate was higher for the former (62% vs 42%), but without being statistically significant difference. The mean number of involved extranodal sites was similar in the two groups. Patients with BCL2 rearrangement appeared to have less aggressive disease than those with germline BCL2 (low/low-intermediate risk 75% vs 47%) and a slightly better 3-yr survival rate (70% vs 41%) but again the difference was not significant. Both groups with or without 6(q) deletion had similar clinical characteristics and outcome. The four patients with c-MYC rearrangement had aggressive disease and did poorly. A comparison between patients with BCL6 or BCL2 or del6(q) or no alterations failed to show any clear differences in clinical characteristics or in the outcome.

The analysis of molecular lesions in B-DLCL may be useful for better diagnostic definition; further studies are required to define the prognostic role of genetic lesions in B-DLCL better.

075
Lymphoproliferative disorders in heart transplant recipients: definition of molecular approach to treatment

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Post-transplantation lymphoproliferative disorders (PTLD) develop in approximately 2% to 6% of cardiac transplanted patients. The incidence is likely to increase with the constantly rising number of organ transplant recipients and the use of new potent immunosuppressive agents. PTLD histologically encompass a spectrum ranging from reactive-looking proliferation to non-Hodgkin lymphoma (NHL), morphologically indistinguishable from those observed in non-immunocompromized patients. The genomic integration of the Eptstein-Barr virus (EBV) seems to play a central

role in the pathogenesis of PTLD. The natural history of this disease is usually characterized by a rather indolent phase sustained by polyclonal proliferation of B lymphocytes and a subsequent rapidly progressive phase characterized by the selection of a truly neoplastic clone, even though the histologic diagnosis is often unable to distinguish these phases. Most importantly, the down modulation of the immunosuppressive regimen along with antiviral therapy might be of clinical value in the treatment of the indolent, polyclonal phase while the clinical outcome of patients treated by relatively intensive chemotherapeutic regimens is poor. Therefore the molecular definition of these lymphoproliferative disorders is of crucial importance for the correct diagnosis and treatment of these patients. We report our experience in 5 heart transplanted patients who developed NHL (2 diffuse large cell lymphoma (DLCL), 2 low grade marginal zone lymphoma (MZL) and 1 Burkitt lymphoma) between 12 and 24 months after transplantation. In these patients the integration of the EBV genome was evaluated through PCR amplification of the internal repetitive fragment (Bam H1 W-fragment) and the diagnosis of NHL was confirmed by the demonstration of IgH gene rearrangement by Southern Blot and/or polymerase chain reaction (PCR) with consensus primers and polyacrylamide gel electrophoresis resolution. In spite of histologically based diagnosis of NHL no chemotherapy was given in two cases (one DLCL and one MZL) in which no molecular evidence of a clonal disease was obtained and in one case of low grade MZL with clonal rerrangement. In these three patients, the reduction of immunosuppression and treatment induced a relatively durable reduction of lymphadenopathy. On the other hand, the chemotherapy schedules used for the treatment of the clonal evolutions in two patients (one DLCL and one Burkitt lymphoma) were found to be highly toxic and the clinical outcome was poor. In conclusion this preliminary experience suggest: 1) that the histologic diagnosis of NHL after transplant should always be associated with clonality studies by molecular techniques; 2) the distinction between monoclonal and polyclonal PTLD may be helpful to design the treatment strategy and to avoid severe toxicity associated with most chemotherapeutic programs; 3) the molecular approach may be helpful to investigate whether long lasting polyclonal PTLD always precedes a truly neoplastic phase of NHL. Finally, the results of chemotherapy suggest the urgent need of alternative therapeutic strategies possibly based on biological response modifiers and or other immunologic approaches.

076
The "international index" is useful in the prognostic evaluation of patients with HIV-related non-Hodgkin's lymphoma (NHL)

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Among patients with diffuse large cell NHL, the *International index* (*II*) (Shipp 1993) is able to identify 4 subgroups with significantly different complete remission (CR) rates and survival. Aggressive NHL frequently develops in HIV-infected subjects. Its treatment is still controversial, but some patients can achieve cure with aggressive chemotherapy (CT) programs. We therefore evaluated the prognostic usefulness of *II* in a series of patients with systemic HIV-related NHL diagnosed at our Center from December 1985 to June 1996.

Of 79 consecutive cases, 5 were excluded because they refused therapy and 5 because pretherapy LDH level was not available. The characteristics of the 69 evaluable patients were those typical of a series of unselected patients with HIV-related NHL (stage IV: 71%; extranodal disease: 86%; diffuse large cell histology: 67%; mean $CD4^+$ lymphocyte count: 135/cmm; preexisting AIDS: 33%). The distribution of the 4 *II* risk groups was as follows: low: 5 (7%); low-intermediate: 12 (17%); high-intermediate: 16 (23%); high: 36 (52%). The degree of immunodeficiency significantly correlated with *II*. Indeed, the mean $CD4^+$ lymphocyte count/cmm was 313, 230, 151, and 72, respectively in the low, low-intermediate, high-intermediate, and high risk group ($p=0.0085$) and the % of patients with preexisting AIDS was 0%, 17%, 44% and 39%. Of 69 patients, 49 (71%) were treated with aggressive chemotherapy (ProMACE-CytaBOM) and 46 were evaluable for response. The percentage of patients treated in the different *II* groups with worsening prognosis was 100%, 67%, 75% and 67% and the % of CR was 100%, 88%, 50% and 29%, respectively ($p=0.0001$). Actuarial 5-year survival significantly differed, being 71% for low, 47% for low-intermediate, 50% for intermediate-high, and 13% for high risk subgroup in treated patients ($P=0.0006$) and 71%, 31%, 47% and 5%, respectively, in the entire series ($p<0.0001$).

Conclusions. 1) the *II* significantly discriminates subgroups with different CR rates and survivals also among patients with HIV-related NHL; 2) the prognosis of patients not belonging to the high-risk group, treated with aggressive CT, is similar to that of corresponding HIV-negative patients; 3) however the majority of HIV-positive NHL belongs to the high risk group, thus accounting for the worse prognosis of HIV-related NHL compared to HIV-negative NHL; 4) the degree of immunodeficiency is significantly related to the *II* risk group, suggesting that it may contribute to the aggressive clinical presentation of lymphoma.

077
Ifosfamide, epirubicin and etoposide (IEV) plus rhG-CSF for relapsed/refractory lymphoma. A safe regimen for induction of remission and mobilization of progenitor cells

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Background. High-dose chemotherapy with peripheral blood stem cell (PBSC) support may be considered the treatment of choice for aggressive non Hodgkin's lymphoma (NHL) patients (pts) with *chemosensitive* relapse or refractory disease.

Purpose. To determine the efficacy of the IEV regimen in providing a satisfactory response rate and good progenitor stem cell mobilization.

Patients and Methods. From January 1996 to February 1997, 11 pts with relapsed or refractory aggressive NHL (5 diffuse large B cell, 4 peripheral T cell, 2 anaplastic large cell) were treated with a combination of ifosfamide 2500 mg/m^2 i.v. days 1-3, epirubicin 100 mg/m^2 i.v. day 1 and etoposide 150 mg/m^2 i.v. days 1-3 (IEV), followed by 5 µg/kg rhG-CSF from day 5 to the last day of progenitor cell collection. Each course was repeated every 21 days for a total of 3 cycles. Median age of the pts was 38 years (range 10-55), M/F ratio was 6/5. All pts were treated with a third generation regimen as first line chemotherapy. Eight pts relapsed after a median follow-up of 17 months (range 3-32), 2 pts were considered not responders and 1 patient was in partial remission (PR) after the first line chemotherapy. Three pts had received prior radiation therapy (2 mediastinum, 1 cervical nodes); three pts had bone marrow involvement at relapse.

Results. The 33 courses of IEV chemotherapy were administered on an outpatient basis. No dose reduction was necessary in any pts. Five pts (45%) achieved a complete response (CR), 6 pts (55%) a PR for an overall response (PR+CR) of 100% to IEV regimen. During the 33 courses, 5 febrile episodes occurred; 3 red blood cell transfusions were necessary, while no platelet transfusions were required. Progenitor cells were mobilized after the third course of IEV in 10/11 (91%) pts. In the 10 pts, the peak of CD34$^+$ cells in PB (a median of 59 CD34/µL) was present after a median of 10 days (range 9-13 days) from the starting of the IEV regimen. In all pts, one apheresis was enough to collect a median of 4.3×10^6 CD34/kg (range 2-10.7). Autograft and PBSC reinfusion was performed in 10/11 pts at a median time of 93 days (range 57-116) from the beginning of the IEV regimen. The patient who did not yield a sufficient PBSC relapsed 2 months after IEV chemoterapy and, therefore, was not considered elegible for high-dose chemotherapy. Five pts are in continuous CR after a median follow-up of 8 months (range 2-14); 3 pts relapsed after a median follow-up of 6 months (range 3-11) and it is too early for the evaluation of response in 2 patients.

Conclusions. IEV regimen plus rhG-CSF provides an excellent response rate in refractory/relapse aggressive NHL pts. This regimen can be given on an outpatient basis with an acceptable toxicity. In our experience this procedure allows good progenitor cell mobilization, reducing the time from the IEV regimen to the high-dose therapy.

078
Small non-cleaved cell lymphoma and mature B-cell acute lymphoblastic leukemia in adults and children: results with the 89-c-41 (NCI) protocol

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Between September 1989 and July 1995, 49 adults (age < 65 years) and children with small non-cleaved cell lymphoma (SNCL) and B-cell acute lymphoblastic leukemia (B-ALL) were treated with the National Cancer Institute protocol 89-C-41. In 1991 the protocol was extended to another 6 Italian hematologic institutions. Patients were stratified into high and low risk groups (Magrath stratification). High risk patients received 4 alternating cycles of two different drug combinations (regimen A and B): regimen A (CODOX-M) comprises fractionated doses of CTX, HD-MTX, ADR and VCR, while regimen B (IVAC) consists of ifosfamide, VP-16 and HD-ARA-C. Low risk patients received 3 cycles of the CODOX-M regimen. Both drug combinations included intrathecal therapy. Forty-six out of 49 patients (94%) achieved complete remission (CR); EFS is 71% at 5 years. A worse prognosis has been recorded in adults compared to children (EFS 55% vs 90% at 2 years, p=0.008). The 5-year EFS rate of SNCL (30/31 high risk) is 83% with a better prognosis in children compared to adults (88% vs 77%). EFS of B-ALL is 48% at 2 years. Hematologic and extrahematologic toxicity were acceptable.

The encouraging results and the feasibility of the 89-C-41 protocol confirm that this regimen can be applied to both adults and children with SNCL and B-ALL. The protocol is still open to patient accrual; an update will be discussed at the meeting.

079
FDG-PET and serum CA125 are reliable, non-invasive tools for staging and monitoring gastrointestinal localizations of lymphoma

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Background. Current staging procedures underestimate the extranodal extent of lymphoma, expecially gastrointestinal, and are almost always unable to detect sidewall invasion and involvement of serosae. In our experience, positron emission tomography (PET) is more accurate than Gallium-67 or computerized tomography (CT) scans in depicting and monitoring abdominal and extranodal disease (*Eur J Nucl Med, in press*). Moreover, we found raised levels of serum CA^{125} (sCA^{125}) in most patients with extranodal disease who had overt or suspected mesothelial involvement; variations in sCA125 levels closely reflected regression or progression of disease (*J Clin Oncol, in press*).

Aims. 1) to verify whether whole body (WB) PET imaging is useful in detecting gastrointestinal localizations of lymphoma and identify primary or multicentric disease, with the aim of optimizing treatment strategy; 2) to evaluate the reliability of PET imaging and sCA125 in monitoring disease status, with the aims of reducing the employment of invasive endoscopic procedures and identifying recurrence early.

Patients and Methods. Fourteen patients with endoscopic and biopsy proven lymphomatous localization in the gastrointestinal tract, were submitted to baseline and follow up WB PET with fluorine-18 deoxyglucose (FDG) (370 Mbq iv, WB imaging between 30 and 75 min after injection) and serial sCA125 level measurements. Concomitant CT and endoscopic findings were available. All the patients received chemotherapy and were evaluable for response.

Results. At diagnosis, WB PET showed increased FDG uptake in all the patients in the gastrointestinum, concordant with endoscopic findings and regardless of histologic grading; in two patients PET identified further lesions in the small bowel, undetected by other instrumental procedures. Such findings changed the therapeutic approach from gastric resection to systemic chemotherapy. WB PET simultaneously detected for each patient all lymphoma lesions uptaking FDG. CT scanning detected gastrointestinal localizations only in 10/14 patients. sCA^{125} levels were elevated in 10 patients with CT-observed esovisceral involvement and were normal in four other patients, with disease apparently confined to the gastric wall. In the five patients who achieved a complete remission both PET and endoscopic findings became negative and sCA^{125} normalized in the three patients with raised baseline values. In the four patients who obtained partial remission, PET showed persisting, although decreased, abnormal FDG activity in all cases, endoscopic survey was positive in 2 patients, and CT scan in only one patient; CA125 persisted unchanged (2 abnormal, 2 normal). Five patients had minor responses and developed progressive disease with the sCA125 concentrations increasing above the normal range in all of them; PET imaging, performed only in one patient, showed a strong uptake.

Conclusions. FDG WB PET is able to stage and monitor gastrointestinal localizations of lymphoma accurately, suggesting lesions (such as small bowel localizations) undetected by other procedures, and residual disease after treatment associated with normal CT. Serum CA125levels paralleled response to treatment and could be a harbinger of disease progression.

080
Front-line CEVOP-B + rhGM-CSF with 96-hour infusion of EpiADM and VP16 in aggressive non Hodgkin's lymphoma. A phase II study

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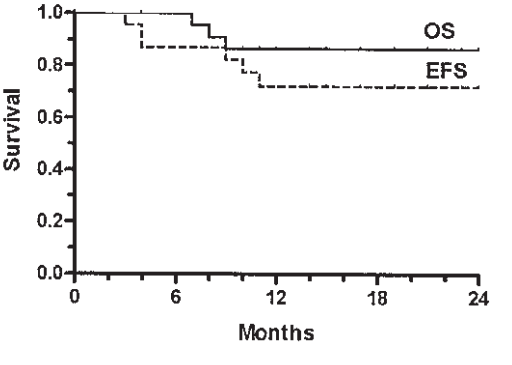
Rationale. CEVOP-B for untreated aggressive NHL (F-J cat.WF) uses a protracted 96h-infusion of Epi-ADM and VP16 in an attempt to circumvent the P-gp mechanism of MDR-related resistance (10 to 20% of untreated aggressive NHL).

Study design. Two-stage optimal design according to Simon. Null hypothesis: complete response (CR) rate $\leq 70\%$; alternative hypothesis: CR rate $\geq 90\%$; α error=0.05, β error=0.20. Six cases are required in the first stage with 5/6 CRs, and 27 cases in the second stage with at least 23 CRs.

Treatment plan. Five total courses, with three-week intervals, of cyclophosphamide: 750 mg/m^2 i.v. d 1, vincristine: 1.4 mg/m^2 i.v. d 1, VP16: 75 mg/m^2/d and Epi-ADM: 17.5 mg/m^2/d as continuous infusion over 96 h (dd 1-4), bleomycin: 15 mg i.v. d 5, PDN: 60 mg/m^2 PO dd 1-7; rh-GM-CSF 5 mcg/kg/d s.c. (dd 8-14); fluconazole: 100 mg/d and ciproxacine: 1 g/d (dd 8-21). Intrathecal methotrexate was added according to histology, and RT (35 Gy) delivered to



bulky mediastinal disease.

Patients. In March 1997, the recruitment of patients was completed. At present, 23/27 pts are evaluable for response (M/F = 14/9; median age=42; stage I-IIE and/or bulky=9; stage III-IV=14; high LDH=14/23; ≥ 2 E sites = 7/23; bulky nodal or mediastinal disease= 6/23).

Results. Twenty CRs (87%) and two PRs (9%) were achieved, and one patient (NHL WF cat. J) had CNS progression after the 4th cycle. No toxic death occurred. After a median follow-up of 14 months (range, 4-24). Overall survival (OS) and event free survival (EFS) were 86 % and 72%, respectively. Grade IV WHO neutropenia occurred in 52% of courses with median ANC of 320/μL (range, 70-1,300) at nadir and complete recovery to ≥ 500/μL within 2-4 days; Grade IV thrombocytopenia occurred in 3% of cycles with median count at nadir of 114,000/μL (range, 19,000-214,000). Other side effects were: grade I-III anemia (46%, only one patient required transfusional support), grade I-II mucositis (34%), grade I-II infection (20%). Delays or dose reductions were needed in only 16/115 cycles (14%), due to extrahematologic reasons.

Conclusions. CEVOP-B is a feasible, well tolerated and very effective program.

081
Fludarabine, cyclophosphamide, and dexamethasone (FLUCYD) for the treatment of advanced low-grade non-Hodgkin's lymphoma

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Experimental data indicates that fludarabine prevents repair of DNA damage induced by alkylators. The aim of this study was to evaluate efficacy and toxicity of the combination of fludarabine (Flu) + cyclophosphamide (Cyclo) + dexamethasone (D) in indolent NHL. Twenty-one pts with advanced indolent NHL were treated with Flu 25 mg/m^2/d i.v.+ Cyclo 350 mg/m^2/d i.v.+D 20 mg/d i.v. in 3-day courses, repeated every 4 wks up to a max. of 8 courses. Treatment was postponed if PMN <1000/μL and/or PLT <100,000/μL, and withdrawn if hematologic toxicity caused a delay >3 wks. TMP-SMX 2 tablets/day × 2 days/week was given as *Pneumocystis carinii* infection prophylaxis. Patients' characteristics: median age 52 (43-73); M/F 12/9; histology: follicular 9; lymphocytic 5; lymphoplasmacytic 5; mantle-cell 2; refractory: 4; relapsed after a CR: 2; in progression after a PR: 15; stage IV: 18; with bone marrow involv.: 18; median of marrow infiltration: 60% (range 5-85%); median number of regimens pre-FLUCYD: 2 (1-4); previous CHOP: 18.

Results. Twelve of 21 pts (57%) responded, wth CR achieved in 4 and PR in 8. The median number of courses to reach maximum response was 4 (3-6) for pts achieving CR and 4 (2-4) for those achieving only a PR. Five pts were unresponsive after 2-6 courses, and 3 went off study after the 1st course because of prolonged hematologic toxicity (>3 wks); 1 pt died during cytopenia after the 1st course. Two pts progressed to high-grade NHL: 1 off therapy after a PR, and 1 during treatment after initial response.

Toxicity. Of 85 courses the percentage of courses performed with a delay of 1 or 2 weeks because of hematologic toxicity (PMN <1000/μL and/or PLT <100,000/μL) was 35% for the 2nd and 3rd course, 69% for the 4th course, 73% for the 5th course. The pts who left the study prematurely because of prolonged hematologic toxicity were: 3 after the 1st course, 1 after the 2nd, and 1 after the 3rd. Fourteen percent of courses were complicated by infection or FUO.

Conclusions. This study demonstrates that the combination FLUCYD is an efficacious salvage treatment for patients with advanced indolent heavily pretreated NHL. It may broaden therapeutic options in the earliest phases of the disease.

082
Hepatitis C virus (HCV) and non-Hodgkin's lymphoma (NHL): a case-control study comparing clinical features and response to treatment in a consecutive series from a single institution

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A growing body of evidence points to the fact that HCV, the major etiological factor of type II *essential mixed cryoglobulinemia*, is implicated in lymphomagenesis. We had prospectically evaluated 146 patients (pts) affected by NHL observed at our institution between 1994 and 1996, tested at diagnosis for HCV antibody by Elisa test, confirmed by Riba II and/or HCV amplification-RNA; all pts were HIV negative. Differences between NHL-HCV+ and NHL-HCV- groups are presented on the table in next page.

Conclusions. mean age of HCV infected pts was older than pts of the control group and NHL in HCV infected pts showed a more aggressive histology and a more frequent extranodal involvement at diagnoses. Despite this biological behavior response to treatment was comparable with control group. Long-term follow up studies are necessary to assess the ultimate outcome of these pts.

	n. NHL HCV+ (%)	*n. NHL HCV- (%)*	*p value*
Patients observed	38 (26)	108 (74)	
Mean age (range)	65 yrs (27-79)	57 yrs (21-89)	0.002
Male/female	17/21	58/50	0.34
B symptoms	5 (13)	26 (24)	0.24
PS (ECOG) 0-1	27 (71)	82 (75)	0.55
Bulky disease	7 (18)	29 (27)	0.30
Stage			
I-II	16 (42)	41 (38)	0.65
III-IV	22 (58)	67 (42)	
Histology (Real/WF)			
Extranodal m-zone/E	4 (10)	5 (0.5)	
Diffuse large B-cell/G-H	25 (66)	48 (44)	0.01
Others	9 (24)	55 (65.5)	
Primary extranodal	24 (63)	29 (27)	
Only nodal	10 (26)	78 (72)	0.0001
IPI Score			
0-1	12 (31)	47 (43)	
2	10 (26)	28 (26)	0.35
>2	16 (43)	33 (31)	
Response to treatment			
G+H (CR)	12 (48)	23 (48)	0.99
A-E (CR+PR)	7 (53)	44 (73)	0.14

083
Cytofluorimetric analysis of lymph node suspensions allows early diagnosis and application of the REAL classification

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We analyzed the immunophenotype of cell suspensions obtained from lymph node biopsy fragments taken from 120 subjects undergoing biopsy because of suspected lymphoma. The samples were cytofluorimetrically analysed using a panel of front-line markers: κ/λ, CD 19, CD3, CD4/8. If clonality was suspected (κ/λ or CD4/CD8 >3 < 0.5), the sample was analysed using the following panel of markers: SIg, CyIg, CD1c, 5, 10, CD11a-c, 23, 25, 30, 43, 45, CD49c-d e FMC7 for B forms; CD1a, 2, 7, 30 e CD4RO for T forms. Light chain Ig restriction was found in 91 pts (group 1); 10 pts showed increased B cells (CD10 > 40%), without chain restriction (group 2); 8 pts showed increased T cells (CD3 >80%) with a normal (3 pts, group 3) or very high CD4/CD8 ratio (5 pts, group 4); 11 pts had a normal T/B ratio (group 5). Histological analysis revealed B-cell non-Hodgkin lymphoma (B-NHL) in 93 cases (90/91 pts in group 1+1 pt in group 2 + 2 pts in group 3); T-NHL in 5/5 pts in group 4; Hodgkin lymphoma in 5 cases (1 in group 3 + 4 in group 5); reactive lymphoadenitis in 15 pts (1 in group 1 + 9 in group 2 + 5 in group 5); metastases of other tumours in 2 pts in group 5. Consequently, at least in the case of B-NHL, screening cytofluorimetric analysis of lymph node suspensions is very highly sensitive, specific and diagnostically accurate (> 95%). In the context of B-NHL, the application of the complete panel made it possible to develop an algorithm (CD5 neg. → SIg ++/+/± → CD10 +/– → CD23 and CD43 +/–; CD5 pos. → SIg ++/+/± → CD23 +/– → CD49c and CD1c +/–), which directs an immunophenotypic diagnosis towards a histotypic diagnosis according to the REAL classification. Cytofluorimetric diagnosis of the histotype was accurate in lymphocytic lymphomas, large-cell lymphomas (even in the presence of marked plasmacytic differentiation), $CD10^+$ centrofollicular and mantle-cell lymphomas; it was not suitable to define the histotype in 31/91 cases, including immunocytomas, marginal zone and $CD10^-$ centrofollicular NHLs.

084
Characterization of t(11;14) translocation in mantle cell lymphoma with fluorescent *in situ* hybridization

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Characterization of chromosome abnormalities in leukemia and lymphoma have contributed to the understanding of the molecular basis of these neoplastic diseases. In addition, in many cases the abnormalities are considered diagnostic or have prognostic value. Genes involved in many of these chromosomal aberrations, have been identified and characterized. In the case of a non-Hodgkin lymphoma called mantle cell lymphoma (MCL), the t(11;14)(q13;q32) and the protooncogene BCL-1 (cyclin D1) have been involved. About 70% of MCL are associated with the t(11;14). However, because of the limits of the cytogenetic analysis and the different breakpoints at the molecular level, it is possible that the actual frequency of association has been understimated. In our study, with the use of an artificial yeast chromosome spanning the entire area where the rearrangements occur, in fluorescent *in situ* hybridization experiments, we detected the BCL1 translocations in 14 of 14 patients with clinical and immunologic features of MCL. Our data provide evidence that the detection of t(11;14) could be diagnostic for MCL. Since this

translocation is associated with a poor prognosis lymphoma, its detection may help to make a correct diagnosis as well as evaluate residual disease, which is critical for planning a rational chemotherapy regimen.

085
The nucleotide mutations in variable regions of heavy chain genes of immunoglobulins in HCV-associated immunocytomas are indicative of antigen selection

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It is known that patients affected by HCV infection frequently have clonal B-cell expansion in peripheral blood mononuclear cells. The monoclonal population generally secrete monoclonal IgM with rheumatoid factor (RF) activity. Recently, a high prevalence of HCV infection has been shown in patients affected by immunocytomas, indicating that this virus is able to determine both benign and malignant B-cell proliferation. To investigate the role of HCV in the pathogenesis of lymphomas more thoroughly, we determined the sequences of clonally rearranged variable regions of heavy chain (V_H) genes of immunoglobulins (Ig) from B-lymphocytes of 8 cases of immunocytomas. All cases had a cryoprecipitable IgMk component with RF activity. The V_H regions were obtained by RT/PCR amplification with primers from V_H FrameWork1 and from constant regions 1 of heavy chain (C_H1) genes of IgM (Cμ) IgG (Cγ) and IgA (Cα). From 5 to 12 independent clones were sequenced from each amplification. The tumor V_H region sequences were associated with Cm sequences in 7 cases, while in one the same V_H sequence was detected in both Cμ and Cγ transcript, indicating that a subset of neoplastic B-cells had undergone Ig heavy chain isotype switching. The V_H region sequences obtained shared 94.7-99.3% homology with the corresponding germline genes (Table 1). These differences with respect to germline configuration are likely to represent somatic mutations since the same nucleotide changes are present in a second set of RT/PCR, cloning and sequencing reactions. The distribution of replacement (R) and silent (S) mutations in the V_H regions showed a high R/S ratio in CDRs and low R/S ratio in the FWs indicating antigen stimulation and selection. Intraclonal diversity among the tumor-derived V_H sequences was seen in all cases, suggesting ongoing mutational events in the neoplastic clones. Since malignant cells express Ig with RF activity, our findings indicate an ongoing process of somatic mutations in the V_H regions of these regions of these Ig, suggesting a role for chronic antigen stimulation (probably by immunocomplexes containing HCV) in the development of HCV-associated lymphomas.

					R/S Mutations		
Pts.	*Ig class*	*V_h family*	*V_h gene*	*% homology*	*FW*	*CDR*	*$J_h$gene*
SEL	IgM+IgG	VH3	DP-51	94.9%	2/5	6/1	J_h 6b
LC3	IgM	VH1	51p1	98.3%	2/1	2/0	J_h 3b
LC4	IgM	VH4	VH4.21	96.2%	3/3	4/1	J_h 2
SS	IgM	VH1	51p1	99.3%	0/1	1/0	J_h 4b
SEG	IgM	VH1	51p1	96.3%	2/3	3/3	J_h 4b
MS	IgM	VH3	DP-47	94.2%	4/7	7/1	J_h 5a
ND	IgM	VH1	51p1	92.9%	3/5	8/1	J_h 4b
LC2	IgM	VH1	51p1	97.0%	2/5	1/1	J_h 4b

CHRONIC LYMPHOPROLIFERATIVE DISORDERS AND MYELOMA

086
Fludarabine (FLU) versus chlorambucil and prednisolone (CHL+P) in the first line therapy of B cell chronic lymphoid leukemia (B-CLL) Preliminary results of the randomized multicentric study

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We report the preliminary results of an Italian multicenter randomized study of first line therapy of active B-CLL. Twenty seven Departments of Hematology are taking part in the trial which began in September 1994; in the study the treatment with FLU is compared to the classical association CHL+P. The end points are: 1) percentage of responses (CR+PR), 2) DFS, PFS, overall survival and 3) toxicity. Eligibility criteria are: active B-CLL with an intermediate (Rai's stage I and II) or high risk (stage III and IV) , age between 18-70 years old. Informed consent is required. FLU is administered I.V.in about 30 minutes with the schedule of 25 mg/sm for 5 consecutive days every 4 weeks for at least

six courses. CHL is given orally in pulsed doses of 30 mg/sm on day 1 and 15 in association with P, given i.m. at the dosage of 40 mg/sm on days 1-5 and 15-19 of each course, every 4 weeks for 6 courses. Patients in CR at the end of the sixth course receive 2 more courses; patients in PR receive 3 more courses and are evaluated for survival; patients in PD after 3 courses or in SD after 6 leave the study and are evaluated for survival. No maintenance therapy is given. Responses are evaluated according to the NCIWP criteria. Up to date 128 patients have entered the study: 65 in the FLU arm and 63 in the CHL+P arm. In FLU arm 45 patients are male with a M/F ratio of 2.25 the median age is 59 (range 37-70); 46 were in the intermediate risk group (Rai I+II) and 19 in the high risk group (Rai III+IV); 24 (36.9%) had lymphocytosis over 50,000, 36 (55.4%) β_2-microglobulin over 2.5 μg and 39 (49.2%) had bone marrow histology of the diffuse type: In CHL+P arm 42 patients out of 63 are male with a M/F ratio of 2.0, the median age is 59 (range 35-70); 43 were in the intermediate risk group (Rai I+II) and 20 in the high risk (Rai III+IV); 29 (46%) had lymphocytosis over 50000, 30 (47,6%) β_2-microglobulin over 2.5 μg and 35 (55,5%) had bone marrow histology of the diffuse type. Up to date 91 patients concluded the study: 48 in the FLU arm and 43 in the CHL+P arm. In FLU arm we observed 25 CR (52.1%), 11 PR (22.9%), 3 SD (6.2%) and 3 PD (6,2%); 6 patients were not evaluable (12.6%). In the CHL+P arm we observed 14 CR (32.6%), 13 PR (30.2%), 5 SD (11.6%) and 5 PD (11.6%); 6 patients (14.0%) were not evaluable. Toxicity was mild in both treatments: in the FLU arm we observed 14 serious adverse events (III=7; IV=7) and 1 death; in the CHL+P arm there were 20 serious adverse effects (III=16; IV=4) and 1 death. Our results confirm that FLU is more effective than CHL+P. In our experience CHL+P gives better results than those reported from recent studies, but no conclusions should be drawn until the study is concluded.

087
Proposed new scoring system (MCSS) for immunophenotypic diagnosis of mature B-cell chronic leukemias

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We evaluated the diagnostic role of immunophenotype (IP) in a series of 411 patients (pts) with mature B-cell chronic leukemias of extrafollicular origin (CD10 neg.). The panel of cytofluorimetric markers and MoAbs consisted of SIg/CyIg (α, γ, μ, δ, κ and λ), FMC7, CD1c, CD5, CD11c, CD19, CD23, CD25, CD49c (clone P1B5) and CD49d. Positivity for the markers was expressed as grade 0 (< 20% of $CD19^+$ cells); grade 1 ≥ 20 < 60%; grade 2 ≥ 60%. The intensity of SIg and FMC7 was evaluated by analyzing the log scale distribution of fluorescence in comparison with controls, and considered *dim* (low or moderate) when the positive peak fell within the first or second logarithmic percentile, and *bright* when it fell within the third percentile or beyond. Frequency evaluation of the various IP patterns using the P3M Block Clustering program (J. Hartigan e L. Engelman) made it possible to identify some modal patterns and weigh the discriminating capacities of the tested markers.

We found three markers that have led us to propose the following *Marcora Center Scoring System* (MCSS). Using the MCSS, 69.1% of our pts (Group 1) had a score of 3.5- 5; 12.2% (Group 2) of 2.5-3; 2.9% (Group 3) of = 2; and 15.8% (Group 4) of < 2 The Group 1 pts had clinical and cytomorphological characteristics compatible with a diagnosis of classical CLL. Group 2 pts had characteristics compatible with CLL although, as the number of cases of atypical morphology was significantly higher than in Group 1 (p=.002), we can consider them as variant CLL. Group 3 pts had typical mantle cell lymphoma; and the disease in Group 4 pts included the leukemic form of immunocytoma and splenic marginal zone lymphoma (with or without villous lymphocytes), HCL and prolymphocytic leukemia.

Marker	*Score*			
	0	*0.5*	*1*	*2*
SIg intensity	bright	-	-	dim
CD5 grade	0	1	-	2
CD23 grade	0	1	2	-

We found CD49c expression and FMC7 intensity useful as additional markers. 0/100 of the cases from Groups 1+2 had rearranged bcl-1 or bcl-2 loci; bcl-1 rearrangement was found in 5/11 group 3 pts. The application of the MCSS may simplify the diagnostic definition of mature B-cell leukemias and facilitate the design of clinical and biological studies.

088
Efficacy of maintenance therapy with human lymphoblastoid α-interferon in hairy cell leukemia (HCL)

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The value of maintenance therapy with α-interferon in HCL for responding pts. has not yet been deter-

mined. We update here the results obtained in 25 pts followed at our Institution since May 1985 as part of a multicenter study by the *Italian Cooperative Group for Hairy Cell Leukemia* to evaluate the efficacy of human lymphoblastoid α-interferon in the treatment of HCL.

Patients were treated with a dose of 3MU daily by subcutaneous injection until complete or satisfactory partial remission, followed by randomization to observation vs maintenance at a single weekly dose of 3MU. The effectiveness of the treatment was assessed every 3-6 and 12 months by hematologic studies and bone marrow (BM) trephine, according to the *Consensus Resolution criteria of the 2nd International Workshop on HCL* (Leeds Castle, 1986).

Twenty-two out of 25 pts enrolled in the study were randomized: 10 in the maintenance (median duration of induction therapy 9.3 months, range 4.8-15.4) and 12 in the observation arm (median duration of induction therapy 12 months, range 4.8-15.4). Ten patients, all in the observation arm, have relapsed, requiring further therapy after a median interval from randomization of 18.6 months(range, 4.4-56). At the time of relapse these pts showed a re-expansion of HCL, with an increase in % BM hairy cells (HCs) (mean, 7 vs 62%) and hairy cell index (HCI = %HCs x %BM cellularity/10,000) (mean, 0.03 vs 0.28). A significant difference was found in the BM changes observed after randomization in the two arms: 1) in the observation arm (median followup 20.3 mos, range 4.4-109.5) there was a worsening of BM status over the first 18 months (mean % BM HCs, 6 vs 36%; mean HCI, 0.03 vs 0.3) with need for further therapy; 2) in the maintenance arm (median follow-up 113.6 months, range 16.7-134.3), the leukemic infiltrate after an initial increase over the first 18 months (mean % BM HCs, 4 vs 32%; mean HCI, 0.02 vs 0.09), showed little subsequent change (mean % BM HCs and mean HCI at 24, 36, 48, 60, 72, 84, 96, 108 and 120 months < 20% and 0.06, respectively).

Our data suggest that maintenance therapy at a dose of 3 MU/week is effective to increase the duration of remission, preventing or slowing down the BM re-expansion of the residual HCs.

089
Spontaneous and drug-related apoptosis in early and advanced chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder characterized by progressive accumulation in bone marrow and lymphoid tissues of neoplastic cells with low proliferative rate and reduced susceptibility to apoptosis. The availability of simple and reproducible *in vitro* assays to measure apoptosis prompted us to evaluate either spontaneous (SA) or drug-induced (IA) apoptosis in peripheral lymphoid cells isolated from CLL patients at different stages of disease. Apoptosis was measured by flow cytometric assay based on Forward Scatter (FW-SC) and Right Scatter (RT-SC) changes (*Cytometry 1992;13:785*). Apoptosis was also confirmed by ISEL and Annexin V techniques. Peripheral mononuclear cells, isolated by F/H density gradient centrifugation and depleted by adherent cells, were obtained from 33 B-CLL patients, of both sexes, ranged 50-86 years (mean: 68), 23 of whom were untreated and with early disease (0, 1, 2 Rai) and 10 with advanced CLL (3, 4 Rai) and off treatment for at least 30 days before the *in vitro* assay. Apoptotic cell rate, expressed as a percentage, was evaluated at the beginning (time "0") and after 72 h. of culture, either in standard conditions (RPMI 1640 + FCS 10%) or in presence of chlorambucil (CLB, 10 μM), fludarabine (FAMP, 3 μM), prednisone (PDN, 0.8 mg/mL) and CLB + PDN.

	Early CLL	*Advanced CLL*	*p-value*
Spontaneous apoptosis	35.45±21.76	19.17±29.63	0.033
FAMP	88.95±26.5	62.8±227.6	0.021
CLB	47.36±228.85	24.02±210.53	0.064
PDN	62.6±224.67	48.04±220.43	0.23
CLB+PDN	78.8±23.95	32.45±218.87	0.005

Results at 72 h. showed that: a) both SA and IA induced by FAMP, CLB and CLB+ PDN were significantly higher in early than in advanced disease; b) the most relevant apoptotic response was always driven by FAMP, either in early or in advanced disease; c) the association of CLB+PDN was more effective than CLB alone, especially in early CLL. When considered case by case, SA and IA showed a wide range of values in both early and advanced disease. An adequate follow-up will address: 1) whether variable SA (range: 4-83% in early disease and 7-38% in advanced CLL) could correlate with *in vivo* time to progression of the disease; 2) whether IA could be predictive of the response *in vivo* to the same chemotherapeutic agents employed *in vitro*.

090
Atypical B-CLL: a cytogenetic and interphase cytogenetic study

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To define better the cytogenetic profile of atypical CLL, conventional chromosome analysis (CCA) and interphase fluorescence in situ hybridization (FISH) studies, using a *BCL1* YAC probe, a 13q14 cosmid probe and a chromosome-12-specific pericentromeric probe, were performed in 57 patients, drawn from 260 patients with CLL. Twelve patients had the t(11;14)(q13;q32) as shown by CCA and by FISH and evidence of t(11;14) was provided by FISH in two additional cases with apparently normal karyotypes. Concomitant 13q14 deletions were detected by FISH in 11 out of 14 *BCL1*+ cases and +12 was seen in four cases. Recurrent primary chromosome changes in 43 *BCL1*-negative cases were: +12 in nine cases, 13q14 aberrations in five cases, 11q anomalies in three cases, 6q21-23 abnormalities and 4q aberrations in two cases each, other non-recurrent chromosome changes in six cases. Six additional patients without detectable chromosome changes at CCA were shown to carry +12 and/or 13q14 deletions at interphase FISH. Thus 47 out of 57 atypical CLLs (82.4%) had a detectable cytogenetic aberration. Among 43 *BCL1*-negative cases, +12 was associated with 13q14 anomalies in three cases, one of which also had an 11q abnormality. Other associations, seen in one case each were: 13q14 deletions with a 6q anomaly, 11q anomaly with a 13q- and a 7q- chromosome, and a 6q anomaly with 7q- and +12. On hematologic grounds BCL-1+ patients shared some features with leukemic mantle cell lymphoma; they showed relatively elevated prolymphocyte counts, with splenomegaly in some cases, in the absence of lymph node or visceral involvement throughout the course of the disease. Early administration of chemotherapy was necessary in *BCL1*+ cases at a median of 16 months after diagnosis. Among *BCL1*-negative cases, therapy-demanding disease was recorded in 19 cases with +12, 13q14 deletions 11q and 6q21-23anomalies, with a two-month median interval between diagnosis and start of treatment, as compared with a 24-month median interval in the remaining patients with normal karyotype or non-recurrent chromosome changes. We arrived at the following conclusions: a) atypical CLL has a high incidence of chromosome anomalies; b) FISH increases the sensitivity of CCA in the detection of t(11;14), 13q14 deletions and +12; c) the presence of complex karyotypes, with various combinations of so called primary chromosome anomalies suggests that the development of sequential chromosome changes, rather than any single specific anomaly, may underlie leukemogenesis in this subset of CLL; d) chromosome changes are clinically significant.

091
Immunophenotypic subclassification of B-cell chronic lymphocytic leukemia (B-CLL): clinical and prognostic analysis

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From January 1988 to December 1992 we prospectively studied 84 B-CLL pts with a panel of monoclonal antibodies detecting B-cell (CD19; CD20; CD21; FMC-7), B-cell associated (CD10, CD11c, PCA1), T-cell (CD2, CD3, CD4, CD5, CD8) and HLA-DR antigens.

Surface membrane immunoglobulins and mouse rosette assays were performed in all cases. Antigen expression was determined by immunofluorescence and flow cytometry using a FACS. B-cell associated or T-cell antigen expression was considered positive if more than 20% or 30% of the gated cells stained positively. A high proportion of mouse rosettes (>30%) was considered a prerequisite for patient accrual into the study.

To determine the clinical and prognostic significance of the co-expression of T-cell or B-cell associated antigens and of the lack of CD5 antigen, we gathered pts into different groups. The cohort of $CD5^+$ pts (n=74, 88%) was classified into four groups according to the expression of T (CD2, CD3, CD4, CD8) or B-cell associated (CD10, CD11c, PCA1) antigens: group I ($CD5^+$, T Ag^-) (n=51; 61%), group II ($CD5^+$, T Ag^+) (n=23; 27%), group A ($CD5^+$, B Ag^-) (n=52; 62%), group B ($CD5^+$, B Ag^+) (n=22; 26%). The $CD5^-$ pts were considered as a separate group ($CD5^-$) (n=10; 12%).

These groups were compared according to clinical and laboratory features, FAB subtypes, Binet and Rai staging systems, disease progression and survival. Group I had a lower leucocyte count and group B a higher incidence of splenomegaly. Typical CLL morphology was strongly associated with group I and group A immunophenotype, whereas mixed morphology was more frequent in group B and in CD5- pts. Of interest there was a higher incidence of splenomegaly, B and C Binet stage and a higher leukocyte count in mixed morphology CLL than in typical morphology CLL. The expression of T-cell Ag or B-cell associated Ag did not affect disease progression or survival. The 4-year actuarial risk for progression was higher in the CD5- group (90%) than in group I-II (30%) or in group A-B (35%) (P=.02). The 5-year survival was lower in the CD5- group than in the CD5+ groups (51%v.65%, p=n.s.)

Conclusions: 1) the expression of T-cell or B-cell associated antigens shows a relationship with clinical and laboratory characteristics of disease at presentation, but is not associated with a bad prognosis; 2) the lack of CD5 antigen seems to identify a subgroup of B-CLL with morphologic and clinical differences and with more rapid progression.

092
Autoimmune hemolytic anemia in chronic lymphocytic leukemia (CLL): a retrospective study of 55 cases

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Autoimmune hemolytic anemia (AHA) is a well known complication of chronic lymphocytic leukemia (CLL), but its pathogenesis is still unclear. We carried out a retrospective study on 1155 CLL patients observed over 12 years at our institution to evaluate the incidence of AHA, its clinical and immunohematologic features and their correlation with disease state and therapy.

AHA was documented in 55 CLL patients (5%), 43 males and 12 females (M/F ratio: 3.6), with a median age of 65 years. The diagnosis of CLL and AHA were made at the same time in 38 cases (69%). The median value of hemoglobin was 8.7 g/dl (range: 4-11 g/dl). Autoantibodies against red blood cells were of the IgG class in 43 cases (78%) and of the IgM class in 12 (22%). At the time of AHA diagnosis, 39 patients (71%) were untreated, while 16 (29%) were on treatment, 13 (24%) with chlorambucil and prednisone (C+P) and 3 (5%) with fludarabine and prednisone (F+P). When the treated patients of the whole series (575 pts) were analyzed, the incidence of AHA was 2.9% (13/470) among patients treated with C+P and 2.8% (3/105) among those treated with F+P. All patients received steroids and 46 also alkylating agents. A hematologic response, evaluated at the third month of therapy, was achieved in 85% of the 40 evaluable patients. Twenty-nine patients had died. The most frequent cause of death was infection (43%). Our results suggest that progressive CLL is itself an important risk for the onset of AHA. However, other conditions of immunodepression, such as those related to therapy with fludarabine or alkylating agents, may have different and adjunctive roles in the pathogenesis of this severe complication.

093
Combination of fludarabine, Ara-c, mitoxantrone and dexamethasone for the treatment of advanced chronic lymphoproliferative disorders

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On the basis of the biochemical modulation noted between fludarabine and cytarabine, and of the synergistic cytotoxicity of fludarabine with other DNA-damaging agents, such as mitoxantrone, 33 patients with advanced and pretreated lymphoproliferative disorders, 16 with chronic lymphocytic leukemia (CLL) and 17 with low grade lymphoma (NHL) were treated with a regimen including fludarabine, cytarabine, mitoxantrone and dexamethasone. The median age of the treated patients was 47 years; all NHL patients were in stage IV, while 11 CLL patients were in stage II and 5 in stage III-IV. We treated 17 relapsed patients (9 CLL; 8 NHL) and 16 patients unresponsive to previous therapy (7 CLL; 9 NHL). The combination regimen consisted of: fludarabine (25 mg/m^2 i.v. at 0, 24th and 48th h), cytarabine (1 g/m^2 i.v. at 4th h), mitoxantrone (10 mg/m^2 i.v. at 6th h) and dexamethasone (20 mg i.v. on days 1 to 3). The last 16 patients received an additional dose of cytarabine (1 g/m^2 at 28th). All patients were treated on an outpatient basis. Bactrim and acycloguanosine prophylaxis were administered. Twenty-six patients have been assessed for response (13 CLL; 13 NHL). A response was documented in all CLL patients (9 CR; 4 PR) and in 10/13 NHL patients (4 CR; 6 PR). Eight responding patients (7CLL; 1 NHL), have subsequently been submitted to autologous hemopoietic stem cell transplantation (7 PBSC; 1 BM). The main treatment-related toxicity was myelosuppression. The infection rate was lower than expected. No atypical infections were observed. Our preliminary results suggest that the combination of fludarabine, cytarabine, mitoxantrone and dexamethasone is an effective cytoreductive regimen that can be safely administered on an outpatient basis to patients with recurrent or unresponsive chronic lymphoproliferative disorders.

094
Increased levels of soluble CD27 in B-cell chronic lymphocytic leukemia

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CD27 is a transmembrane disulfide-linked homodimer belonging to the nerve growth factor receptor (NGFr) superfamily generally expressed on leukemic B-chronic lymphocytic leukemia (CLL) cells. The shedding in the serum of this molecule provides a useful disease-marker in CLL (*von Oers et al., Blood 1993, 82:3430*). In order to validate and extend further these preliminary observations we studied 82 previously

untreated $CD5^+$ B-cell CLL patients in whom serum levels of soluble CD27 (sCD27) were measured at diagnosis using a sandwich enzyme-linked immunosorbent assay (Compact soluble CD27 Elisa kit, Laboratory of the Netherland Red Cross). Levels of sCD27 were significantly higher than those in a healthy control population (2868.2±3217 U/mL versus 246.5±77.1 U/mL; p = 0.01). Despite a lack of statistical significance (p = 0.08) the amount of sCD27 was lower in patients with typical (i.e., $CD5^+$, $CD23^+$) CLL than in those with atypical CLL (i.e., $CD5^+$, $CD23^-$) (2730±2853 U/mL vs. 4624.8±5045 U/mL). Increased levels of sCD27 reflected clinico-hematologic parameters representative of tumor mass such as Binet clinical stage (stage A, 2433.1±3021.3; stage B, 2905.7±1673; stage C, 5372.0±5210; p = 0.02), bone marrow (BM) histology (non-diffuse, 2043.8± 1817 U/mL; diffuse, 3436.6±2987.9 U/mL; p = 0.01) and absolute peripheral blood (PB) lymphocytosis (r = 0.428; p < 0.001). As far as serum markers claimed to be associated with clinical stages and disease-activity are concerned, we chose to correlate with sCD27 the followings: β2-microglobulin (β2M), lactate dehydrogenase (LDH) and tumor necrosis factor-α (TNF-α). The first is a marker suitable for assessing tumor burden, the second a marker of cell death and the third a member of the NGFr superfamily. Interestingly, each of these serologic markers correlated with sCD27 (β2M, r = 0.508, p < 0.001; LDH, r = 0.446, p < 0.001; TNF-α, r = 0525, p < 0.001) thus suggesting that sCD27 may play a role in the differentiation and/or selection of leukemic B-lymphocytes. Finally, on the basis of our results it seems that sCD27 is a highly specific and suitable marker for CLL. It is not clear whether very high levels of sCD27 are particular to patients with atypical CLL whose immunological features (i.e., $CD5^+$, $CD23^-$) suggest a possible diagnosis of leukemic mantle cell lymphoma (MCL).

095
Triggering of CD40 antigen inhibits fludarabine-induced apoptosis in B chronic lymphocytic leukemia cells

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We analyzed the effect of the anti-CD40 monoclonal antibody (mAb) G28-5 on the apoptosis induced by fludarabine in B chronic lymphocytic leukemia (B-CLL) cells. In cultures of cells obtained from the peripheral blood of 13 patients and incubated with fludarabine (0.8 μg/mL), 31.7±2.3% and 48.1±8.6% of apoptotic elements were detected in flow cytometry at, respectively, day 3 and 5. In 9 of these samples, the addition of mAb G28-5 to the cultures reduced the apoptosis percentages by at least 35% (mean value: 59.8±6.4%). The differences between the apoptosis parcentages in cultures with or without anti-CD40 mAb were highly significant (p<0.0025) in a paired t- test. Since the CD40 antigen activates NF-kB/Rel transcription factors in B cells, and NF-kB/Rel complexes can inhibit cell apoptosis in some systems, we investigated whether the anti-apoptotic effect of mAb G28-5 on fludarabine-treated cells could be related to modulation of NF-kB/Rel activity. As expected, B-CLL cells displayed significant levels of nuclear NF-kB/Rel activity in electrophoretic mobility shift assays (EMSA); p50, RelA and c-Rel components of the NF-kB/Rel protein family were identified in these complexes. Following a 20 hr exposure to fludarabine, NF-kB/Rel complexes were no longer detectable in the nuclei. The addition of anti-CD40 mAb to the cultures restored the nuclear levels of NF-kB/Rel complexes. The NF-kB/Rel inhibitor, dichloroisocoumarine (DCIC), blocked both the NF-kB/Rel induction and the cell rescue from apoptosis by mAb G28-5. These results suggest that the triggering of the CD40 antigen by its ligand *in vivo* could affect the apoptotic effect of fludarabine on B-CLL cells and that its neutralization, or the use of NF-kB/Rel inhibitors, could enhance fludarabine activity.

096
Autologous circulating progenitor cell transplantation as first line treatment for multiple myeloma

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Starting from May, 1991, 52 untreated myeloma patients entered a multicenter pilot study in 6 Italian Centers: 2-3 monthly cycles of VAD followed by CY, 7 g/sm + G-CSF (Granulokine, Roche) 5 mcg/kg b.w./day c.i. for 14 consecutive days, to mobilize and collect PBSC. The subsequent conditioning regimen was melphalan (60 mg/sm) + busulfan (16 mg/kg/t.d.) followed by G-CSF. As maintenance IFN was given, at the dose of 3 M.U. t.d. 3 times a week, until relapse. The median age of the patients was 49 (33-61). Forty-one patients, out of the 52 enrolled, were submitted to PBSC collection, while 39 received the conditioning regimen plus PBSC. All the 39 patients evaluable for PBSCT showed at least an objective

response, with 15 (37%) CR, (disappearance of serum MC by immunofixation and < 5% plasma cells in the bone marrow). Considering all the 52 enrolled patients for an intention to treat evaluation, 75% of the patients responding, with 29% achieving CR. Eight patients progressed during the VAD (7)-HDCY (1) phase, while 18 out of the 39 transplanted have relapsed. The actuarial overall and event-free survivals are 56% and 30% respectively, projected to 76 months, while the actuarial response duration is 46% projected to 67 months. Toxicity was very low: white cells and platelets rose to >1,000/mmc and >50,000/mmc, respectively, after a median period of 11 and 14 days from transplant. Two patients, both in relapse, died one on day +70, one on day +155, one due to candida pneumonia, the other because of acute CMV hepatitis. A median of 3 aphereses were performed for each patient with a median number of $CD34^+$ cells and CFU-GM yielded of 15.75×10^6/kg b.w. (range: 1.5-81.5) and 21.6×10^4/kg b.w. (range: 2.1-180.7), respectively. In conclusion, this treatment appears to be feasible, with low toxicity, suggesting that it could have an important role to play as an aggressive treatment for myeloma, increasing the duration of survival of patients with this disease.

097
Allogeneic stem cell transplants for multiple myeloma. The Bologna experience

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The present report contains an analysis of a series of 58 patients with active, symptomatic MM who received myeloablative therapy followed by allogeneic transplants of hemopoietic stem cells from HLA-identical siblings. Twenty-two patients had stable chemotherapy-sensitive disease, while the remaining 36 either had failed to respond or progressed while on conventional chemotherapy. Conditioning treatments were unfractionated TBI + chemotherapy in 36 patients and the BU-CY 4 regimen in the remaining 22. GVHD prophylaxis was performed with T-cell depletion ± CsA in 28 patients and Csa ± MTX in the other 30. Grade III-IV acute GVHD was documented in 6 patients. The overall frequency of CR, as defined by the disappearance of M protein by immunofixation analysis, was 36% of all patients and 54% of those who could be evaluated (i.e surviving >90 days). Forty-one patients died, 26 (49%) of transplant-related causes (most frequently, GVHD and infection) and 15 of MM. The 4- and 8-year projected probabilities of survival were 30% and 11%, respectively. Nineteen patients out of 39 (49%) who entered either CR or PR following engraftment had signs of progressive disease, mostly within the first 2 years after transplant. However, late relapses also occurred between 3 and 5 years, after which time there was an apparent plateau in the remission curve. Indeed, 36% of patients were projected to be long-term, disease-free survivors, with 5 of them (4 of whom received the BU-CY 4 regimen) remaining in CR at 52, 66, 81, 95 and 158 months after transplant. A multivariate analysis was performed to identify the most important variables affecting transplant outcomes, including post-engraftment attainment of CR, early death, overall survival and relapse or disease progression-free survival. Chemosensitive disease (p=0.003) and female sex (p=0.01) were significant predictors of post-transplant attainment of CR (93% and 78% probability, respectively). Chemosensitive disease significantly (p=0.009) influenced the relapse or disease/progression-free survival (5-year projected: 65%), whereas patients with Durie and Salmon stage I-II had significantly (p=0.01) lower probability of dying from any cause (8-year projected survival: 47%).

It is concluded that sustained duration of CR is possible following allogeneic stem cells transplants for MM. Patients with chemosensitive disease and low myeloma cell mass are reasonably the best candidates for allogeneic transplantation and should be primarily considered for future clinical trials.

AUTOLOGOUS BONE MARROW TRANSPLANTATION

098
Hematologic reconstitution (HR) after PBPC autotransplantation: comparison between programmed cryopreservation and uncontrolled-rate freezing at –80°C

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Some studies indicate that there is no great difference in the ecovery of hemopoietic progenitors (HP) after either uncontrolled-rate freezing (URF) or programmed cryopreservation. However the clinical impact of these two different techniques has not been sufficiently studied, particularly as regards the differences in the hemopietic recovery (HR) and the clinical outcome after an adequate follow-up.

In our study we compared the HR after PBPC autotransplantation in 28 patients (group A) and 30 patients (group B) from whom the HP were cryopreserved with URF by putting the bags directly in a freezer at –80°C (group A) or into a programmed-rate freezer (group B). The same cryogenic mixture (DMSO 10% plus albumin 4%, final concentration) was used in both groups and the storage was always performed at –196°C in liquid nitrogen.

After the conditioning regimen groups A and B (who were matched for the main clinical characteristics) received respectively a median number of 1.2 (0.5-7.2) vs 1.9 (0.3-13.3) MNC$\times 10^8$/kg (p=ns), 80.4 (15-410) vs 59.8 (13-286) CFU-GM$\times 10^4$/kg (p=ns) and 5.9 (2-26.5) vs 6 (1-51.3) CD34$^+\times 10^6$/kg (p=ns). The median follow-up after autotransplantation was 14 months (range: 2-32).

The HR was fairly similar in the two groups: 11 (8-15) days for absolute neutrophil count (ANC) > 500/μL and 13 (9-37) days for platelet count > 20,000/μL in group A vs 10 (8-14) days for ANC > 500/μL and 12 (8-18) days for platelet count > 20,000/μL in group B. In group A two and five patients never achieved the value of 50,000/μL and 150,000/μL platelets, but it should be noted that three of them had an early hematologic relapse of disease.

The probability analysis showed that 95% of patients obtained ANC > 500/μL at day 13 and 14 (p= 0.2330), a platelet count > 20,000/μL at day 32 and 18 (p=0.1413), a platelet count > 50,000/μL at day 80 and 33 (p=0.2311) and a platelet count > 150,000/μL at day 166 and 100 (p=0.5521) in groups A and B, respectively.

We did not observe any significant difference as regards the duration of neutropenia, duration of antibiotic therapy, days of hospitalization or RBC or platelet transfusions.

In conclusion, our data suggest that the uncontrolled-rate freezing can be considered as a good alternative to the traditional controlled-rate method.

099
PBPC mobilization with DHAP regimen followed by autotransplantation in 33 lymphoma patients after first-line chemotherapy failure

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We transplanted 33 lymphoma patients mobilized with DHAP followed by G-CSF after failure (relapse or not complete remission: CR) of first-line chemotherapy. Their age ranged between 18 to 63 years (median 50); 13 were female and 20 male; 26 were affected by non-Hodgkin's lymphomas and 7 by Hodgkin's disease. A median of 3 (range: 1-5) DHAP courses had been given before PBPC transplantation; twenty-four hours after the last dose of cytarabine, G-CSF (Granulokine, Roche) was administered at 5 μg/kg/day until leukapheresis (LK). After DHAP chemotherapy 13 patients obtained CR, 12 a partial remission (PR) and 8 had disease progression. The PBPC collections were carried out when the circulating CD34$^+$ cell count was >28/μL starting on day +13 on average (range: 11-18).

A median of 5.8$\times 10^6$/kg (0.7-22) CD34$^+$ cells and 40$\times 10^4$/kg (7.3-208) CFU-GM was collected; afterwards patients received myeloablative therapy (BEAM in 15 cases, thiotepa-melphalan in 8, TBI in 4, CVB in 3 and mitoxantrone-melphalan in 2 cases.

We observed two toxic deaths during the first 100 days (1 due to interstitial pneumonia and 1 to CMV infection); the engraftment was rapid and complete in all patients; the hemopoietic reconstitution was characterized by 11 days to reach neutrophils >500/μL; 14 days to reach platelets >20,000/μL and 23 (16-44) days to reach platelets >150,000/μL; patients were generally discharged 15 days after the PBPC reinfusion. After transplantation 23 patients were in CR and 6 showed disease progression ; at present, with a median follow-up of 12.5 months (2-75), 17 pts are still in CCR, 9 died of disease progression, 2 have stable disease and 3 have recently received a third line treatment. In conclusion, DHAP followed by G-CSF proved to be very effective in mobilizing PBPC; the autotransplantation of DHAP+G-CSF mobilized BPC is simple, cheap and effective and the overall toxicity of this sequence was acceptable. Finally the duration of the whole salvage sequence was very short (< 4 months).

100
Autologous PBSC (PBSCT) vs autologous bone marrow transplantation (ABMT) after ICE-NOVIA induction/consolidation in acute myeloid leukemia

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Eighty-nine acute myeloid leukemia patients were submitted, in our center between January 1993 and September 1996, to remission induction and consolidation, identical in all cases, consisting of ICE (idarubicin: 10 mg/sqm days 1, 3, 5; aracytin 100 mg/sqm/day by continous perfusion, days 1-10; vepesid 100 mg/sqm/day, days 1-5) and, after complete remission (CR) obtainment, NOVIA (novantrone 12 mg/sqm/day, days 4, 5, 6; aracytin 500 mg/sqm/12

hours, days 1-6). Out of 85 evaluable cases, 74 patients obtained CR, 11 were resistant. Up to now, eleven patients have been HLA matched with a familiar donor and have been submitted to allogeneic bone marrow transplantation, 43 have been autografted. Bone marrow was harvested in 23 cases, in a range of time varying from 1 to 6 months from CR (mean 3); ABMT followed in a mean of 3.8 months (range: 3 to 8) from CR. 21 patients were submitted to G-CSF (5 mg/kg/day s.c.) mobilization starting 1 day after NOVIA administration, provided that cytogenetic and cytologic analysis had previously confirmed the achievement of CR after ICE; an adequate amount of PBSC was obtained from 20 of 21 patients, with a mean of 2 aphereses. 20 patients were then submitted to peripheral blood stem cell transplantation (PBSCT) in a period of time ranging from 2 to 6 months after CR. The conditioning regimen was identical for the two groups: busulfan 1 mg/kg/6 hours for 4 days, from -7 to –4; cyclophosphamide 60 mg/kg/day for 2 days, from –3 to –2). ABMT treated patients did not differ from an historical group of cases previously treated at our Institution for recovery of PMN and platelets [days to 500 PMN/mL: 36.4 (range: 14-81); 20,000 Plts/mL: 95.4 (range: 14-327); 50,000 Plts/mL: 145 (range: 17-265)]. At present, with a mean follow up of 23 months, 14 patients are in continuous CR; 9 have relapsed. In the PBSCT group the periods of recovery to 500 PMN/mL (17.8 days, range 11-46), to 20,000 Plts/mL (21 days, range 11 to 42) and 50,000 Plts/mL were significantly shorter than for ABMT treated cases ($p<0.05$). At present, with a median follow up of 13 months, 13 cases are in continuous CR and 7 have relapsed. In the PBSCT group we observed a significant advantage for platelet transfusion and days of hospitalization. Disease free survival was 40% at 40 months for ABMT and 58% at 30 months for PBSCT ($p=0.03$).

101
Myelodysplastic syndromes after autologous stem cell transplantation

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Myelodysplastic syndromes (MDS) are currently considered late complications of autologous bone marrow stem cell transplantation (ASCT), but have rarely been reported in allogeneic transplants. In our center, 64 patients underwent bone marrow biopsy (BMB) and cytogenetic analysis at fixed intervals before and after ASCT. Non-clonal cytogenetic abnormalities appeared in 12 patients during the year following ASCT, unaccompanied by any clinical or morphological signs of MDS; six patients showed clonal abnormalities and among these two (one with monosomy 5, one with monosomy 7) developed full-blown MDS. The karyotype in the other 46 patients remained normal, although MDS was diagnosed in three of them on clinical and morphological grounds. In brief, five of the 64 patients (three females, two males; median age: 34 years) developed a clinically evident MDS 4-60 months (median 14) after ASCT (for HD in four cases and AML in one), without presenting any karyotypic abnormalities before transplantation. One female patient with HD underwent bone marrow harvesting during second complete remission and was transplanted when her bone marrow was aplastic and showed no signs of disease. Three of the patients (died of leukemic transformation) presented a BMB picture of MDS with an excess of blasts, and two MDS with fibrosis (one died as a result of bone marrow insufficiency; the other is still living in an untransformed state).

Our experience confirms that MDS should be considered late complications of ASCT. The fact that all of the patients had a normal karyotype before transplantation underlines the leukemogenic role of the conditioning therapy, even if it is not possible to exclude the possibility that other factors related to the underlying disease and/or previous treatments may be involved. From a clinico-pathological point of view, we have observed two entities: one with an excess of blasts, characterized by rapid leukemic evolution; the second with fibrosis, characterized by a slow progression towards bone marrow insufficiency.

102
Mobilization and transplantation of Philadelphia (Ph^1)-negative peripheral-blood progenitor cells (PBPC) in chronic myelogenous leukemia

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Allografting is the only treatment shown to be curative for CML. The main problem is that this approach is limited to a minority of young patients with an HLA-identical sibling. Patients not eligible for allografting could be treated with IFN-α which can be useful in terms of long-term survival only in patients achieving at least major cytogenetic remission (MCyR). In those patients without HLA compatible donors or those not cytogenetically responsive to IFN-α, the option of mobilization/autografting procedure is being developed (*Carella et al. Bone Marrow Transpl. 12:267, 1993*). Since July 1989, 147 adult patients with Ph'-positive CML in different phases of disease entered our protocol. Sixty-six patients in

blastic (BP) or accelerated phases (AP), pretreated with IFN-α and cytogenetically unresponsive to this drug, and 81 other patients in chronic phase (CP) were mobilized. Among the latter group, 33 untreated patients were mobilized in early CP (ECP).

The following table refers to 114 patients in BP, AP and late CP, all pretreated with IFN.

	Blastic phase (n=38)	*Accelerated phase (n=28)*	*Chronic phase <1 year (n=26)*	*Chronic phase >1 year (n=22)*
PBPC Ph-negative	8 (21%)	5 (17%)	11 (42%)	4 (18%)
MCyR	3	3	6	7
Ph-neg.+ MCyR	11	8	17 (65%)	11 (50%)
High-grade non hematologic toxicity	14 (63%)	10 (35%)	5 (19%)	7 (32%)
Procedure-related deaths	5 (13%)	2 (7%)	1 (4%)	--

PBPC: peripheral blood progenitor cells; MCyR: major cytogenetic remission

Thirty-three patients with a median age of 46 years not previously treated with IFN-α, were recently mobilized with ICE or mini-ICE protocols (*Carella et al., J Clin Oncol, 15:1575, 1997*). In the early phase of recovery a median of 4 leukophoreses were performed. Nineteen patients (58%) achieved a complete cytogenetic remission (CCyR) while 7 patients (21%) achieved a MCyR. Overall response was 26/33 patients (79%). There were no patient deaths from procedure-related or other causes. To date, 20 patients have undergone autografting either as Ph'-negative (15 patients) or in MCyR (5 patients). The high-dose therapy consisted of busulphan (14 patients) or IVT protocol (idarubicin, VP-16 and single dose TBI) (6 patients). Nineteen patients are alive from 3 to 41 months after autografting (median 18 months); 5 patients maintain a CCyR (3+, 6+, 18+, 23+, 25+ months) and 7 patients MCyR (3+, 5+, 8+, 18+, 21+, 30+, 30+ months). No procedure related deaths occurred. After engraftment, all patients were treated with IFN-α + low-doses IL-2. In conclusion, the *in vivo* mobilization technique employed in our Unit has been demonstrated to be a safe procedure. It resulted in a high selection of Ph'-negative or MCyR cells in the blood if these cells are harvested after Idarubicin-containing regimens in patients in early CP not pretreated with IFN-α. After reinfusion, these cells were able to engraft and sustain CCyR/MCyR in 60% of patients.

103
Differential expression level of some adhesion molecules on CD34⁺ steady-state BM and CD34⁺ mobilized hemopoietic progenitor cells

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Whether CD34⁺ hemopoietic progenitor cells circulate or remain nested within the bone marrow (BM) microenvironment depends on the presence and function of cell adhesion molecules (CAMs). Changes in the patterns of CAM expression may reflect mechanisms of hemopoietic stem cell mobilization. Since the CAMs VLA-4, VLA-5, ICAM-1, LECAM-1 and LFA-1α mediate cell-cell and cell-matrix interactions, their expression rate on either CD34⁺ steady-state BM or CD34⁺ mobilized cells was investigated in 14 patients affected by malignant hematologic disorders (4 HD, 6 NHL, 1 AML, 3 MM), with no active disease and undergoing autologous PBSC transplantation. PBSC mobilization was obtained by either chemotherapeutic treatment (cyclophosphamide or idarubicin plus cytosine arabinoside) followed by G-CSF or G-CSF plus GM-CSF alone. The analysis was performed by biparametric flow cytometry and the CAM expression levels were expressed as either percentages of positive cells or quantified by calculating the antibody binding capacity (ABC) units per CD34⁺ cells.

Table 1.

	BM		*PBSC*	
	%	*ABC x 10^3*	*%*	*ABC x 10^3*
VLA-4	86.03±17.48	31.4±5.5	60.11±33.52*	40.4±24.3
VLA-5	34.92±27.13	29.4±18.2	38.35±32.39	36.5±24.3
ICAM-1	28.77±26.68	38.3±13.9	39.57±35.87	48.2±27.1
LECAM-1	52.50±25.75	68.4±30.9	25.25±21.83°	29.9±7.3#
LFA-1α	57.95±23.95	53.3±21	62.89±25.21	74.2±52

*p-values (PBSC vs. BM): *p=0.02; °p=0.01; #p=0.0007*

In our study the antigens VLA-4 and LECAM-1 were expressed at a significant lower percentage on CD34+ mobilized PBSC than on steady-state BM CD34⁺ cells. When we considered the ABC units per CD34⁺ cells, only LECAM-1 showed a significantly higher amount of ABC on CD34⁺ steady-state BM cells than on mobilized ones. In contrast, no significant differences were found for the other adhesion molecules. Our results suggest that a low VLA-4 and LECAM-1 expression rate on mobilized CD34+ cells could be of relevance in the mechanisms of hemopoietic progenitor cell peripheralization.

104
Immunomagnetic selected CD34⁺ autotransplant for treatment of severe LES

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Recently ABMT was proposed as a possible strategy for the treatment of severe or poor-prognosis

autoimmune diseases in light of results from animal models and incidental reports from patients with autoimmune disease secondary to hematologic malignancies. We performed an autologous $CD34^+$ immunomagnetic selected autotransplant in a 16-year-old girl affected by non responding LES with immunomediated anemia and thrombocytopenia. The patient was mobilized with CTX 4 g/m^2 + G-CSF 5 µg/kg/day after informed consent by the parents and approval by the ethical committee had been given.

On day +9 a leukapheresis procedure was performed ($CD34^+$: 0.10138×10^9/L; WBC: 7.4×10^9/L). Immunomagnetic selection using Isolex 300 (Baxter) ensured a $CD34^+$ cell yield of 42% with a purity of 90.76% with a three log T-cell depletion. Selected product was resuspended in 10% DMSO solution and stored in liquid nitrogen. A second unselected leukapheresis product was stored as back-up. The conditioning regimen consisted in CTX 50 mg/kg at day -6 and -5 + GAL 10 mg/kg on day -4, -3,-2; +PDN 1 g day -3, -2, -1. The total $CD34^+$ cell count reinfused at day 0 was 3.88×10^6/kg. $CD3^+$, $CD4^+$ and $CD8^+$ cell counts reinfused were respectively 2.18×10^4/kg, 1.02×10^4/kg and 1.8×10^4/kg. ANC $>0.5\times10^9$/L and PLT $>20\times10^9$/L were achieved respectively on day +5 and +7. At the time of writing the patient is well with a normal blood count without any treatment. Our experience demonstrates the feasibility and the safety of $CD34^+$ autotrasplant for treatment of autoimmune diseases; however, a longer follow up is necessary to evaluate the efficacy of this new treatment strategy.

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105
Autologous platelet support in patients with breast cancer receiving high-dose chemotherapy and circulating progenitor cell transplantation

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Purpose. To evaluate the possibility of supporting the phase of thrombocytopenia following high-dose chemotherapy (HDC) and circulating progenitor cell (CPC) transplantation by autologous platelet concentrates (PC).

Patients and Methods. PC were collected from 12 patients undergoing HDC and CPC transplantation for high-risk breast cancer (BC). A single plateletpheresis was performed at rebound after high-dose cyclophosphamide (HDCY), when platelet count exceeded 250×10^9/L. PC were cryopreserved in 5% DMSO after controlled-rate freezing and stored in liquid nitrogen. *In vitro* studies of cryopreserved platelets (aggregation, ATP release and change of mean platelet volume induced by EDTA) along with tumor detection by reverse transcriptase-polymerase chain reaction (RT-PCR) for cytokeratin 19 (CK19) were performed. When platelet counts dropped below 20×10^9/L following HDC (thiotepa 600 mg/m^2, L-PAM 160 mg/m^2) and CPC transplant ($CD34^+$ cells $>5\times10^6$/kg), PC were thawed in a 37°C water bath, centrifuged to remove DMSO, resuspended in autologous plasma and reinfused within one hour.

Results. Large quantities of platelets were harvested in all patients (median 6.5×10^9, range 4.9-12). *In vitro* studies showed well preserved platelet function as compared to that in both fresh platelets and standard PC. Nine out of 12 patients received autologous PC, one did not require platelet support and two were not transfused with autologous platelets due to the detection of CK19 mRNA in the apheresis product. At the time of transfusion most of the patients were febrile (>38°C) and had mucositis >G2. The median number of platelets reinfused was 3.3×10^{11} (range 2.5-5.1) with a median loss during the freeze-thaw-wash procedure of 37%. Autotransfusion was able to maintain platelet count above 20×10^9/L in all patients, with a corrected count increment (CCI) > 7.5 in 6 of 9 cases. Only one patient required an additional transfusion due to epistaxis, which occurred when the platelet count was greater than 20×10^9/L. No side effects related to PC infusion were recorded. Eleven control patients who received the same HDC and a similar number of $CD34^+$ cells required a total of 12 allogeneic PC units.

Conclusions. Large doses of autologous platelets can be collected easily and safely administered to support the period of thrombocytopenia in patients with BC receiving HDC and CPC support. Autologous PC in these patients can abrogate the risks deriving from allogeneic platelet transfusion.

106
Primary resistant or relapsed Hodgkin's disease (HD): relevance of high dose chemotherapy with autologous stem cell transplantation (ASCT) in a retrospective study

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Patients with primary resistant or relapsed HD often fail to achieve complete remission with conventional chemotherapy or subsequently relapse. Better results, with failure free survival (FFS) rates of 40 to 50% are reported using high dose chemotherapy with ASCT. Between 1986 and 1996 32 patients with refractary or relapsed HD received high dose chemotherapy followed by ASCT. Three patients were in partial remission, 7 primary resistant after first line chemotherapy, 22 in relapse (1 chemoresistant and 21 sensitive), of which 10 occurring < 12 months from first CR. Fifteen patients were previously treated with only a first line chemotherapy (MA/MA or ABVD), 14 with two lines and 3 with three regimens. Median age was 23 yrs (range 19-39), 19 males and 13 females, 4 PL, 22 SN, 5 CM, 1 DL. Median time from diagnosis to transplant was 22 months (6-161). Relapsed patients received almost one course of standard chemotherapy before ASCT. Nine patients were transplanted with bone marrow, 11 with peripheral blood stem cells (PBPC) and 12 with both. Myeloablative regimens were: CBV in 11 patients, BEAM in 9 and melphalan+mitoxantrone in 12. Median time to reach 500 neutrophils was 15 days and to 50,000 platelets 23 days. Only one toxic death and four cases of severe infection occurred. Complete remission was obtained in 27 patients (79%). At three years overall suvival (OS) is 40%, disease free survival (DFS) 51% and failure free survival (FFS) 28%. Clinical characteristics at the time of transplantation did non influence FFS. FFS was affected by the status of disease at the time of transplantation: refractory or chemoresistant relapsed patients had the worst prognosis, with FFS of 12% at 8 months; patients in partial remission had a FFS of 66% and those with sensitive relapse 53% at three yrs. First complete remission duration did not influence FFS. In conclusion high dose chemotherapy is feasible with low toxicity. This procedure improves the outcome of patients in partial remission or in sensitive relapse after conventional chemotherapy. This approach does not seem to offer an advantage to refractory patients and alternative approaches need to be investigated in this poor prognosis subset of patients.

107
Magnesium chloride (MC) for the prevention and the management of mucositis after allogeneic BMT and autologous hemopoietic stem cell transplantation (HSCT)

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Severe mucositis after high-dose chemotherapy continues to be a prominent clinical problem with no known efficacious remedy. Because of a fortuitous clinical observation and of previous experimental and clinical data, we included MC solution for the management and the prevention of mucositis caused by conditioning regimens. This report is based on 48 consecutive patients (MC group) (mean age 44.7±11.8) prepared for autologous or allogeneic HSCT using conditioning regimens including various chemotherapeutic drugs. Moreover, 77 transplanted patients (mean age 36.4±14.8) are evaluated as historical controls. A total of 41 (38.8%) patients in the control group had a mucositis score III-IV compared to 2 occurrences of mucositis in the 48 MC treated cases (4.2%) (p=0.001). In addition, there was a significant difference between the historical series and MC group in the duration of mucositis (p=0.0001) and in the mean interval from transplant to the onset of mucositis (p=0.015).

Multivariate regression indicated underlying disease, transplant type, source of stem cells, fever duration and mucositis prophylaxis as significant predictive factors of the mucositis score. Furthermore, matched case-control analysis confirmed the lower score of toxicity (p=0.0001) and shorter duration of mucositis (p=0.0010) in the MC group. The mechanism of mucositis reduction by MC is unclear. One possibility is that magnesium exerts a regulatory role on inflammatory cytokine production, probably by altering the Ca2:Mg2 ratio.

108
PCR-detection of residual tumor cells after high-dose chemotherapy: comparison between PBPC and bone marrow harvests

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For a long time, it has been speculated that PBPC represented a tumor-free source of stem cells. In our study, we tested the never proved hypothesis that PBPC were less frequently contaminated by tumor cells than bone marrow harvests (BMH). The grafts were collected after a high-dose chemotherapy program which included a mobilizing treatment with cyclophosphamide 7 g/sm, followed by G-CSF (5 µg/kg). Overall, 216 PBPC and 66 BMH were evaluated (41 patients with low/intermediate grade non-Hodgkin's lymphoma [NHL] and 25 with multiple myeloma [MM]). All NHL patients had a marrow involvement by morphological and/or PCR analysis. Minimal residual disease was assessed by PCR, using the bcl-1, bcl-2 or

IgH gene rearrangements as tumor cell markers (sensitivity 10^{-4}- 10^{-6}). Forty-one of 216 PBPC and 19 of 66 BMH were found to be PCR-negative.

Pts	all PBPC neg.	BMH neg.	PBPC+BMH neg.	only PBPC neg.	only BMH neg.
NHL	10/41	17/41	9/41	1/41	8/41
MM	2/25	2/25	2/25	0/25	0/25

Conclusions. PBPC are not less contaminated than BMH cells.

109
CD34+ selected PBSC autograft in CLL: preliminary results of a multicenter study

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CLL patients with advanced disease have a poor prognosis with a 3 year median survival. The high response rate achievable with FAMP allows the planning of high dose therapy followed by autologous transplantation in those patients aged < 60 years. Also in this setting the use of PBSC can offer advantages but tumor contamination of the graft becomes a main issue. Based on this background we have started a prospective, non randomized multicenter study using $CD34^+$ selected cells in patients with >B stage responding to FAMP. The mobilization of $CD34^+$ cells is obtained by CY 4 g/m^2 + lenograstim 5 µg/kg and followed by positive selection with an immunoabsorption technique. The myeloablative regimen consists of busulfan 12 mg/kg and melphalan 140 mg/m^2. A close monitoring of the response at clinical, histologic, phenotypic and molecular level is planned. Up to now 18 patients have been enrolled; of these 8 underwent collection and selection procedures and 7 have already been transplanted. At mobilization-transplantation time 7 were in CR and one in PR. A median of 383.5×10^6 $CD34^+$ cells were collected after 1-4 aphereses. After positive selection the median of $CD34^+$ cells was 143×10^6 with a recovery of 30% (10-80%) and a depletion of $CD5/20^+$ cells >99%. At transplantation the patients received a median of 2.25 (0.8-2.8)$\times10^6$/kg purified $CD34^+$. $CD5/20^+$ cells infused ranged from 46 to 0.03×10^6/L. All the patients engrafted. Median time to PMN > 0.5 and 1.0×10^9/L was 11 and 13 days respectively; a platelet count >25×10^9/L was reached on day16. Our data show that in chemosensitive CLL an adequate number of $CD34^+$ can also be effectively mobilized after FAMP and that autotransplantation with $CD34^+$ selected cells is feasible. Molecular studies of minimal residual disease (Ig gene rearrangement) will be presented at the meeting.

110
Matched-pair analysis of peripheral blood stem cell transplantation (PBSCT) versus autologous bone marrow transplantation (ABMT) in Hodgkin's and non-Hodgkin's lymphomas: an update of the EBMT lymphoma registry study

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We report the updated results of the matched-pair analysis on patients with Hodgkin's (n=1299) and non-Hodgkin's (n=1915) lymphomas registered in the EBMT Lymphoma Registry aimed at assessing short and long-term advantages of PBSCT over ABMT.

A preliminary analysis of prognostic factors for PFS showed an influence (multivariate analysis) of status at transplant for NHL, sex, size of largest mass at transplant, status at transplant and conditioning regimen for HD. The pair analysis was carried out matching NHL and HD patients separately by their prognostic factors. Additionally, NHL were matched for histology, while both HD and NHL patients were matched for date of transplant. With this method 454 patients were matched in the NHL group, 256 in the HD group. In a previous paper (*J Clin Oncol 1997;15:509-17*) we reported an unexpectedly better OS and PFS for ABMT vs. PBSCT patients in the HD group with no difference in OS or PFS in the NHL group. This appeared to be justified by an increased relapse/progression rate with PBSCT in the HD group (58.6% vs. 40.0% with ABMT). Hematologic recovery occurred significantly faster with PBSCT both in HD and NHL patients. After that report, the follow-up observation has been extended by a year, and the analysis of outcome redone. Relapse/progression rate at 4 years was superior with PBSCT both in HD (51.1% vs 43.6% with ABMT, p=0.0218) and in NHL (56.9% vs 45.8%, p=0.0259). Accordingly, overall surivival and PFS were better with ABMT, both in HD and NHL, but the difference between PBSCT and ABMT became statistically significant in favor of ABMT only in HD for PFS (p=0.048). The poorer results with PBSCT are unexplained and should be confirmed with randomized studies.

111
Effect of AG957, a BCR-ABL-specific tyrosine kinase inhibitor, on chronic myelogenous leukemia progenitor cells

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Chronic myelogenous leukemia (CML) is a clonal disorder of the hematopoietic stem cell characterized by a chimeric BCR/ABL gene giving rise to a 210-kD fusion protein ($p210^{BCR/ABL}$) with in-creased protein tyrosine kinase (PTK) activity. AG957 is a tyrphostin known to specifically inhibit $p210^{BCR-ABL}$ activity. We evaluated the effects of AG957 on the *in vitro* growth of CML-derived multipotent (CFU-Mix), erythroid (BFU-E), granulocyte-macrophage (CFU-GM) and long-term culture-initiating cell (LTC-IC) progenitors. Preincubation (30 min) of $CD34^+$ CML cells with AG957 (1-100 µM) induced a dose-dependent suppres-sion of colony growth. AG957 doses inducing 95% (ID95) growth inhibition of CFU-Mix, BFU-E and CFU-GM were 78, 86, and 85 µM, respectively. AG957 (>50 µM) exerted a significant growth suppression of CML LTC-IC. Normal progenitors were sig-nificantly less inhibited than CML progenitors. DNA electrophoresis demonstrated that preincubation of the BCR-ABL-transfected Mo316 cell line with AG957 (100 µM, 24 hours) induced apoptosis. To increase the apoptotic effect of AG957, exposure of CML $CD34^+$ cells to AG957 was followed by incubation with Fas ligand (1 µg/mL). This treatment resulted in a significant increase of colony suppression. In CML patients at diagnosis, individual colonies were analyzed for the presence of BCR/ABL mRNA by reverse transcription polymerase chain reaction (RT-PCR). Preincubation with AG957 (50 µM) markedly reduced the percentage of CFU-GM expressing the hybrid BCR/ABL mRNA (control samples: 100%, AG957-treated samples: 33%). In con-clusion, our data demonstrate that: (a) AG957 strongly inhibits CML, but not normal, primitive and committed progenitors; (b) the apoptotic effect of AG957 is signifi-cantly enhanced by Fas ligand; (c) AG957-induced destruction of BCR/ABL positive progenitors suggests the use of this tyrphostin for *in vitro* purging.

ALLOGENEIC BONE MARROW TRANSPLANTATION

112
Bone marrow long-term initiating cells are decreased after allogeneic bone marrow transplantation

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We investigated bone marrow (BM) hematopoietic progenitor cells in 37 normal donors and in 20 patients who had been successfully allotransplanted from 1 to 8 years before testing. Transplanted patients had normal blood counts and bone marrow cellularity at the time of the study. Methods included flow cytometric evaluation of CD34+ cells and colony assays for colony forming-unit cells (CFU-C) and long-term culture initiating cell (LTC-IC) measurement. In the LTC-IC assay, the relation between cell input and the output of secondary colonies after 5 weeks of culture was linear, allowing enumeration of the LTC-IC number from the number of mononuclear cells (MNC) plated. By limiting dilution analysis performed in normal donors and in transplanted patients, we determined that approximately 4 colonies were generated by a single LTC-IC; this value was used to extrapolate LTC-IC number from the secondary colonies obtained. $CD34^+$ cells were decreased 3 to 4-fold in the transplanted patients compared to normal donors ($594\pm85/10^5$ total nucleated cells vs 2219 ± 271). Primary CFU-C were decreased 2.1-fold ($22.3\pm3/10^5$ MNC plated vs 55 ± 4), while a 6-fold decrease of LTC-IC was observed in the BM of transplanted patients compared to that of normal donors ($0.3\pm0.07/10^5$ MNC plated vs 1.9 ± 0.4). We found that BM LTC-IC cell number correlated with concurrently determined BM $CD34^+$ cells ($r=0.954$) and BM primary CFU-C cells ($r=0.810$). We conclude that: i) BM stem cell compartments, as measured by the LTC-IC assay, are severely and permanently depressed following bone marrow transplant. The hematopoietic progenitor reduction is less evident if measured by $CD34^+$ cells or CFU-C assay; ii) a complete hematopoietic reconstitution after successful transplant may be sustained by a limited stem cell pool.

113
Comparable outcome of allogeneic bone marrow and peripheral blood cell transplant in adults with hematologic malignancies

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We report the outcome of 111 patients grafted in our Unit between January 1994 and January 1997 from HLA identical sibling donors. 53 patients received unmanipulated peripheral blood cell transplants (PBCT) and 58 received unmanipulated bone marrow grafts (BMT). Patients were prepared with cyclophosphamide (CY) and fractionated total body irradiation (TBI)I (n=64) or CY and thiotepa (n=47). Graft-versus-host disease (GvHD) prophylaxis consisted of cyclosporin A (CyA) and methotrexate (MTX). BMT and PBCT patients were comparable for diagnosis, age, gender, interval from diagnosis and disease phase.

PBCT patients had faster neutrophil engraftment (day +14 vs day +15, p= 0.002), and less days on antibiotics (12 vs 14, p=0.01). The number of days in hospital, total days of neutropenia, acute GvHD, chronic GvHD, and CMV infections, were comparable. Actuarial 3 year transplant related mortality (TRM) was 19% vs 30% (p=0.1), survival was 46% vs 52% (p=0.1), and relapse was 40% vs 45% (p=0.6).

These data suggest that the outcome of allogeneic BMT and PBCT are similar in patients with hematologic malignancies.

114
Allogeneic hemopoietic stem cell transplantation (HSCT) for patients with high risk acute lymphoblastic leukemia (ALL): favourable impact of chronic graft-versus-host disease (cGVHD) on survival and relapse

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Between 1978 and 1996, 170 patients with acute lymphoblastic leukemia (ALL) and a median age of 22 years (1-49), underwent an allogeneic hemopoietic stem cell transplant (HSCT) from HLA-identical siblings (n=149), family mismatched donors (n= 18) or unrelated HLA matched donors (n=3). Of these patients 92% had high risk ALL at diagnosis, 33% were in first remission (CR1) and 85% received an unmanipulated HSCT with cyclosporin-methotrexate for graft-versus-host disease (GvHD) prophylaxis. After a median follow-up of over 6 years, 59/170 patients are alive.

The actuarial 10 year survival is 53%, 38% and 20%, for patients respectively in CR1, CR2 or advanced phase. The main causes of death were leukemia (n=44), acute GvHD (n=15) and infections (n=10).

The actuarial survival of patients with (n=39) or without (n=64) cytogenetic abnormalities, grafted in CR1/CR2 was respectively 45% and 48% (p=0,5).

For CR1/CR2 patients there was a significant reduction of transplant related mortality (TRM) after 1992 (35% vs 10% p=0.01), but no reduction of leukemia relapse (38% vs 30%, p=0.7). In multivariate analysis the presence of chronic GvHD was the most important favorable prognostic factor for survival (p<0.0001) and relapse (p<0.0001).

This study confirms that long term survival can be achieved with HSCT even in ALL patients with cytogenetic abnormalitites; transplant mortality has been reduced making the procedure safer, whereas leukemia relapse is unchanged: the latter is significantly influenced by the occurrence of chronic GvHD. Immune intervention post-HSCT may be considered to address this problem.

115
Neurologic complications in allogeneic bone marrow transplantation

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We retrospectively evaluated the neurologic complications in 349 consecutive allogeneic bone marrow transplants performed in patients (pts) affected by hematological malignancies (196) or non-malignant diseases (153). Early neurologic complications, defined as those occurring during the first 100 days following the transplantation, occurred in 39 patients and were encephalopathy (49%), seizures (38%), cerebrovascular events (8%), peripheral neuropathies (5%).

Encephalopathy was correlated to hyponatremia (Na=110 mmol/L) in one pt, to hyperammonemia in 2 patients, to CsA toxicity in 11 patients, six of these showed typical CsA neurotoxicity consisting of cortical blindness, ataxia and somnolence. In 5 patients the etiology of encephalopathy was undefined. Three subjects experienced seizures during the conditioning therapy with busulfan despite phenobarbital prophylaxis, and one patient, affected by aplastic anemia, during the course of CTX. Seizures were correlated to

hyponatremia (Na=121 mmol/L) in one patient, to hyperpyrexia (T=40°C) in another patient, and to CsA in 9 patients. Cerebrovascular events comprised a sudden massive cerebral hemorrhage, a fatal stroke, and a subdural hematoma successfully treated with conservative measures. The peak occurrence of neurologic complications was in the 3-4th week after transplant. Mortality for neurologic events was 17% of overall early transplant related deaths. The survival in the first 100 days after transplant was 87% in patients without neurologic complications, 78% in patients with isolated seizures or peripheral neuropathies, 27% in patients with encephalopathy or cerebrovascular events ($p<0.001$).

Age, sex, diagnosis, prophylaxis for CNS relapse (prior cranial radiation therapy or intrathecal chemotherapy) before transplant, conditioning regimens (TBI-containing versus busulfan-containing regimens versus other regimens), GvHD prophylaxis (CsA versus CsA+ MTX), acute GvHD ≥ 2, GvHD therapy (LD-PDN versus HD-PDN) were evaluated as risk factors for neurological complications. Univariate analysis identified the following risk factors: prophylaxis for CNS relapse ($p<0.04$), development of acute GvHD ($p<0.03$), and use of HD-PDN for acute GvHD therapy ($p<0.01$). In multivariate analysis, prophylaxis for CNS relapse and use of HD-PDN for GvHD therapy were indipendent predictors of neurologic adverse events.

116
In vivo treatment of severe refractory acute GVHD by antioxidant N-acetylcysteine

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Acute graft-versus-host disease (GVHD) is thought to be initiated by alloreactive type 1 T cells that secrete inflammatory cytokines (TNF-α, IL-1). Efficient T cell proliferation requires costimulation via CD28/B7 or other pathways.

With regards the potential immunomodulating effects of any one of a variety of antioxidant compounds including glutathione, dithiothreitol or N-acetylcysteine (NAC), on TNF production and on co-stimulating molecules (CD80) inhibition, we started a new protocol in which cyclosporin-A (CSA) is associated with NAC infusion in patients affected by severe (III-IV grade) GvHD refractory to conventional therapy (CSA and 6-methylprednisolone).

Between August 1994 and June 1996 four patients: 2 AML (one in 1st complete remission, one relapsed after alloBMT), 1 ALL (2nd complete remission), and 1 chronic phase CGL, received NAC associated with CSA treatment for refractory severe acute GvHD (3 pts grade IV, 1 pt grade III) following related allo-BMT (1 case) and allo-peripheral stem cell transplantation (3 cases). The NAC was administered through a venous catheter at the dosage of 150 mg/kg in 250 mL of saline solution over a period of 30 minutes; then 50 mg/kg of NAC was given over a period of 4 hours for 3 weeks. All the patients received NAC associated with CSA (3mg/kg) i.v.

In all the patients treated we observed an improvement in clinical parameters. In particular, manifestations of GVHD disappeared completely in two patients. One patient received a 21-day NAC infusion; the therapy was interrupted in two patients, one after 7 days and 8 hours because of fatal hemorrhagic complications (cerebral and bowel) and in one after 72 hours due to pulmonary infection. All the patients showed, at the FACS analysis performed 12 h after NAC infusion, a significantly lower percentage of CD8 positive T cells, CD80 and CD25 positive cells.

We observed, *in vivo,* a synergic inhibitory effect on co-stimulatory molecules mediated by NAC e CSA. These data indicate that the effects observed following NAC and CSA administration could be beneficial in the treatment of severe GvHD.

117
Search for unrelated umbilical cord blood (UCB) units for transplantation of high risk leukemic patients

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Searches for unrelated UCB units were conducted for 55 high risk leukemic patients if there was neither marrow donor nor time to find a suitable donor in BMDWW; searches were addressed to the New York, Milan, Düsseldorf and Paris Cord Blood Banks. The objective was to find an UCB unit with proper cellularity (cryopreserved nucleated cells ≥ 1.5×10^7/kg b.w. of the recipient) and HLA matched (≥ 4/6 loci after DRB1 high resolution typing).

For 41 of 55 patients (74%), after a median time of 7 days (1-534), we found 146 units which were HLA matched for ≥ 4/6 loci by serologic typing (HLA class I) and low resolution DNA typing (HLA class II).

Cellularity criteria reduced the number of suitable UCB units to 83 (57%) on which DRB1 high resolution typing was performed. Finally, according to our criteria, 24 units were fully eligible for transplantation in 19 of the 55 patients (35%). Median time from the

start of the search to full eligibility was 37 days (15-482). After a median time of 76 days (49-106) from the beginning of the search, 10 of the 55 patients (18%) have been transplanted with unrelated UCB, so far. After a median follow-up of 5 months from transplantation and 7 months from search, 2 patients relapsed and died of the disease, 1 died in complete remission (sepsis) and 7 are still alive in complete remission. Actuarial probability of relapse-free survival from transplantation is 60% at 18 months. Actuarial probability of survival from search is 55% in the transplanted patients and 38% in non-transplanted patients (not significant).

We conclude: 1) 35% of high risk leukemia patients have found a suitable source of stem cells for transplantation through searches in Cord Blood Banks; 2) transplantation has been performed within 4 months of the search in alla cases; 3) larger groups of patients should be analyzed to compare the outcome of transplanted to non-transplanted patients.

118
Screening tests for predicting the extensive c-GVHD after allo-BMT

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This prospective study was aimed at evaluating the predictive value of some screening tests for the subsequent development of extensive chronic GvHD (c-GvHD). The study was performed on a series of patients submitted to allo-BMT by using univariate time to an event analyses. Forty patients entered the study; 31 of them with a minimum follow up of 250 days and without evidence of extensive c-GvHD at day +100 were analyzed. They had received the same schedule of post transplant immunosuppressive therapy. The following screening tests were performed at day +100: physical examination, complete blood counts, liver function tests, skin biopsies, Schirmer's testing, pulmonary function abnormalities, previous history of acute GvHD, donor recipient sex matching, previous CMV infection, presence of autoantibody, occurrence of limited c-GVHD. Age as predictive factor was analyzed as a continuous variable. None of the above parameters seems statistically predictive of c-GvHD. We noticed a trend towards developing c-GvHD in patient aged > 30 years, patients who had a previous CMV infection, or who had pulmonary function abnormalities. P values and HR were respectively: p = 0.07; 0.05; 0.06; HR = 0.155; 0.156; the large confidence interval means more patients to be enrolled.

119
Peripheral blood stem cells (PBSC) allograft. GITMO experience

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In May 1996 we recorded 86 patients as having been transplanted with peripheral blood stem cells (PBSC) in 7 institutions of the GITMO network. The patients, suffering from advanced onco-hematogical diseases were transplanted with PBSC of siblings either identical or mismatched for one antigen (2/86 pts.), mobilized with growth factor, overwhelmingly G-CSF (85/86). Median age was 42 years (range 3-57). In 6 out of 86 patients PBSCs were used as a second transplant. Diagnosis was ANLL in 22 patients, ALL in 17, CML in 16, NHL in 10, HD in 3, MM in 2, MDS in 12, myelofibrosis in 3 and SAA in 1. Conditioning regimens were Cy-TBI 9 patients, Bu-Cy in 28, thiotepa-Cy in 39 and others regimens in 10. GvHD prophylaxis was cyclosporin-A alone in 8 cases, methotrexate alone in 1 case and Cy-A plus MTX in 77. A median of 2 leukoaphereses (range 1-4) allowed the harvest of a median of 12.8×10^8/kg mononuclear cells, 7.45×10^6/kg $CD34^+$ cells and 4.05×10^8/kg $CD3^+$ cells. All patients achieved a stable engraftment of neutrophils of 0.5×10^9/L at a median of 14 days (range 8-29), while time to platelets more a 20×10^9/L was 14 days (range 9-153). The rapid recovery was associated with a median of 6 days of fever and a median of only 6 packed red cell transfusions and 4 platelet transfusions. Acute GvHD > II was present in 39/86 patients (45.3%), while chronic GvHD was evident in 59/83 (67%) patients at risk and was extensive in 24 (33%). Overall survival has not yet reached the median after 33 months with 19 patients still at risk after 21 months. The event free survival reached the median at 17 months, while none of 19 patients still at risk has died or relapsed. The disease free survival never reached the median with 62% of patients at risk. For overall survival and event free survival there was a significant difference for patients transplanted in standard versus high risk. Transplant related mortality was 21/86 patients (24.4%), while disease related mortality was 11/86 patients (12.79%) without any significant difference between the two risk groups (p=0.12). These data do not differ from data obtained with bone marrow transplantation; we await the for results of prospective trials still in progress.

120
Role of mixed lymphocyte cultures in the prognosis of GVHD

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Mixed lymphocyte cultures (MLC) are considered one of the first screening tests for the compatibility of bone marrow transplantation between HLA identical subjects and between unrelated donor and recipient.

"One way" and "two way" MLC are distinguished thus:

1. "One way". One of the two lymphocyte populations is irradiated and plays the role of antigen whereas the other population contains the proliferating cells (relative response-RR).
2. "Two way". Both lymphocyte populations examined are able to proliferate.

In this study we evaluated the correlation between the MLC results and the development of acute GVHD (AGVHD). With this aim we retrospectively examined both "one way" and "two way" MLC in 105 couples of subjects with an HLA identical donor admitted to an allogeneic BMT. The results of the two way MLC, expressed in counts per minute, were related to different cultures of recipient, donor and control (not HLA identical cells compared to recipient and donor cells) as follow:

A. "two way" counts x 100/(recipient cells not irradiated + control cells irradiated counts)+(donor cells not irradiated+control cells irradiated counts).

B. "two way" counts x 100/(recipient cells not irradiated+donor cells irradiated counts)+(donor cells not irradiated+receiving cells irradiated counts).

The RR and the "B" formula did not have significant results in the AGVHD onset evaluation, while the results of the two way "A" formula could be considered significant ($p<0.001$) for values > 136%. Though the "two way" MLC can not substitute other more indicative tests such as CTLp analysis this study showed that if can be an easy and fast evaluation of the probability of AGVHD development at the time of the first selection of related and unrelated donors.

121
Cord blood stem cells transplant in β thalassemia major patients: preliminary data

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The use of cord blood is a new therapeutic approach in *non conventional* therapy for β thalassemia major patients. We report our experience of cord blood stem cell transplantation in two β thalassemia major transfusion dependent patients (one male and one female). Clinical and hematological features are listed in the table below.

Case	*ß gene mutations*	*Age at transplant (years)*	*Ferritin level*	*Weight (kg) (ng/mL)*	*Hepatomegaly (cm)*
SAM (F)	-87/IVS1-1	3.2	900	12.5	2
MA (M)	IVS1-110/IVS1-6	5.7	800	21	1

Both patients were the first child of their family and for the second pregnancy their respective mothers underwent prenatal diagnosis that confirmed fetuses were not affected. Prenatal diagnosis was performed in our Centre by CVS at 10 weeks gestation. At birth of the sibling cord blood was collected (see table below) and cryopreserved in Milan's Cord Blood Bank.

Case	*Volume of cord blood (mL)*	*WBC total ($x10^6$)*	*CFU-GM total ($x10^3$)*	*$CD34^+$ total ($x10^6$)*
SAM	50	532	274	
MA	86	812	430	3.65

The patients were HLA identical to HLA typed on cord blood so they underwent cord blood transplant in Pavia after a conditioning regimen consisting of busulphan, thiotepa and cyclophosphamide.

Case SAM also received bone marrow stem cells (1.2×10^8/kg) because the number of cord blood stem cells was considered not to be sufficient. She is now at +210 days and has Hgb 11.6 g/dL, WBC 7,000/mm^3 and PLT 222,000/mm^3. Case MA is at +75 days and has Hb 10.6 g/dL, WBC 5,000/mm^3 and PLT 101,000/mm^3. Both patients have no sign of GVHD and are under prophylaxis with cyclosporin (10 mg/kg/die).

We think that when cord blood transplantation is possible it offers many advantages with respect to bone marrow transplantation. There are some limitations due essentially to the volume of cord blood collected and to the weight of the patients undergoing transplantation.

122
Umbilical cord blood (UCB) transplant from unrelated mismatched donors in patients with high risk (HR) leukemia

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Eligibility for UCB unrelated transplant in 55 HR leukemia patients (pts) was evaluated considering

patient criteria [*disease stage: HR-1st CR or >1st CR or relapse with < 30% of blasts; *absence of marrow donor or no time for search in BMDWW; *autologous bone marrow rescue]; and *UCB unit criteria* [*HLA matched or < 2 loci mismatched after DRB1 high resolution typing; *number of cryopreserved NC >1.5×10^7/kg b.w. of the recipient]. On the base of these criteria, at least one unit was identified for each of 41 candidates with a median of 7 days (range 1-534); high resolution DNA typing of DRB1 could define within a median of 37 days (range 15-482) full eligibility for 19 pts; 10/19 underwent transplant after a median time of 76 days (49-106) from the beginning of search. The underlying disease of these 10 pts with a median age of 9 years (range 2-16) and median body weight of 30 kg (range 12-67) was AML=2 (1 CR1, 1 CR2), and ALL=8 (1 CR1, 3 CR2, 3 relapse, 1 Ph+Acc.P). All patients received UBC unit from unrelated 1 locus (n=8) or 2 loci (n=2) HLA mismatched donors. All pts were prepared with an identical conditioning regimen consisting of F-TBI, VP16, CTX and ALS. CSA and 6-MPr were administered for GVHD prophylaxis. The median dose of infused viable cells was: NC 2.6×10^7/kg (range 1.4-7.9), CD34$^+$ cells 3.4×10^5/kg (range 1.6 - 8.9) and CFU-GM 2×10^4/kg (range 1-13.9). At DNA polymorphism study, the hematopoiesis was full chimeric in 9 of 10 pts at day +20 and in 7 of 10 pts at day +35. Three pts showed no hematologic engraftment (2 spontaneous autologous reconstitution, 1 BM rescue); 3 pts (2 relapse ALL, 1 CR-HR-AML) died because of relapse (n=2) or sepsis (n=1); 5 pts developed grade I acute GVHD. At a median follow-up of 9 months (range 1-22), 7 of 10 (70%) pts are alive in CR. Full chimeric hemopoiesis is present in 5 of these 7 pts. The following preliminary conclusions can be drawn: 1) the short time for the search is a relevant advantage for pts with high risk leukemia; 2) DNA polymorphism studies are particularly useful for monitoring and predicting engraftment; 3) the availability of cryopreserved autologous marrow is mandatory; 4) an early autologous hematopoietic reconstitution after unrelated mismatched UCB transplant is possible.

INFECTIONS IN IMMUNOCOMPROMIZED HOST

123
Early discharge and home care management of therapy-induced neutropenic aml patients

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Home care management of infections in low risk neutropenic patients with cancer has been shown to be a safe and effective alternative to hospitalized care, leading to an improvement of the quality of life, being more cost-effective and freeing inpatient beds in oncologic institutes.

A *Home Care Unit-ROMAIL* (HCU) has been implemented in 1994 at our Hematological Center in Rome. Our HCU is composed of several physicians and nurses and a welfare officer. A doctor on continuous duty is available 24 hours a day, all year round. Of 280 patients with hematologic malignancies, most of them in advanced phase, who received domiciliary assistance, 12 were discharged early still in a therapy-induced neutropenic phase (absolute neutrophil count (ANC) < 500 cells/mm^3). Seven of the patients were males and 5 females, with a median age of 41 years (range: 21-60). All of them were *in-patients* and affected by AML: 4 patients received induction treatment, 7 patients received consolidation treatment and one patient autologous bone marrow transplantation. Informed consent was given before discharge. The median interval from the last chemotherapy was 22 days (range: 10-40). The median ANC was 30 cells/mm3 (range: 0-300). All patients were in good condition (median Karnofsky status was 70, range 60-80). Nine patients were discharged early to their homes while still on antibiotic intravenous therapy (median time 3 days, range: 1-15), 1 patient while still on amphotericin-B treatment and 2 patients while taking prophylactic oral antibiotics (ciprofloxacin).

The median number of home care days was 7 (range: 2-15). The patients on intravenous therapy (8 on antibiotic treatment and 1 on antifungal treatment) continued their therapy at home without clinical complications until ANC exceeded 500 cells/mm^3 (median ANC 1.000 cells/mm^3, range: 700-3800). Three patients (25%) were re-hospitalized: 2 patients developed recurrent fever, 1 patient showed worsen-

ing of general conditions for prolonged cytopenia. The 2 patients readmitted for recurrent fever had brief and uncomplicated hospitalization. Five (41%) patients required red cell and platelet transfusions. The total number of home care days was 77; the medical and nursing accesses average was 0.72/day and 0.59/day respectively. No life-threatening medical complications occurred in our study group of patients.

Our preliminary data seem to indicate that close medical surveillance and ready availability of emergency care makes possible home care management of therapy-induced neutropenic patients while on antibiotic treatment. However further studies are required for better identification of the criteria for safe and effective early discharge strategy also for patients at risk of developing infections during the neutropenic phase.

124
Revisited indications for bone marrow biopsy in HIV-infected patients

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HIV-infected patients frequently undergo bone marrow biopsy for different reasons: 1) to investigate a persistent and unexplained fever, with microbiological cultures too; 2) to diagnose cytopenias; 3) to diagnose and to stage lymphomas. From 1986 to 1996 we performed 350 bone marrow biopsies in HIV patients: cytological, histological and microbiological studies were carried out on the specimens. Most of biopsies, approximately 85%, showed unspecific findings: hypo/hypercellularity with dysplastic features of one or more hematopoietic lineages (dysmegakaryopoiesis was almost always present), associated with an increase in lymphoid cells (interstitial/paratrabecular aggregates/nodules) and plasma cells.

Fifty bone marrow biopsies (14.3%) were positive, 27 (6,6%) for hematological neoplasms (17 non-Hodgkin's lymphomas NHL, 6 Hodgkin's diseases HD, 2 multiple myelomas, 2 B-ALL) and 23 (6.6%) for infections (16 disseminated mycobacterial infections, 1 CMV, 1 cryptococcus, 1 leishmania, 4 granulomas).

Persistent and unexplained fever was the main indication for biopsy (70%); cytopenia the reason for 22% and lymphomas for 8%. In lymphoma patients, bone marrow biopsies were performed for staging in 23 cases (17 NHL, 6 HD) while in 7 cases (5 NHL, 2 HD) they allowed the diagnosis. Hypoplastic bone marrow was found in 40% of HIV patients, generally due to combined myelotoxic drugs. Apart from lymphoma staging, bone marrow biopsy is a useful procedure in the investigation of a persistent and unexplained fever: 1) to diagnose mycobacterial infections, where the biopsy is the most rapid procedure which allows diagnosis of disseminated infection and enables antimycobacterial therapy to be initiated before culture results; 2) to search for malignant lymphoma, since the bone marrow biopsy can be, though rarely, the initial site affording the diagnosis. In cytopenic patients, apart from cytopenias due to mycobacterial infection and hypoplastic marrow, bone marrow biopsy showed unspecific dysplastic features which had no relevance to therapeutic strategy.

125
Invasive pulmonary aspergillosis (IPA) in hematologic neoplasms: clinical manifestations and TC radiographic findings

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Invasive pulmonary aspergillosis (IPA) is most commonly seen in hematologic patients. The two major predisposing factors are neutropenia and high-dose corticosteroid therapy. In neutropenic patients development of pulmonary infiltrates may initially be absent, owing to the paucity of the inflammatory response, and fever may be the earliest manifestation of pulmonary aspergillosis. The radiographic pattern is variable; sometimes chest X ray may be normal when studied in the acute phase. Early computerized tomography chest scan (CT) findings include the *halo sign*, a distinctive zone or halo surrounding a round pulmonary mass, having an attenuation lower than that of the center of the mass. The CT halo sign appears early in the course of infection during bone marrow aplasia, before air-crescent formation or cavitation. In severely neutropenic patients its presence strongly suggests IPA. Since 1987 we have studied 42 patients with IPA (23 AML, 11 LAL, 3 MDS, 5 NHL), 39 with severe neutropenia (neutrophils $< 0.5\times10^9$/L) and thrombocytopenia (platelets $< 20\times10^9$/L), Diagnoses were autoptic in 16 patients. Chest X ray showed a single nodule in 4/42 cases (9%), multiple nodules in 10/42 (23%), a single infiltration in 7/42 (16.6%), multiple infiltrations in 13/42 (31%), cavitations in 11 /42 (26%), and mycetomas in 7/40 (16.6%). Three patients had fatal hemophtysis. Bronchoalveolar lavage was performed in only 16 patients because of clinical deterioration and/or severe thrombocytopenia and was positive in 8 (50%). At onset of infection chest X ray was negative in 6/42 (14%) patients. CT scan was performed in 19 patients and showed extension of pulmonary mass and/or cavita-

tion not seen on the chest X ray in 12/19 (63%) patients. In conclusion, in leukemic patients and in candidates for BMT the early presumptive diagnosis of IPA can change the therapeutic program; prompt chest CT provides a non-invasive method of establishing or substantiating the early diagnosis of IPA, often at a time when radiographic chest film findings are non-specific, fungal cultures remain negative and biopsy procedures are prohibited by the thrombocytopenia and compromised respiratory status.

126
Encephalitis in patients with acute leukemia. An unusual cause of death in complete remission

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We describe the clinical course and outcome in 4 patients with acute leukemia (2 AML and 2 ALL, M/F 2/2, average age 64 yrs, range 54-71). All patients, who were in hematologic complete remission, developed tremors, lack of coordination, gait disturbance, memory loss and progressive impairment of consciousness until coma. The median time between the onset of neurological signs and death was short, 21 days (range 7-50). In all patients we did not observe systemic or CNS hematological relapse. Microbiological studies of serum and liquor were performed. Tests for bacteria, CMV, Herpes simplex and zooster, Papova, JVC, Cocksackie B1-B6, measles, *Rubeola*, *Criptococcus*, *Toxoplasma gondii*, *Aspergillus* and *Candida* were all negative, on the histopathological specimens, too. There were no metabolic abnormalities. Brain CT scan, performed in all patients, showed: *ex vacuo* cephalohydrocele and cortico-subcortical degenerative processes. EEG showed diffuse slow activity. Only 2 patients had high fever (>38°C). At autopsy seromeningitis was demonstrated in only one case. At microscopic histology all patients had the same changes: multifocal leucoencephalitis with spongiform changes of the medullary center, diffuse gliosis with astrocytosis and microglial proliferation with a large nucleus and an inclusion body. We were not able to identify the nature of these inclusions neither by immunohistochemistry or *in situ* hybridization.

Encephalitis in patients with hematologic malignancies is rarely reported in literature. Usually *Herpes zoster* is responsible for necrotic encephalitis and Papova-virus for multifocal leukoencephalitis. More recently rare cases of subacute spongiform post-transfusional encephalitis have been reported. In our series none of the patients had a clinical or pathological picture that suggested any of these diseases. In conclusion the dramatical and fatal outcome of our patients suggested the possibility of an encephalitis of unidentified causes. Further studies on larger numbers of patients are needed to evaluate the real impact of this complication.

THALASSEMIA AND HEMOGLOBINOPATHIES

127
HB bronte and HB Maddaloni-Caserta: two new α_2 globin gene alleles in families from Southern Italy

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We studied molecular defects causing α-chain variants in families from Southern Italy. Blood samples were collected in EDTA-K3; hematologic parameters were obtained by standard methods; hemoglobin was analyzed by HPLC, electrophoresis and isoelectrofocusing; DNA was purified from white blood cells with a salting-out method; the DNA sequences of the α-globin genes was identified by PCR amplification and direct sequencing of ssDNA.

Eight hemoglobin variants were characterized and identified by DNA sequencing. Out of them, one is common, five are rare and most probably due to de novo mutations, two are new. Hb J-Oxford or α15(A13)Gly→Asp – frequently detected in Southern Italy – was found to be due to the α1 cod 15 GTA→ATA substitution. Out of the rare hemoglobins, Hb G-Waimanalo or α64(E13)Asp→Asn was found to be due to the α1cod 64 GAC→AAC mutation; Hb O-Indonesia or α116(GH4)Glu→Lys was due to α1 cod 116 GAG→AAG; Hb Prato or α31(B12)Arg→Ser was due to α2 cod 31 AGG→AGC; Hb Stanleyville-II or α78(EF7)Asn→Lys was due to α2 cod 78 AAC→AAA; Hb Sun Prairie or α130(H13)Ala→Pro was due to α2 cod 130 GCT→CCT.

The two new hemoglobins were due both to new alleles of the α2-globin gene. The first new allele *α2 cod 93 GTG→GGG* caused the synthesis of the α93 (FG5)Val→Gly variant chain. The new hemoglobin, named Hb Bronte, was 6% of peripheral Hb instead of the expected value of around 25%; this reduction is most probably due to molecular instability, considering that the region of the mutation is essential for

Hb molecule assembly. A similar defect is presented by Hb Nottingham due to a mutation in the same position (FG5) of the β-globin chain. The other new allele *α2 cod 26 GCG→ACG* caused the synthesis of the α26(B7) Ala→Thr globin variant and of a new hemoglobin, named Hb Maddaloni-Caserta. This hemoglobin was 3% of the total Hb and was associated with reduction of the MCV and MCH. The low percentage is most probably due to decreased production of mRNA for the Hb Variant caused by the G→A substitution in a cryptic splicing site at codons 25-27 (ATG↓GTGCGG) (ATG↓GTACGG).

This mutation might cause the activation and preferential utilization of the cryptic splicing site instead of the IVS-I normal 5' splicing site at codon 30 (GAG↓GTGAGG). This molecular pathogenesis appears to be similar to that shown by Hb E.

128
Genotype and clinical correlation study of 126 patients with thalassemic syndromes (β/β, β/δβ, s/β thalassemia) from the province of Reggio Calabria

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The β globin gene defect was studied in 67 thalassemia major (TM), 16 thalassemia intermedia (TI), 18 β/δβ or β/Hb Lepore and 25 Sickle/β (S/β) thalassemia patients, for a total of 126 patients with a mean age of 22.9 years. The vast majority (95%) of defects were identified by a commercially available standardized assay (β globin strip A, Nuclear Laser Medicine) based on the reverse dot blot method. This assay allows the identification of 9 β globin gene defects, namely c39, IVS1-110, IVS2-745, IVS1-1, IVS1-6, IVS2-1, -87, HbS, HbC.

The remaining mutations were identified by ARMS or DGGE techniques. The frequency of thalassemic mutations in patients originating from the province of Reggio Calabria was as follows: IVS1-110 30.5%, c39 23.5%, IVS1-6 12.8%, IVS2-745 11.8%, IVS1-1 8.6%, IVS1-5 1.1%; IVS2-1, IVS1-2, c8, c44, –101 were demonstrated in one chromosome each (0.5%) while in 1.1% of cases the mutation was not identified. The different genotypes and the presence of β^+ mild mutations (–87, IVS1-6 and –101) were correlated to the main clinical features (age, trasfusion requirement, iron overload, organ complications, etc.). In TM patients, the presence of a β^+ mild mutation was associated with a more favourable clinical course. All TI patients presented at least one β^+ mild defect. Nine β/δβ patients were transfusion-dependent and all bore a β° or β^+ severe mutation. Out of 25 S/β patients only 2 cases had a mild defect (namely -87 and δβ) and both cases were diagnosed in adulthood (60 and 45 years, respectively).

In conclusion: 1) the commercially available standardized assay appears useful and reliable; 2) IVS1-110, -87 and IVS2-745 mutations have a specific high frequency in our geographic area; 3) the genotype is related to the clinical behaviour of these patients.

129
Unstable variants Hb Gun Hill, Hb Koln and Hb Sun Prairie: variability of clinical hemolytic disorders

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Unstable hemoglobin variants cause hemolytic chronic anemia, but the clinical and metabolic disorders are quite different and are correlated with the molecular defects and with the globin genotype patterns of the patients. We analyzed β- and α-globin genes and globin chain synthesis *in vitro* in three patients and report here correlation of genotype and phenotype.

The first patient, male, two years old, was moderately pale. No clinical, metabolic or hematologic disorders were detected. Bilirubin was 0.5 mg/dL, serum-iron 78 μg/dL, ferritin 25 ug/mL, Hb 11 g/dL. HPLC revealed an abnormal hemoglobin (11%) and increase of Hb F (10%). DNA sequencing of the β-globin gene indicated that the patient was heterozygous for a *de novo* deletion of codon 91-95 causing the synthesis of Hb Gun Hill characterized by the loss of Leu-His-Cys-Asp-Lys amino acids. This variant chain is incapable of binding heme groups but its conformation is sufficiently intact to permit combination with α-chain and the formation of a molecule which is partially stable.

The second patient, male, 10 years old, was affected by anemia and had jaundice and splenomegaly. He had a hemolytic crisis characterized by dark urine and increase of jaundice. Hb was 10.1 g/dL, reticulocytes 11%, bilirubin 3 mg/dL. Heinz bodies and RBC alterations were not present. Hemoglobin analysis by HPLC or electrophoresis did not reveal abnormal hemoglobins. DNA analysis indicated that the child was carrier of a *de novo* mutation, the GTG→ATG substitution at β-codon 98 leading to Hb Koln; the Val→Met substitution renders the molecular structure unstable.

The third patient, male, 25-year-old, had been

affected by jaundice for about 10 years and had hepatospenomegaly. Hematologic pattern was of α-thalassemia type (Hb 12.8 g/dL, MCV 67 fL, MCH 21 pg, Hb A2 2.2%); there were 3% of reticulocytes , bilirubin ws about 5 mg/dL. Abnormal hemoglobins were not detected by HPLC or electrophoresis. DNA analysis revealed that the patient was compound heterozygous for α^+ thalassemia (-α3.7) and α2 cod 130 GCT→CCT leading to the Ala→Pro substitution and to Hb Sun Prairie. The father and one brother were carriers of the Hb variant, but had only a slight increase of bilirubin and no clinical symptoms. Hb Sun Prairie is unstable because – as expected – the introducion of a Pro residue in the middle of a helix disturbs molecular stability.

These observation suggests that hemolytic alterations are mild in the carriers, but are increased in association with α-thalassemia because of the high relative percentage of chain variant.

130
Triplicated α gene and heterozygous β thalassemia: clinical studies

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Triplicated α gene gives an excess α globin chain synthesis resembling a β thalassemia mutation. Asymptomatic when in a heterozygous state, the ααα gene in association with a β thalassemia mutation may give a variable intermediate phenotype.

In this paper we report hematological, molecular and clinical data from 23 Apulia adult patients who are β thalassemia carriers; 21 of these patients are also heterozygous and 2 homozygous for the triplicated a gene. In all these patients ααα gene is anti-3.7. The two patients with homozygous ααα gene are both also carriers of a β° thalassemia mutation and present a variable clinical β thalassemia intermedia phenotype. Seventeen of the 21 patients heterozygous for the ααα gene are β° carriers; they have extremely variable clinical and hematological features; in fact, one is a β thalassemia major patient and transfusion dependent, 11 patients have clinical β thalassemia intermedia with quite variable features and 5 have a β thalassemia carrier phenotype.

The other 4 patients with heterozygous ααα gene are, in contrast, β^+ carriers; 2 of them show a β thalassemia carrier phenotype whereas the other 2 have β thalassemia intermedia.

Fetal hemoglobin is increased in all these patients but at present neither this increase nor the imbalanced a\nona globin chain synthesis or the different β thalassemia mutations (β° or β^+) can explain the variability of the clinical features.

131
Survey of sickle cell disease in Italy

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Objectives. The present study was designed to determine the distribution and severity of sickle cell disease (SCD) in Italy.

Design. For the collection of data, a questionnaire was sent to all Italian centers of Pediatrics and Hematology.

Results. A total of 696 cases were reported. The distribution of registered patients shows that, although the S gene originated mostly in Sicily and Southern Italy, 20% of patients with SCD now live in Central and Northern Italy. Forty-four patients (6%) had non-Italian parents. The types of SCD reported were as follows: compound heterozygotes HbS-β thalassaemia, (S-Th, 518 cases); homozygotes for HbS, (S-S, 149 cases); compound heterozygotes HbS and another abnormal hemoglobin (21 cases). The population of patients with SCD is younger than the general Italian population. More than 90% of patients have had no crises or only a limited number, namely, up to 6/year. Infections ranged between 0 and 6/year. Splenomegaly was reported in 28% and 80% of adult patients with S-S and S-Th, respectively. The prevalence of gallstones was 48%.

Conclusions. The survey established that 1) sickle cell disease is widely distributed in Italy; 2) while the clinical spectrum is extremely variable, severe forms are infrequent; and 3) the clinical condition of patients with S-Th is generally less severe than that seen in patients with S-S.

THROMBOSIS AND HEMOSTASIS

132
Prevalence of gene mutation predisposing to thrombophilia in patients with venous thromboembolism

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We carried out a screening for inherited thrombophilia in 247 patients with venous thromboem-

bolic disease consecutively referred to our center (M/F 112/135, median age 36.5 yrs, range 10-65). In all patients we measured antithrombin III and protein C levels (amidolytic methods), total and free protein S levels (ELISA); moreover, we searched for factor V 1691G→A mutated allele (FV Leiden, cause of activated protein C resistance) and for factor II 20210G→A mutated allele. In 192 of such patients we searched for the MTHFR 677C→T mutated allele, possible cause of hyperhomocysteinemia in homozygous carriers. These mutated alleles were also investigated in a control group of 144 individuals (M/F 59/85). Deficiency of coagulation natural inhibitors has been detected in 15 patients (AT III=2, PC=11, PS=2) (6%). The homozygous mutation MTHFR C677T was detected in 36 patients (18.7%) and 24 controls (16.6%), appearing as a gene polymorphism with no effect on the venous thrombotic risk. The mutation FV G1691A (factor V Leiden) was found in 41 patients (37 heterozygotes and 4 homozygotes) (16.5%) and in 3 controls (2 heterozygotes and 1 homozygote) (2%); the mutation FII G20210A was found in 16 (heterozygous) patients (6.4%) and 3 (heterozygous) controls (2%). Another 4 patients (1.6%) were carriers of a double defect FV G1691A + FII G20210 A, not detected in any control. In our experience an inherited predisposition to thrombophilia can be detected in at least 30% of the patients with venous thromboembolic disease.

133
Risk of recurrence in patients with deep vein thrombosis and heterozygous mutation for factor V Leiden

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We retrospectively studied 119 patients (M/F 51/68) with heterozygous factor V Arg506Gln mutation (factor V Leiden) and previous deep vein thrombosis (DVT) confirmed by objective methods. In 79 of them (66%) a risk factor was present. The total time of follow-up after the first thrombotic event was 902 years (median 5 years). Each patient was matched with a control individual with previous DVT and paired for sex, age at the clinical onset, type of DVT (idiopathic or with a concurrent cause), total years of follow-up; in each control thrombophilia was previously ruled out by laboratory investigation. Forty-four Leiden individuals (36.9%) and 33 controls (27.7%) had recurrent DVT; idiopathic episodes were 32 (26.8%) in the Leiden individuals and 22 (18.4%) in the controls. The incidence /100 pt-years of first recurrence was not significantly different between the two groups (see Table below).

In both groups the incidence of idiopathic recurrence was higher in the patients with a first idiopathic event than in those with an associated risk factor for DVT, yet not significantly. In conclusion the occurrence of a first DVT does not imply that secondary prophylaxis with oral anticoagulants should be lifelong in heterozygotes for factor V Leiden.

Recurrence of DVT (/100 pt-years)	*cases*	*controls*
overall (in all the subjects)	6.15	3.98
idiopathic (in all the subjects)	4.47	2.65
idiopathic (after idiopathic DVT)	8.87	6.08
idiopathic (after DVT with risk factor)	2.86	1.20

PLATELETS AND MEGAKARYOCYTES

134
Thrombopoietin-stimulated *ex vivo* expansion of megakaryocyte progenitors of human cord blood

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Megakaryocyte growth and differentiation are governed by a number of growth factors, most prominently the *c-mpl* ligand, thrombopoietin (TPO), whose role on hematopoietic cell expansion is not yet clearly established. In previous studies we have defined the experimental conditions for hematopoietic cell expansion in stroma-free liquid cultures in the presence of various cytokines. We have now studied the role of TPO alone or in combination with other growth factors (FLT3-ligand [FL], IL3, *c-kit*-ligand [KL], and IL6) on the megakaryocyte pathway from $CD34^+$ cord blood cells. Twenty thousand highly purified $CD34^+$ cord blood cells were cultured in *short-term* liquid cultures (up to 21 days) in the presence of medium, TPO [10 U/mL (Zymogenetics)] and/or the following growth factors: FL [50 ng/mL (Immunex)], KL (50 ng/mL), IL6 (10 ng/mL), IL3 (10 ng/mL), which were added alone or in various combinations at the beginning of the cultures and then replaced twice a week.

At days 3, 7, 14 and 21 the cultures were demidepopulated by removal of one half the culture volume which was replaced by fresh medium, TPO and the other growth factors. Cells of the harvested medium were assayed for CFU- and BFU-Mk-derived colony formation. TPO alone was unable to sustain CFU- and BFU-Mk for longer than 2 weeks. The KL- or FL-supplemented cultures allowed an increased output (after 21 days FL+TPO and KL+TPO output was 10 fold the input number). The IL6-supplemented cultures were less effective in sustaining *ex vivo* megakaryocyte expansion. The IL3-supplemented cultures allowed a better expansion (after 21 days of cultures the IL3+TPO output was 20 fold the input number). The best expansion was seen with the combination of TPO+IL3+FL ±KL (30 fold the input number after 21 days of liquid cultures). In the presence of KL+TPO±FL±IL3, BFU-Mk were also detectable for up to 2 weeks. These findings will help in designing an ex vivo expansion protocol for Megakaryocyte progenitors for the management of post-transplant thrombocytopenia.

135
Platelets from patients heterozygous for the deficiency of 2MeS-ADP binding sites have a secretion defect

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Background. Two unrelated patients with severe deficiencies of platelet binding sites for the adenosine diphosphate (ADP) analogue 2MeS-ADP have been described (*Cattaneo et al, Blood 1992; Nurden et al, J Clin Invest 1995*), whose platelets aggregate poorly to ADP. In one of them, platelet secretion was shown to be abnormal. We showed that platelets with primary secretion defect (PSD) [characterized by abnormal secretion, normal granule stores, thromboxane A2 production and ADP-induced primary wave of aggregation] have moderate deficiency of binding sites for 2MeS-ADP. Our hypothesis is that the full complement of ADP receptor(s) is necessary for normal platelet secretion, and that some PSD patients are heterozygotes for the severe defect of platelet ADP receptors. *Subjects.* Two sisters (MG and IG, aged 48 and 56y) with lifelong histories of abnormal bleedings, and a young boy (GL, 13y), son of MG with no history of abnormal bleedings. Their bleeding times were prolonged (MG: 15 min; IG: 20 ; GL: 13; normal range <8 min). All had normal platelet count, serum TxB2, platelet granules content, and coagulation tests. *Methods.* Platelet aggregation and secretion induced by ADP (4 µmol/L), collagen (2 µg/mL), PAF-acether (0.2 µmol/L) or U46619 (0.5 µmol/L) (lumiaggregometer). The specific binding of [^{33}P]2MeS-ADP to washed platelet suspensions was measured as described (*Gachet et al, Br J Haematol 1995*). *Results*. The ADP-induced primary wave of aggregation was abnormal in MG and IG, normal in GL. The table shows the results of binding and secretion studies.

	2MeS-ADP binding	*ATP secreted/10^8 platelets*			
Subjects	*sites/platelet*	*ADP*	*U4669*	*collagen*	*PAF-acether*
MG	225	0	0	0.07	0.03
IG	240	0	0	0.14	0
GL	430	0.01	0	0.71	0.08
Normal range	530-1102	0.02-1.4	0.1-1.1	0.2-3.2	0.06-2.5

Conclusions. This study of a new family with the platelet disorder characterized by defective binding of 2MeS-ADP supports our hypothesis that the full complement of platelet ADP receptor(s) is necessary for normal platelet secretion and that some patients with PSD are heterozygous for the defect.

Authors index

Name, abstract number(s)

A

Abate G, 079, 080
Accorrà F, 126
Adorno G, 096
Aglietta M, 014, 134
Agostini F, 017
Albano F, 054
Alessandrino EP, 008, 016, 048, 116, 118,119, 120
Alfinito F, 095
Alimena G, 051, 059, 061
Allione B, 050
Almici C, 006, 027
Aloia C, 079
Amabile M, 012
Amadori S, 041, 042, 043, 096
Amaranto P, 077
Andriani A, 096
Andrizzi C, 048, 123
Annaloro C, 033, 053, 101
Annino L, 055, 056
Anselmo AP, 066
Antinori A, 126
Arcese W, 017, 117, 122
Arcieri P, 133
Aricò M, 057
Ariola C, 035
Aronica G, 041, 042, 043
Artusi T, 010, 025
Astolfi M, 108
Astori C, 008, 016, 081, 118
Attardo G, 065
Attolico I, 054, 062
Audisio E, 050, 074
Avanzi GC, 007
Avanzini P, 055, 065
Avvisati G, 096, 133
Azzarà A, 034, 107

B

Baccarani M, 039
Bacigalupo A, 045, 113, 114, 119
Bagnara GP, 018, 019
Baldini L, 011, 065, 083, 087
Balduini A, 008, 016, 120
Balduini C, 105
Bandini G, 097, 119
Barbui T, 020, 048, 052, 073, 075
Bardi A, 029, 031, 051, 084, 090
Barozzi P, 010
Barulli S, 015, 071
Battaglia M, 105
Battistini R, 061
Bavaro P, 115
Bellesi G, 066
Bellio L, 088, 091
Bellucci R, 004, 005, 077
Beltrami G, 045
Benedetti F, 048
Benni M, 097
Bergonzini S, 010
Berisso G, 113, 114
Bernasconi C, 008, 016, 081, 088, 091, 116, 118, 120
Bernasconi P, 008, 016, 051, 116, 118, 120
Bernasconi S, 028
Bertini M, 068, 070, 073, 074, 106
Bertolli V, 101
Bertoncelli MC, 106
Bertone A, 039
Betrò P, 060
Biaggi G, 116, 118
Bianchi A, 067
Bigoni R, 029, 031, 051, 084, 090
Biti G, 066
Bizzoca R, 062
Bizzoni L, 077
Bo M, 091
Boccadoro M, 108
Boccomini C, 050, 070, 106
Boccuni PN, 023, 058, 112
Boecklin F, 122
Boiocchi M, 082
Bonacorsi G, 010, 025
Bondì F, 104
Bonfichi M, 008, 016, 118, 120
Bonini A, 119
Bonsi L, 018
Bordonaro R, 082
Bottarel F, 007
Botto B, 070, 073, 074, 106
Bragardo M, 007
Brama M, 059
Brancorsini D, 071
Brandozzi G, 071
Breccia M, 017, 060
Bregante S, 113, 114
Brera C, 008, 016
Breviario B, 075
Broccia G, 064
Brugia M, 071
Brugiatelli M, 065, 128
Bruno A, 041, 042, 043
Brusamolino E, 081
Buccisano F, 041, 042, 043
Buffa R, 083
Bugliosi R, 096
Bullrich F, 090
Burrone O, 085

C

Caberlon S, 091
Cacciola E, 089
Cadeo GP, 076
Caggese L, 124
Cagossi K, 010
Cairoli R, 125
Caldera D, 116, 118
Callea I, 049
Calvi R, 070, 074, 106
Campanale D, 130
Campanella B, 066

Candoni A, 039
Canepa L, 045
Canevari A, 088, 091
Cantonetti M, 066
Cantoni S, 124
Cantore N, 047
Cantori I, 099
Capalbo S, 086, 109
Capelli D, 015
Capello D, 074
Capone F, 047
Caporale R, 018
Capucci A, 036
Caracciolo D, 067, 068
Caramatti C, 027
Caravita T, 096
Carbone A, 074, 082
Carella AM, 032, 045, 048, 102
Carestia C, 127, 129
Carlo Stella C, 020, 006, 027, 048, 111
Carluccio P, 062
Carmini D, 017, 117, 122
Caroli Costantini M, 124
Carosi GP, 076
Carrabba M, 037
Cartoni C, 123
Carulli G, 034
Casari S, 076
Casorelli I, 132
Cassibba V, 086
Castelli G, 088, 091
Castellino C, 067, 068
Castello A, 088
Castoldi GL, 029, 031, 051, 084, 090
Casula P, 064
Cataldi I, 071
Cattane A, 076
Cattaneo C, 037
Cattaneo M, 135
Cavalieri E, 066
Cavigliano P, 051
Cavo M, 097
Cazenave J-P, 135
Cedrone M, 059
Cedrone M, 123
Celesti F, 059
Celesti L, 032, 102
Centurioni R, 069, 099
Cerretti R, 092, 093
Cerri R, 045
Ceschini E, 071
Cherasco C, 067, 108
Chiaretti S, 078
Chisesi T, 069
Chiurazzi F, 086, 095
Chiusolo P, 132, 133
Ciattaglia G, 071
Cilloni D, 006, 027, 111
Cimino G, 061, 117, 122
Cimino R, 058
Cinciripini A, 015
Cinieri S, 073
Cinque F, 108
Ciocca Vasino MA, 070
Clavio M, 045, 086
Clementi M, 071
Cocco PL, 064
Colombi M, 065
Colombo A, 116, 118
Coluzzi S, 092
Condemi R, 107
Congiu M, 069
Console G, 049, 107
Consoli U, 089, 103
Conter V, 057
Contino R, 054, 062
Contu A, 069
Coppi MR, 054
Coppola L, 061, 063
Corazzelli G, 079, 080
Cordone I, 004
Corradini P, 067, 068, 108
Corso A, 081
Cortelezzi A, 033, 037, 053
Cortellazzo S, 073
Cortese D, 107
Costantini S, 095
Cox MC, 041, 042, 043
Crea AMR, 128
Crescenzi S, 092
Crescimanno A, 104
Cristiani S, 037
Cro L, 011, 083, 087
Croce CM, 084, 090
Crugnola M, 081
Cudillo L, 096
Cuneo A, 029, 031, 051, 084, 090
Curzi L, 098

D

Damasio E, 045, 086
Damiani D, 039
Da Prada GA, 105
Da Prato I, 046
D'Ardia S, 050
D'Ascola DG, 128
Dattola A, 049
De Angioletti M, 127, 129
De Chiara A, 080
De Cuia MR, 059
D'Elia GM, 060
de Fabritiis P, 004, 005
De Felice L, 017, 117, 122
De Gregoris C, 123
Dejana A, 032, 102
Della Cioppa P, 112
Della Volpe A, 037, 053, 101
Dell'Olio M, 094
Del Poeta G, 041, 042, 043, 055, 096
Del Prete S, 015
Del Vecchio L, 023, 058, 112
Dentamaro T, 096
De Paolis MR, 054
De Propris MS, 004, 005, 077
De Renzo A, 063
De Rosa G, 112
De Rosa V, 080
De Sanctis V, 072, 077, 123
De Stefano V, 132, 133
De Vita S, 082

Dianzani C, 007
Dianzani U, 007
Di Bartolomeo P, 115, 119
Dibenedetto SP, 057
Di Cataldo A, 013
Di Girolamo G, 115, 119
Di Girolamo R, 127, 129
di Marzo Capozzi F, 047
Dini D, 025
Di Noto R, 058
Di Paola MC, 003, 009
Di Pucchio T, 017
Di Raimondo F, 021, 055
Disca S, 103
Dominioni F, 017
Donelli A, 109
Donisi A, 076
Donno A, 054
Dotti GP, 020, 075

E

Efremov DG, 085
Elia L, 059, 117, 122
Emanuelle E, 029
Epiceno AM, 041, 042, 043
Equitani F, 126
Ermacora A, 039
Evangelisti L, 018

F

Fabbiano F, 055
Facchetti F, 036
Falda M, 048, 050, 055, 073, 106, 119
Fanin R, 039
Fanni Canelles M, 085
Farabegoli P, 012
Federico M, 065
Ferrara F, 058
Ferrari A, 055, 056
Ferrari MG, 010
Ferreli A, 064
Filosa G, 071
Finazzi G, 052
Fiocchi R, 075
Fiore M, 062
Fiorillo MT, 128
Fioritoni G, 096
Fiumara P, 089, 103
Foà R, 004, 092, 093
Formica V, 089, 103
Fortuna A, 100
Frassoni F, 032, 102
Freilone R, 070, 073, 074, 106
Frigeri F, 080
Fugazza G, 032, 114

G

Gachet C, 135
Gaidano G, 074
Galimberti S, 046
Galliano M, 070
Gallicchio M, 007
Gallo E, 068
Galvagno F, 021
Gamberi B, 003
Gammaitoni L, 007, 014
Gandolfo GM, 094
Garau D, 006, 020, 027, 111
Garetto L, 134
Gargantini L, 081
Gavarotti P, 067, 068
Geromin A, 039
Giacca M, 009
Giachetti R, 111
Giangiacomi M, 071
Gigante C, 121
Ginaldi L, 065
Giona F, 056
Giorgiani G, 121
Giovannini M, 072, 123
Girelli G, 092
Girmenia C, 061, 123
Giustolisi R, 089, 103, 109
Gobbi M, 045, 086
Gobbi P, 065
Goldstone AH, 110
Gozzetti A, 097
Gregorj C, 035
Grignani F, 073
Grimaz S, 039
Grossi A, 018, 019
Gualandi F, 113, 114
Guardabasso V, 131
Guffanti A, 083
Guglielmi C, 017, 072, 117, 122
Guglielmo P, 089, 103

H

Hagemeijer A, 029

I

Iacone P, 096
Iacopino P, 048, 049, 107, 109
Impera S, 089
Incagliato M, 114
Indrizzi L, 069
Iori AP, 117, 122
Irrera G, 107
Isaza A, 045
Ivanovski M, 085

K

Kim S, 030
Klersy C, 118

L

Lacerra G, 127, 129
Ladetto M, 108
Ladogana S, 055
Lamacchia M, 062
Lamanda M, 056, 093
Lambertenghi Deliliers D, 033
Lambertenghi Deliliers G, 033, 038, 048, 053, 101
Lamberti A, 095
Lamparelli T, 113, 114
Landonio G, 124
Lanfrancone L, 020
Larocca LM, 126
Lasagni L, 019
La Starza R, 051

Latagliata R, 028, 035, 060, 061
La Targia ML, 065
Laurenti L, 117, 122
La Verde G, 093, 096
Lazzarino M, 081, 088, 091
Lecchi A, 135
Lemoli RM, 097, 100
Leone G, 005, 022, 096, 126, 132, 133
Leoni P, 015, 069, 071, 078, 086, 098, 099, 109
Lerma E, 032, 102
Levato D, 094
Levato L, 094
Levis A, 055, 070, 106
Levitzki A, 027, 111
Liberati AM, 073
Lisci A, 004, 005
Liso V, 054, 062, 109
Li Volti S, 013
Locatelli F, 040, 050, 073, 106, 119, 121
Lo Coco F, 055
Lombardi L, 011
Lombardi R, 135
Lombardini L, 018
Lombardo M, 065
Lo Pardo C, 058
Lovisone E, 073
Lucia MB, 022
Luciano L, 023, 024
Luppi M, 010
Luzi G, 060

M

Maciejewski JP, 001, 002, 024, 030
Magno M, 062
Mainolfi C, 079
Maiocchi MA, 091
Maiolo AT, 011, 037, 065, 083, 087
Maiorana A, 010
Majolino I, 069, 096, 109, 110, 119
Malcovati L, 118
Mancini M, 051, 059
Mancini S, 071, 098
Mandelli F, 055, 056, 066, 072, 078, 092, 093, 096, 123
Manfroi S, 100
Mangianti S, 100
Mangoni L, 006
Mannella A, 094
Mannucci PM, 133, 135
Marasca R, 010
Marcacci G, 047
Marcenò R, 096
Mariani G, 104
Mariani G, 109
Marin L, 039
Marmont AM, 113
Marmont F, 050, 073, 106, 119
Marocolo D, 036
Marseglia C, 008, 016, 120
Marsico S, 115
Martelli M, 072, 077, 093, 096
Martelli MP, 072, 077
Martinelli G, 012, 097, 116, 118
Martinelli I, 133
Martino M, 107
Mascolo MG, 017
Masera G, 057
Masi M, 041, 042, 043
Masia MC, 098
Masolini P, 039
Massa M, 116
Massarelli G, 064
Mastrullo L, 129
Masullo C, 126
Matera R, 094
Matteucci C, 051
Mattia L, 107
Maurizi Enrici R, 066
Mauro FR, 004, 092, 093
Mazza P, 086
Mazza U, 074
Mazzaro C, 085
Mazzucconi MG, 028
Mecucci C, 051
Meloni G, 048, 055, 060, 096
Mengarelli A, 117, 122
Messina C, 057
Messina G, 107
Mestice A, 054, 062
Miano C, 057
Mianulli AM, 003, 009
Michelutti A, 039
Michieli M, 039
Miglino M, 045
Milone G, 021, 109
Miniero R, 057, 119
Mininni D, 054
Minotti C, 133
Minotto C, 031
Modugno E, 130
Moioli MC, 124
Moleti L, 123
Moleti ML, 078, 122
Molica S, 065, 086, 094, 109
Monda VM, 080
Monni A, 064
Montanari M, 098, 099
Montefusco E, 060
Montefusco V, 012
Montillo M, 078, 081
Morabito F, 049, 107
Morandi S, 069
Morano SG, 061
Mordini M, 119
Mordini N, 113, 114
Morosato L, 040
Morra E, 055, 081, 124, 125
Morselli M, 010
Mostarda I, 024
Motta M, 083
Motta MR, 097, 100
Motta T, 075
Mozzicafreddo G, 071
Musolino C, 049, 109
Musso M, 104, 109
Musto P, 094
Muti G, 125

N

Nanni M, 051, 059
Narni F, 109

Negrini M, 084, 090
Neri A, 011, 087
Niscola P, 123
Nobile F, 049
Nobili L, 083, 087
Noris P, 105
Nosari A, 124, 125
Novero D, 068, 074

O

Occhini D, 113, 114
Offidani AM, 071
Offidani M, 098, 099
Olioso P, 115
Olivieri A, 015, 069, 098, 099, 109
Onida F, 101
Onida GA, 064
Oreste PL, 124, 125
Orgiana L, 072
Oriani A, 033, 053, 101
Orlandi E, 081
Orsucci L, 070, 074, 106

P

Paciaroni K, 132, 133
Pagano L, 126
Pagliai G, 018
Pagliai L, 019
Pagnucco G, 008, 016, 081, 088, 091
Palestro G, 074
Palma A, 121, 130
Palmisano G, 069
Palombi F, 072
Palumbo G, 062
Palumbo GA, 021
Palumbo M, 013
Pane F, 012, 024
Pantano A, 106
Paolino F, 106
Papa G, 096
Papalinetti G, 115
Parisi G, 103
Parvis G, 074
Pastano R, 100
Pastore C, 074
Pastore D, 062
Pauri P, 071
Pavesi L, 105
Pearce R, 110
Pedrazzoli P, 105
Pelicci PG, 020
Pelizzari AM, 036
Pellacani A, 100
Pellegrini W, 036
Peraino M, 028
Perona G, 040, 044
Perotti C, 105
Perrone F, 080
Perrone MP, 117
Perrone P, 122
Persico M, 063
Petrini M, 034, 046
Petroni D, 012
Petrou M, 128
Petrucci MT, 035
Petti MC, 028, 035, 060
Pezzullo L, 001
Piacibello W, 014, 134
Pierri I, 045
Pietrapertosa A, 130
Pileri A, 067, 068, 108
Pizzolo G, 040, 044
Pizzuti M, 073
Podestà M, 032, 102
Polizzi V, 104
Pontari A, 049
Porcellini A, 069
Porretto F, 104
Postorino M, 041, 042
Pozzato G, 085
Pozzoli E, 033, 053
Privitera A, 103
Proia A, 004, 077, 093
Proia S, 066
Pruneri G, 083
Pucci G, 107
Pupilli C, 019

Q

Quaini R, 069
Quirici N, 038

R

Rainaldi A, 096
Raiola A, 001, 023, 024, 112
Rambaldi A, 020, 075
Ramirez F, 049
Ranieri P, 130
Rapanotti MC, 059
Recchia A, 055
Redi R, 060
Regazzi E, 006, 020, 027, 111
Remiddi C, 100
Resegotti L, 050, 070, 073, 074, 078, 106
Ribera S, 125
Ricciardi MR, 017, 035
Ricco A, 054
Richelda R, 011
Ricotti G, 071
Rigo A, 040, 044
Rigolin GM, 031, 084, 090
Ripalti A, 009
Risitano AM, 001, 023, 112
Risso M, 045
Rizzari C, 057
Rizzi S, 097, 100
Rizzoli V, 006, 020, 027, 069, 111
Roberti MG, 029, 031, 051, 084, 090
Robustelli della Cuna G, 105
Roccaforte S, 115
Rocchi L, 093
Rocchi M, 011
Rogato A, 039
Romani C, 123
Romano A, 122
Romano MF, 095
Romeo MA, 021, 131
Romitti L, 101
Ronchetti D, 011
Ronco F, 049

Ronconi S, 097
Rossetti A, 107
Rossi A, 073, 128
Rossi E, 045, 132
Rossi Ferrini PL, 019, 086
Rossi G, 036, 067, 076
Rossi V, 133
Rosti GA, 097
Rotoli B, 001, 023, 024, 063, 095, 112
Ruggeri M, 075
Rumi C, 022
Rupoli S, 015, 071, 099
Rupolo M, 082
Rus C, 050
Russo D, 039
Russo F, 079, 080
Russo L, 107
Russo-Mancuso G, 131
Rutella S, 022

S

Sacco C, 074, 082
Sacha T, 102
Saglio G, 007, 012, 055, 074
Sajeva MT, 048
Sala R, 004, 092, 093
Salmaso F, 039
Salvagno L, 069
Salvaneschi L, 105
Salvatore F, 024
Salvi A, 015
Sambataro MP, 013
Sammarelli G, 006, 027, 111
Sanavio F, 014, 134
Santini G, 069, 085, 086
Santini V, 018, 019
Santoleri L, 125
Santonocito A, 103
Santoro A, 109
Santucci MA, 003, 009
Santulli B, 047
Sarina B, 037
Sato T, 001, 002, 030
Savarino M, 025
Savoldo B, 006, 020, 027, 111
Scalone R, 104
Scapoli GL, 029
Scappini B, 019
Schilirò G, 013, 057, 127, 129, 131
Scimè R, 109, 119
Sciorio A, 127, 129
Scognamiglio M, 058
Scoppa G, 080
Scorca A, 062
Screnci M, 017, 117, 122
Sculli G, 049
Sebastio L, 058
Selleri C, 001, 002, 023, 024, 030, 112
Serresi S, 071
Sertoli M, 069
Servida F, 038
Sessarego M, 032, 113, 114
Severino A, 014, 134
Sica S, 022, 048, 096
Silingardi V, 065
Silvestri F, 085
Silvestris I, 037
Simonacci M, 071
Soligo D, 033, 038, 053, 101
Somalvico F, 033
Sorbara M, 128
Spadea A, 028, 060, 061
Spazzapan S, 082
Specchia G, 054, 055, 062
Spinelli O, 020
Spriano M, 045, 086
Stabile F, 125
Stagno F, 089, 103
Stanziola MC, 058
Stellini R, 076
Strippoli P, 018, 019
Surico GM, 057

T

Tabilio A, 006, 055, 096
Tafuri A, 017, 035, 061
Taghipour G, 110
Tagliaferri E, 101
Taibi R, 089
Talamini R, 082
Tamburini A, 041, 042, 043
Tanda F, 064
Tannoia N, 121, 130
Tarella C, 067, 068, 108
Tecchio C, 040, 044
Tedeschi A, 081
Tedeschi L, 069
Terragna C, 012, 097
Testa R, 127, 129
Testa U, 022
Testi AM, 056, 057, 078, 117, 122
Testi R, 046
Testoni N, 012, 051, 100
Todisco E, 078
Tonelli R, 018
Torella R, 063
Torelli G, 010, 025
Torlontano G, 115
Torretta L, 105
Tosi P, 100
Tosti S, 061, 123
Trasarti S, 077
Tribalto M, 096
Trunfio R, 128
Tulissi P, 085
Tura S, 003, 009, 012, 097, 100
Turco MC, 095

V

Vaccario ML, 126
Vaira A, 054
Valbonesi M, 032, 113
Valsecchi MG, 057
Vanelli L, 016, 091
Van Lint MT, 045, 113, 114
Vegna ML, 056
Venditti A, 005, 041, 042, 043
Ventriglia A, 108
Venuta S, 095
Veronese ML, 084, 090

Viero P, 073
Vignetti M, 048
Villa MR, 063
Vinante F, 040, 044
Visani G, 048, 100
Vitale V, 113
Vitelli G, 094
Vitolo U, 068, 070, 073, 074, 106
Vittone A, 012
Vitucci A, 121
Vitulo P, 118
Voena C, 108
Volonterio A, 124
Volpe E, 047
Volpe G, 074
Volpe S, 047
Vulcano F, 117, 122

Y

Young NS, 002, 030

Z

Zaccaria A, 012, 026, 051, 086
Zagonel V, 055, 074, 078, 082
Zallio F, 067, 068
Zamagni E, 097
Zambaldi G, 069
Zanolin E, 040
Zighetti ML, 135
Zikos PM, 114
Zucal N, 083, 087
Zuffa E, 026

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