ABSTRACTS

001
A MATHEMATICAL MODEL FOR THE EVALUATION OF AMPLITUDE OF HEMOGLOBIN FLUCTUATIONS IN ELDERLY ANEMIC PATIENTS AFFECTED BY MYELODYSPLASTIC SYNDROMES: CORRELATION WITH QUALITY OF LIFE AND FATIGUE

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Therapy with Red Blood Cell transfusions and rHuEPO for management of anemia in patients with myelodysplastic syndromes causes recurrent fluctuations in hemoglobin levels. The purpose of this study was to elaborate a mathematical model for the interpretation of hemoglobin fluctuations and to correlate the resulting numerical parameter (*Variaglobin Index*) with quality of life (QoL) and fatigue. We hypothesized that poly-transfused patients may experience increased fatigue besides a decrease in the QoL due to recurrent *down-up-down* hemoglobin excursions. This prompted us to look for a numeric parameter capable of expressing the amplitude of hemoglobin fluctuations and to study the impact of this variable on QoL and fatigue. We set up a mathematical model to evaluate the amplitude of Hb fluctuations during a month. The obtained variable, which we named *Variaglobin Index*, was then correlated with QoL and fatigue. In output the software returned:
(*i*) Numeric value of *Variaglobin Index* (higher values indicate a higher amplitude of Hb fluctuations);
(*ii*) Numeric value of mean Hb observed during the month;
(*iii*) 3D-Graphical plot of Hb fluctuations compared to a plane representing the mean Hb value in each patient.
Thirty-two elderly MDS patients (20 males, 12 females) with a mean age of 73 years (range 64-83) were enrolled in the study. After studying the hemodynamic Hb fluctuations in these patients, we found that lower amplitude of the *Variaglobin Index* was significantly correlated with a better quality of life and less fatigue.
Based on our findings, the QoL in elderly MDS patients can be improved by lowering the amplitude of Hb fluctuations. One approach could be to transfuse patients more frequently but with less RBC units. Treatment with rHuEPO is another alternative and, as indicated in international guidelines, should be recommended in patients with a high probability of positive response.

002
AUTOIMMUNE MANIFESTATIONS AND T-CELL RECEPTOR V BETA REPERTOIRE ANALYSIS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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In recent years much attention has been paid to the role of autoimmunity in the pathophysiology of myelodisplastic syndromes (MS). Moreover, autoimmune phenomena, ranging from clinically overt diseases to serological abnormalities, are often reported in patients affected by MS. In this study we investigated the incidence of autoimmune manifestations in 33 patients with MS (16 RA, 1 RARS, 10 RAEB, 5 CMML, 1 RAEB-t) belonging to the Sardinian population. In addition, in 11 of these patients and in 20 normal subjects the peripheral blood TCR repertoire was evaluated in $CD4^+$ and $CD8^+$ T-cells by flow-cytometry. Noticeably, two-third of the patients showed autoimmune abnormalities, being half of them clinically affected and half exclusively characterised by serological anomalies. When we looked at the TCR repertoire pattern, the BV mean expression did not differ between patients and controls in the $CD4^+$ subset, whilst in $CD8^+$ cells the expression of BV3 and 21.3 turned out to be markedly reduced in MS patients. No statistically significant differences were detected in the number of BV expansions, defined as subfamily expressions higher than the mean +2 standard deviations. Nevertheless, one patient showed an unexpectedly high expression of BV5.1, which represented 75% of the whole $CD8^+$ repertoire. Interestingly, we detected a significantly higher number of BV deletions in MS patients, confined to the $CD8^+$ subpopulation. Such deletions were detected in 50% of the patients, ranging from one to five subfamilies per subject and involving several BV gene products. In conclusion, in our study we found a higher incidence of autoimmune abnormalities when compared to that reported in the literature. Moreover, as the size of the TCR repertoire is related to the immunocompetence status, the restriction of the BV gene usage detected in MS patients may play a role in the immune dysregulation observed in course of MS.

003
IL TRATTAMENTO DELL'ANEMIA DELLE SINDROMI MIELODISPLASTICHE MIGLIORA LA GEOMETRIA CARDIACA

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L'anemia è una complicanza frequente delle sindromi mielodisplastiche (MDS). Recentemente, è stata associata a cambiamenti cardiaci che possono essere responsabili di morbilità cardiovascolare. Fattori emodinamici indotti dall'anemia promuovono una graduale ipertrofia ventricolare sinistra (IVS). Le epoetine inducono risposte eritroidi in circa 40% dei casi cosicchè, da un punto di vista farmacoeconomico, si raccomanda una selezione di pazienti con probabilità di risposta Gli effetti delle epoetine sulla geometria cardiaca non sono stati esplorati. Vengono riportati i risultati preliminari in 20 pazienti anemici con MDS trattati con darbepoetina alfa (DPO). In uno studio di fase II i pazienti hanno ricevuto DPO 150 mcg alla settimana con raddoppio nei non rispondenti per raggiungere Hb=12.0 g/dL. La geometria cardiaca è stata valutata al basale e dopo 6 e 12 mesi con misurazioni ecocardiografiche. La massa ventricolare sinistra indicizzata (MVSI) è stata calcolata con l'equazione di Devereux. I cambiamenti di QoL sono stati misurati con *QOL-E*. L'Hb media basale era 9,0 (range 7,5-10,8) g/dL; nei 3 pazienti con Hb > 10,5 g/dL, la geometria cardiaca era normale in uno e 2 pazienti avevano rimodellamento iniziale mentre, al di sotto di questo valore, 10 (59%) avevano IVS (p=0,038). Il cambiamento mediano di MVSI valutato dopo 6 mesi in 14 pazienti era -4 (range 53–51). Nei 9 pazienti con risposta eritroide si è osservato un miglioramento della geometria cardiaca (p=0,033) e dei 4 pazienti con ipertrofia eccentrica, uno è migliorato e uno ha ottenuto la normalizzazione. La risposta al trattamento era associata a miglioramenti significativi di QoL. *Conclusioni*. Questi dati suggeriscono che le risposte eritroidi nei pazienti con MDS siano associate a miglioramenti della geometria cardiaca e della QoL. Uno studio più ampio è necessario per confermare questi risultati e per integrare la potenziale riduzione del rischio cardiovascolare nella valutazione del costo-efficacia dei trattamenti.

004
C3 ACCUMULATES ON PHN RBCS DURING TREATMENT WITH THE COMPLEMENT INHIBITOR ECULIZUMAB

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Paroxysmal nocturnal hemoglobinuria (PNH) red blood cells (RBCs) inefficiently prevent complement activation and membrane attack complex (MAC) formation due to the lack of the glycosylphosphatidylinositol (GPI)-linked complement regulators CD55 and CD59, resulting in chronic intravascular hemolysis, hemoglobinuria and anemia. Eculizumab (Soliris,™ Alexion Pharmaceuticals, Inc.) is a humanized monoclonal antibody against the complement fraction 5, which inhibits the terminal MAC formation and demonstrated clinical efficacy *in vivo*. Here we present some biological findings from 8 PNH patients on eculizumab at the University of Naples, all included in the ongoing international trial Extension. All the patients showed a direct anti-globulin test positive for C3; subsequently, C3 coating on RBCs was systematically investigated by flow cytometry. All PNH patients showed a substantial portion of C3-coated RBCs; the percentage of C3d+ RBCs did not show any relationship with any clinical data, with the exception of PNH RBC clone size (p=0.005). A two-color flow cytometry analysis using a counter staining with an anti-CD59 demonstrated the presence of three distinct RBC populations: *i*. RBCs with normal phenotype, C3d-negative; *ii*. RBCs with PNH phenotype, C3d-negative; *iii*. RBCs with PNH phenotype, C3d-positive. No C3d-positive RBCs with normal phenotype were demonstrated; in contrast, untreated PNH patients showed the first two populations but no C3d-positive RBCs. Three patients harboring the larger C3d+PNH populations were studied by *in vivo* 51-Cr RBC survival while in suboptimal clinical response to eculizumab and in absence of intravascular hemolysis, showing a markedly reduced RBC half-life (around 10 days), with some excess counts on spleen and liver. We hypothesize that the blockade of C5 by eculizumab prevents intravascular hemolysis, but does not affect C3 deposition and cleavage on red cells membrane, which is not controlled given the absence of CD55. Thus, the increased survival of PNH red cells on eculizumab treatment may allow C3d accumulation on the membrane, possibly leading to extravascular hemolysis in the reticulo-endothelial system via the complement receptors. In conclusion, PNH RBCs surviving to intravascular hemolysis by the complement inhibitor eculizumab are coated in a substantial portion by C3d. The persistent *in vivo* reduced half-life strongly suggests the presence of an additional mechanism of disease such as extravascular hemolysis, eventually explaining the partial efficacy of eculizumab although intravascular hemolysis is controlled.

005
CA15-3 - A MARKER FOR THE MEGALOBLASTIC ANAEMIA?

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Ca 15-3 is a glycoprotein present in the cells of the mammary carcinoma and in some epithelial cells. Is a marker used for the monitoring of the breast and gastrointestinal carcinoma. The megaloblastic anaemia is an anaemia characterized by deficit of absorption of Vitamin B12 and is associated with gastritis atrophic and the target cell is the parietal gastric cell. In our institution, from June 2003 to February 2006, the level of Ca15-3 and of others tumour markers (CEA; Ca125; Ca19.9; a-FETO) they have been tested in the serum of 22 patients (14 male and 8 female with median age of 60 and range of 36-80 years) with *de novo* megaloblastic anaemia, 3 patients were gastrectomized. In all patients has been effected: esophagogastroduodenoscopy and control anti-parietal gastric cells antibody (APCA). Increase level in the serum of CA 15-3 with normal level of other tumour markers have been found in 19/22 patient with median value of 61 U/mL (range 35-100 U/mL) in 3 patients (gastrectomized) the value of CA 15-3 was normal. Besides in 17/19 patients have shown positivity for the APCA, only in two patients has been diagnosed a gastritis atrophic, in the other patients has been observed a normal gastric mucous. After a median observation of 36 months any patient has developed a mammary or gastro-intestinal carcinoma. These results indicate what the increased level of CA 15-3 antigen in patient with megaloblastic anaemia is positively correlated with APCA and with the presence of a normal gastric mucous. These clinical conditions make to suppose the destruction from the APCA of the parietal gastric cells with the liberation of this glycoprotein and this is shown in the 3 patients gastrectomized with presence of APCA and not increased level of the CA 15-3. In conclusion the CA15-3 antigen is probably an specific marker for the diagnosis of megaloblastic anaemia and is probably associated with the destruction of parietal gastric cells.

006
ANEMIE MEGALOBLASTICHE: DIFETTO "FUNZIONALE" DI VITAMINA B12 NEGLI ETILISTI CRONICI

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Le anemie megaloblastiche sono caratterizzate da un difetto nella sintesi di DNA, che può essere causato o da una carenza di vitamina B12 ed acido folico o da farmaci. Abbiamo valutato il tipo di deficit vitaminico in 79 pazienti affetti da anemia megaloblastica, tutti responsivi ad un trattamento sostitutivo con cianocobalamina e/o acido folico. Si è documentato un deficit di vitamina B12 in 68 (86%) pazienti, di acido folico in 5 (6%), un deficit combinato in 3 (4%), mentre in 3 soggetti (4%), etilisti cronici, risultavano nella norma il dosaggio sierico di vitamina B12 nonché di acido folico sierico ed eritrocitario. Questi ultimi 3 pazienti risultavano però responsivi ad un trattamento con cianocobalamina. Dei 68 pazienti con deficit di vitamina B12, 65 erano affetti da anemia perniciosa; in tale popolazione i livelli medi di vitamina sono risultati notevolmente ridotti (59pg/mL±39). Complessivamente, su tutta la popolazione in esame, erano affetti da etilismo 5 soggetti; 3 con livelli sierici di vitamina B12 nella norma, 1 presentava contestualmente anemia perniciosa ed etilismo con livelli sierici di vitamina B12 ridotti, 1 risultava carente di acido folico. Nelle affezioni epatiche, in particolare in quelle alcol correlate, si possono rilevare livelli normali o elevati di vitamina B12, pur in condizioni di carenza tessutale; si ipotizza che tale condizione sia causata da un difetto di captazione e/o ad una incrementata dismissione epatica di cobalamina nonché ad una alterato metabolismo delle proteine di trasporto (transcobalamine). Nell' ambito della popolazione dei soggetti con anemia perniciosa, 3 erano anche affetti da epatite cronica HCV correlata e presentavano comunque ridotti livelli sierici di vitamina B12. Questi dati suggeriscono che in caso di anemia megaloblastica con associata epatopatia alcolica il dosaggio sierico della cobalamina non sia indice attendibile della reale disponibilità della vitamina, realizzandosi in tale contesto un deficit *funzionale*.

007
ACUTE MYELOID LEUKEMIA IN THE ELDERLY, INTENSIVE OR MAINTENANCE THERAPY? OUR EXPERIENCE IN PATIENTS OVER 65 YEARS

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The treatment of acute myeloid leukaemia in elderly with age > 65 years is still debated. In literature numerous studies have valued the feasibility of intensive chemotherapy in these patients. The aim of the study is to value the difference in EFS and OS among 2 groups of AML elderly patients treated with intensive chemotherapy (IC) or maintenance (M). From June 2001 to May 2006 we have treated in our Division 54 AML patients, 30 male and 24 female with median age of 73 years (66-90 years). 27 patients (16 M and 11 F with median age of 71 years) have received intensive chemotherapy (I.C. Flag and MICE) and 27 (14 M and 13 F with median age of 78.5 years) have received maintenance (low dose cytarabine and/or support). In IC group 12 patients (45%) have obtained to complete remission (CR) with to EFS and OS media of 4, 47 and 7, 15 months respectively, the rate of TRM has been of 25%. In the M group the CR has been documented in 8 patients (30%) with to EFS and OS media of 4,22 and 4,94 months respectively (Figure 1-2). This results have shown a best rate of CR in the IC group but the OS and EFS difference is not statistically significant in the two groups (p: 0.7). In conclusion the Intensive chemotherapy has not improved the survival in AML elderly patients. New therapeutics strategy is necessary for to improve the EFS and OS in these patients.

Interesting is the use of specific monoclonal antibodies (anti CD33) in this poor disease especially in maintenance after a CR obtainable with an intensive or low dose chemotherapy.

008
EPIDEMIOLOGIA DELLE EMOPATIE IN CALABRIA DAL 1998 AL 2001

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Obiettivo dello studio è illustrare i dati generali di distribuzione dei nuovi casi di neoplasie ematologiche in Calabria in funzione delle variabili *genere*, *età e provincia di residenza*; si vuole fornire, inoltre, i principali indicatori statistici di incidenza al fine di fare un confronto con i dati dei registri nazionali pubblicati su "Il cancro in Italia. I dati di incidenza dei Registri Tumori. Volume terzo: 1993-1998". Il Registro Tumori calabrese (fonte dati Schede Dimissione Ospedaliera, SDO), dal 1998 al 2001, ha registrato 3702 nuove diagnosi di emopatie maligne: 28,6% LNH, 7,3% LH, 17,5% MM, 23,3% leucemia linfoide, 19,7% leucemia mieloide, 3,6% altre leucemie. Il 55,8% dei pazienti affetti da emopatie sono maschi, il 44,3% femmine con un'età mediana globale di 67,8 anni (0-100,3). Il Tasso Standard (TS) calabrese delle emopatie è pari a 44,6 (x 100.000 ab.). Il valore più elevato si riscontra nelle province di Catanzaro (TS 55.5) e di Crotone (TS 50,1). La tabella in basso mostra una sintesi dei principali dati di incidenza per singola emopatia. Confrontando il TS calabrese delle emopatie, con i tassi delle altre regioni e con la media nazionale, si riscontra che la nostra regione ha un TS più elevato rispetto alla media nazionale per tutte le emopatie ad eccezione del LNH.

Table 1.

Emopatie	*Genere*		*Età mediana*	*TS Calabria*		*TS Italia*	
	*M**	*F**	*(range)*	*M**	*F**	*M**	*F**
LNH	575	484	65,3 (15,6-94,5)	14,00	11,80	16,9	14,3
LH	151	119	44,4 (11,2-98,9)	3,90	2,90	3,1	2,7
MM	340	307	72,3 (25,6-97,6)	7,95	7,29	5,8	5,4
LEUC. LINFOIDE	489	373	69,8 (0,6-100,3)	11,57	8,87	6,2	4,2
LEUC. MIELOIDE	424	306	68,7 (0,0-96,3)	10,12	7,28	4,9	3,8

** M=maschio F=femmina*

009
THE INSTITUTIONAL ACCREDITATION PROJECT FOR THE HAEMATOLOGY WARD AT THE COMMUNITY HOSPITAL IN COSENZA, ITALY

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The Institutional Accreditation (namely regulated by law) is a pre-requisite in providing health care services in the National Health Care System. It entails the verification of the presence of general and structural, technological, organizational and clinical-professional specific requisites in health care wards and services. The Accreditation model will select, within the National Health Care System and for each Region, a Register of qualified health care providers guaranteed for the inquiring committees (Regions), citizens and health care personnel. The Community Hospital in Cosenza has launched, on a regional level, a pilot experimental project concerning Accreditation involving many sectors among which Haematology ward. The objectives of the project are: a) to set the self-evaluation path for those health care wards and services involved in verifying the presence of the already mentioned requisites as well as the implementation of clinical and professional requisites; b) to use the Accreditation model as a tool of constant improvement; to endow health care professionals with clinical governance instruments through the evaluation of one's own performance, benchmarking characterization, critical analysis, and change barriers. The methodology used focused on identifying areas to monitor and develop. Meetings were held with personnel in order to discuss the project and assign tasks and responsibilities; d) to provide consulting for the organization of the final audit; e) to support the operative units involved in the editing of the Accreditation Manual, procedures, interface agreements, general and specific requisites as well as the acquisition of all the documentation necessary for verification. The project consists of a first phase of preparation for the Audit visit complete with Manual and related requisite grids for self-evaluation; a pre-audit second phase with an external commission responsible for checking the completeness and accuracy of the documentation produced. The third phase of the Audit visit entails the final examination of all the evidence produced, a field visit by the Commission along with the editing and restitution of the final report and verification final results.

010
ANALISI DI CLIMA DI UN GRUPPO COOPERATORE MULTICENTRICO NAZIONALE DI ONCO-EMATOLOGIA

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I Gruppi Cooperatori Nazionali Onco-Ematologici nasco-

no con la finalità di promuovere e condurre la ricerca applicata alla clinica nell'ambito delle malattie neoplastiche del sistema emopoietico. Dovendo fronteggiare realtà particolarmente articolate ed eterogenee, essi si configurano di regola in schemi organizzativi piuttosto rigidi, che, pur rendendosi necessari per assicurare lo svolgimento e il controllo ottimali di tutte le diverse fasi dello studio, conferiscono loro una connotazione *verticistica*. Tale strutturazione, pur dettata da specifiche necessità di rigore organizzativo, predispone ad alcuni inconvenienti, specie nei confronti di componenti del Gruppo esclusi dalla gestione diretta dello studio, come avviene, ad esempio, per i Centri periferici. Queste considerazioni hanno condotto alla determinazione di effettuare un sondaggio sulle eventuali criticità esistenti all'interno di un ipotetico Gruppo Cooperatore Onco-ematologico che operi in territorio nazionale, allo scopo di evidenziare la natura e la frequenza di tali disagi e di proporre eventuali misure correttive. L'indagine è stata effettuata nell'ambito di un Master di *Management dei Servizi Sanitari applicati all'Ematologia* tenutosi presso l'ISTUD di Stresa tra il 2005 ed il 2006, utilizzando un questionario articolato in 16 quesiti a risposta multipla, distribuito in 4 differenti Istituzioni Onco-Ematologiche italiane universitarie ed ospedaliere. Il questionario è stato proposto a 75 operatori sanitari coinvolti in sperimentazioni cliniche, 63 dei quali laureati (suddivisi in 29 con ruolo dirigenziale, 12 senza ruolo dirigenziale, 21 in corso di formazione) e 12 operatori non laureati (infermieri, data manager). La prima osservazione emersa è che su gran parte delle risposte si è riscontrata una sostanziale uniformità di opinioni, trasversale alle categorie di appartenenza. Il questionario sembra quindi fornire indicazioni significative che coinvolgono la maggior parte degli intervistati nella comunità scientifica che si occupa di ricerche cliniche. In sintesi, gli elementi positivi della partecipazioni a gruppi cooperativi sono in primo luogo rappresentati dalla possibilità di confrontarsi con altri colleghi e di scambiare informazioni che consentono di migliorare in modo rilevante la qualità dell'assistenza e le conoscenze degli operatori. La partecipazioni a protocolli comporta, come ulteriore elemento positivo, la crescita culturale dei partecipanti ed una maggiore considerazione scientifica, stima e visibilità personale e delle istituzioni di appartenenza. La ricerca di maggiore visibilità scientifica non è però soddisfatta a pieno. I centri minori ed i laureati strutturati, ma senza ruolo dirigenziale, restano, in particolare, in cerca di affermazione e stima da parte del gruppo. Tra le cause di questa insoddisfazione, lo scarso coinvolgimento nella ideazione e proposizione dei protocolli è particolarmente sentito. Migliorare la visibilità scientifica significa inoltre incrementare la collegialità dei diversi autori, che devono essere globalmente rappresentati nelle pubblicazioni scientifiche, le quali dovrebbero anche essere piu' numerose. Un ulteriore modo per soddisfare queste ambizioni è individuato nella possibilità di sviluppare sottoprogetti che possano conferire un maggiore coinvolgimento per singoli partecipanti. Elementi negativi sembrano, in particolare, concentrarsi nella mancanza di un reale dialogo fra i diversi centri. In aggiunta, decisioni verticistiche, conflitti di potere e, talora, scarsa organizzazione, vengono anch'essi percepiti come aspetti non favorevoli.

In generale, i costi non sembrano rappresentare un elemento limitante, anche se talora emerge come la richiesta di maggiori finanziamenti sia cruciale. L'incremento lavorativo indotto dai protocolli risulta essere consistente: la maggior parte degli intervistati si distribuisce su un aumento dei carichi di lavoro pari a circa il 40%. Significativa è la percentuale di intervistati che ritiene importante passare dal volontarismo attuale alla definizione ed alla disponibilita' di figure professionali dedicate (data manager, trial office) che svolgano specificamente il lavoro di conduzione di uno studio clinico. Risulta anche necessario qualificare l'organizzazione delle attivita' con una maggiore attenzione al lavoro di squadra, identificando ruoli e compiti specifici all'interno del gruppo cooperatore e del singolo gruppo partecipante al progetto. Migliorare la partecipazione ai protocolli è ritenuto possibile anche attraverso un maggior numero di incontri periodici ed un piu' esteso utilizzo del mezzo internet per la diffusione e divulgazione dei protocolli. Inaspettatamente, non è considerata rilevante l'opportunità che i trials clinici offrono di poter accedere in anticipo a farmaci che saranno disponibili sul mercato solo dopo 2-4 anni dal momento della sperimentazione.

011
RECIDIVA MENINGEA IN PAZIENTE CON LEUCEMIA ACUTA PROMIELOCITICA: DESCRIZIONE DI UN CASO

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Introduzione. Il coinvolgimento del sistema nervoso centrale rappresenta un evento raro in corso di leucemia acuta promielocitica (LAP).[1] Si riporta qui un caso di recidiva meningea intervenuta in corso di remissione completa molecolare di LAP a rischio intermedio.
Descrizione. Donna di 62 anni nell'agosto 2004 riceve, presso altro centro diagnosi di LAP a rischio intermedio secondo i criteri della stratificazione delle linee guida del protocollo GIMEMA - AIDA 2000 (leucociti < $10x10^9$/L - piastrine < $40x10^9$/L). Trattata secondo tale protocollo consegue remissione completa molecolare e viene avviata a terapia di mantenimento con ATRA, MTX + 6-MP. Nell'agosto 2006 giunge alla nostra osservazione per cefalea, vertigini, nausea, vomito e compromissione grave del visus. Le indagini mostrano, a fronte di un emocromo e di un cariotipo normali, presenza di blasti atipici ad abito promielocitico all'esame del liquor e presenza del riarrangiamento PML/RARα tipo bcr3 nel midollo e nel liquor. Si pone diagnosi di recidiva meningea di APL e si inizia il trattamento intratecale (Methotrexate 12 mg - Ara-c 50 mg – PDN 40 mg) due volte a settimana per quattro settimane; la insorgenza di aracnoidite chimica associata a mancata *clearance* del liquor inducono ad avviare trattamento con radioterapia cranio-spinale e terapia di salvataggio. *Conclusioni*. La manifestazione tardiva di meningosi leucemica in corso di remissione completa molecolare di leucemia acuta proimielocitica induce a riflettere sulla necessità di introdurre l'esecuzione

della rachicentesi diagnostica in pazienti con PML/RARα tipo bcr3 anche se ritenuti a rischio basso/intermedio.

1. Br J Haematol. 2003; 120(2):266-70.

012
IMMUNOPHENOTYPIC DIFFERENCES BETWEEN DIAGNOSIS AND RELAPSE IN ADULT AML

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In acute myeloid leukemia (AML) changes in the antigen expression pattern between diagnosis and relapse have been reported with a frequency of 38-75% in patients with acute lymphoblastic leukemia and 62-91% in patients with AML. In this study we compared the immunophenotypic features of leukemic blast cells from patients with AML at diagnosis and first relapse by flow cytometry in order to detect losses or gains of antigens and their impact on the evaluation of MRD.
The study was performed in 48 adult patients (23 males, 25 females); the distribution according to the FAB classification was as follows: M0 (2 cases), M1 (5), M2 (18), M3 (5), M4 (9), M4eo (2), M5a (5), M5b (2). Immunophenotypic changes occurred in 36 cases (75%). Changes in progenitor-associated antigens (CD34, CD117) included more losses than gains (23% vs. 12%) whereas changes in lymphoid-associated antigens (CD7, CD10, CD19, CD56) included more gains than losses of antigen expression (29% vs. 10%); changes in myeloid antigens (CD11b, CD13, CD14, CD15, CD33, MPO) were almost balanced for loss and gain (25% vs. 35%). Four of 5 cases with t(8;21) underwent changes in antigen expression related to this translocation; particularly, we observed loss of CD19 in 1 case, gain of CD19 in 2 cases and gain of CD19 and CD56 in 1 case; notably, no change was observed in all M3 cases. Morphologically and by cytochemistry differences were identified leading to changes in the FAB subtype in 8 cases. A high frequency of immunophenotypic changes in antigen expression between diagnosis and relapse in AML patients seems to indicate either intrinsic instability of the leukemic clone, or induced by multiple drugs, and suggests the risk of false negatives in the detection of minimal residual disease, particularly if panels with limited numbers of antigens are used.

013
TWO NEW CASES OF ACUTE PROMYELOCYTIC LEUKEMIA FOLLOWING MITOXANTRONE TREATMENT IN PATIENTS WITH MULTIPLE SCLEROSIS

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Over the last few years, several reports have indicated that the incidence of therapy-related acute myeloid leukemia (t-AML) in the population of MS patients treated with mitoxantrone is higher than the risk of *de novo* AML in the general population and the occurrence of acute promyelocytic leukemia (APL) has been well-documented.
We reported two new cases of APL in MS patients following treatment with mitoxantrone. The first patient, a 21-year-old female, started to suffer from progressive-relapsing disease and was treated with beta-interferon followed by mitoxantrone. After 18 months she developed pancytopenia and a diagnosis of APL was formulated. The patient was treated according to the AIDA protocol. Subsequently, the patient underwent autologous hematopoietic stem cell transplantation (HSCT) according to a multicenter experimental protocol which included MS patients with documented rapidly progressive MS over the previous year despite immunomodulating or immunosuppressive treatments. Complete molecular remission was confirmed 6 months after transplantation. The second patient, a 37-year-old female, was treated for progressive-relapsing MS with mitoxantrone. After 5 months, she developed APL. The patient was treated according to the AIDA protocol and underwent autologous HSCT, with complete molecular remission. Little is still known about the pathogenetic mechanisms in t-AML that can lead to the chromosomal translocation t(15;17) and the PML/RAR-alpha fusion transcripts occurring in APL. A recent report suggests that mitoxantrone induces *in vitro* topoisomerase II-mediated cleavage of PML and RARA genes and that this cleavage could be a general mechanism causing the DNA damage that develops after treatment with drugs that target topoisomerase II. These two further cases emphasize the need for doctors to carefully consider the choice of mitoxantrone for the treatment of MS and to include the potential risk of t-AML in each informed consent. Patients treated with this drug require special vigillance and hematological evaluation for several years after discontinuation of therapy.

014
USE OF MYLOTARG IN ELDERLY PATIENTS AFFECTED BY ACUTE MYELOID LEUKEMIA: EXPERIENCE OF A SINGLE UNIT

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Gemtuzumab ozogamicin (GO, Mylotarg) is a recombinant humanized anti-CD33 monoclonal IgG4 antibody that delivers the potent cytotoxin, calicheamicyn, into CD33 expressing cells. In particular GO has been approved for the treatment of elderly patients with AML. Its effect is increased by adding conventional chemotherapy, in particular Ara-C given preliminarly. Our group treated 20 elderly patients affected by AML, 12 relapsed and 8 refractory patients. The mean age was 72 (67-83). 8 relapsed patients received mean-low doses of GO (3mg/sqm) on 1 th and 15 th day with intermediate administration of MICE; 4 relapsed patients received GO (3 mg/sqm) on 1 th and 8 th day plus ARA-C (100 mg/sqm c.i. 1 th- 7 th day); 4 refractory patients received low doses of GO (2mg/sqm) repeated day 1, 3, 5 and after, monthly; 4 refractory patients received low dose of GO (2mg/sqm) weekly for 4 weeks and after, monthly. All relapsed patients deceaded. Two out of eight refractory patients deceaded, whereas six achieved complete remission (CR). The median recurrence-free survival was 6,4 months for patients who achieved CR. Grade 3-4 neutropenia and thrombocytopenia were observed in all patients. Grade 1-2 hyperbilirubinemia was observed in 16/20 patients and grade 3 in 4/20; grade 1-2 hepatic aspartate aminotransferase and alanine aminotransferase elevation was observed in 12/20 patients, grade 3 in 7/20, grade 4 in 1/20. All patients received Defibrotide prophylaxis (10mg/kg/day i.v.) from day -1 to day +30. VOD was never observed in our series. Our experience in a small cohort of patients seems to show a good effectiveness of GO therapy for refractory elderly patients with a acceptable safety profile. Our conclusions are that GO has to be administered using higher doses, but the acquired experience about this problem reports a very good effect of low doses of GO on veno-occlusive disease. Therefore, we have to resort to splitting up of the doses, repeated in short time.

015
EXTRAMEDULLARY INFILTRATES OF AML: BIOLOGICAL AND CLINICAL FEATURES

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Extramedullary infiltration (EMI) of malignant precursor cells in acute myeloid leukaemia (AML) may occasionally be a presenting clinical symptom at onset and may developed at any site in the body but most commonly in the gum, central nervous system (CNS) and soft tissue. There is controversy about the prognostic significance of extramedullary disease in AML. The present study examines the incidence, the biological features and prognostic significance of EMI at diagnosis in adult patients with AML. From January1997 to December 2004, 213 untreated patients with *de novo* AML were studied. According to Grimwade *et al*., 14 patients were in the favourable-risk group, 159 in the intermediate-risk group, 25 in the poor risk group and in 15 karyotipe was not available. All patients had been treated with induction therapy according to the GIMEMA protocols including cytarabine, etoposide and idarubicin (15 pts), or mitoxantrone (15 pts) or daunorubicin (183 pts). Of 213 cases with *de novo* AML, 29(14 %) had EMI at diagnosis. Ten patients (34.8 %) had skin infiltrates, 12 (41.4%) had gum hypertrophy, 5(17.2 %)had CNS involvement and 2 (6.9 %)had soft tissue infiltration. No significant differences in terms of sex, age median, Hb level and platelets count were found between patients with EMI and patients without EMI. The patients with EMI had higher median WBC counts ($27x10^9$/L) than patients without EMI ($8.5x10^9$/L)(p=0.05). The patients with EMI had higher incidence of M4/M5 FAB subtype (62 %) than patients without EMI (27.4 %)(p=0.005). Cytogenetic analysis was performed in patients with and without EMI; none of the abnormal cytogenetic findings was associated with EMI. We evaluated the relationship between the AML blasts surface antigen expression and EMI; the association between CD56/CD4 and CD56/CD14 was more significantly expressed in patients with EMI (35% and 29%, respectively) than without EMI (10.4 % and 6.9%, respectively)(p=0.004, p=0.003). The overall CR rate was 65%; the RC rate was lower in patients with EMI (48.2%) than patients without EMI (76.1%)(p=0.001) and their disease free survival was also shorter (p=0.017); the median duration of CR was 10 and 25 months (range 2-96) in EMI and no EMI group, respectively. Our data show that high WBC count, M4/M5 subtype, CD56//CD4 and CD56/CD14 expression are associated with extramedullary infiltrates of AML at diagnosis; the presence of EMI adversely affects the complete response rate to induction chemotherapy and the OS rate. Analysis of the clinical and biological features in a larger series of adult AML patients is needed to evaluate the allocation in this subgroup in a different or more intensive treatment arm.

016
CUTANEOUS ALTERNARIOSIS IN A PATIENT WITH ACUTE LYMPHOBLASTIC LEUKAEMIA: CLINICAL FEATURES AND DIAGNOSTIC ISSUES

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Cutaneous alternariosis is a rare form of phaeohyphomycosis caused by an opportunistic mold of the genus Alternaria. The clinical pattern is characterised by polymorphic cutaneous or subcutaneous involvement, ranging from localised eczema-like to chronic verrucous lesions. Thus the clinical diagnosis is extremely challenging and histological, cultural and molecular investigations are usually indispensable. More often reported in transplant recipients, cutaneous alternariosis has been exceptionally described in patients with haematologic malignancies. Here we report on a 24-year-old man presenting in September 2005 with a diagnosis of T acute lymphoblastic leukaemia. After an early relapse during the consolidation phase and the failure of the second line treatment, he was started on FLAG-Ida (Fludarabine, Cytosine Arabinoside, G-CSF and Idarubicin) in March 2006. On day +17, during bone marrow aplasia, he developed a painful erythematous-violet nodular lesion on the right foot. The patient was apyretic and all the biochemistry was unremarkable. Noticeably, he had been on antimycotic prophylaxis with Itraconazole 200 mg/die for 3 months. The skin biopsy showed a perivascular inflammatory infiltrate, whilst non-sporulant septate hyphae were observed on the cultural examination. Notwithstanding the lack of a conclusive diagnosis, considering the persistent pancytopenia, a treatment with intravenous Voriconazole 400 mg/die was started. Afterwards, further identification of fungi was based on sequence analysis of PCR product obtained with specific primers for ribosomal RNA Internal Transcript Sequences (ITS1+ITS4). A comparison with available databases allowed the identification of Lewia infectoria, a teleomorphic form of Alternaria infectoria. Noticeably, the lesion showed a slow but complete response to the antimycotic therapy, although the patient underwent an allogeneic stem cell transplant before its complete resolution. The present case confirms the role of Alternaria as possible pathogen in immunocompromised patients. Moreover, the proteiform clinical picture and the uncertainty of routine analyses underline the necessity of molecular methods in the diagnostic pathway.

017
HEPATITIS B AND C INFECTIONS EVALUATION IN HEMATOLOGIC MALIGNANCY

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Background. Few studies has been evaluated the role of hepatitis in development of hematologic malignancy. Aim: We conducted a monocentric based case control study to verify a possible association between myelodisplastic, myeloproliferative , lynphoproliferative disease and hepatitis B and C infections. *Methods*. We analized 334 consecutively registred patients in *San Pellegrino* Hospital in Trani (Italy) during 2005 year with positivity for hepatitis B virus surface (HBs antigen) and/or HCV antibody. Of 334 seropositivity HCV and/or HBV patients hospitalized in different wards (internal medicine, cardiology, nephrology, orthopedy, intensive care, general surgery and hematology), 36 patients were affected by hematologic malignancy. *Results*. In our case control study there was no higher association between seropositivity of HCV or HBV and hematologic malignancy than others disease used as controls, besides the incidence of HCV and HBV infection was comparable in all hospitalized group of provenience. The 36 hematologic patients included in our study, divided in 26 with lymphoproliferative disease (LPD) and 10 with myeloproliferative/myelodisplastic disease (MPD/MDS) presented in LPD group 18 HCV infection and 8 HBV infection and in MPD/MDS group group 9 HCV infection and 1 HBV infection . We found higher incidence of seropositivity of HCV in LPD group and in MPD/MDS group than seropositivity HBV (p: 0.004 and 0.03 respectively , evaluated with T test). *Conclusions*. It may be useful further evaluations to verify if the HCV infection influence the mechanisms of tumorigenesis differently between lymphoid and myeloid malignancy.

018
OSTEOPROTEGERIN AND RANKL IN THE PATHOGENESIS OF OSTEOPOROSIS IN PATIENTS WITH THALASSAEMIA MAJOR

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Osteoporosis represents an important cause of morbidity in Thalassaemia Major patients; the etiopathogenesis is multifactorial and includes expansion of the bone marrow, endocrine disorders, iron overload and genetic factors. Two cytokines, osteoprotegerin (OPG) and RANKL, have recently been identified as important mediators in the pathogenesis of osteoporosis. In this study, the possible role of the OPG-RANKL system in the pathogenesis of osteoporosis in Thalassemia Major is assessed, as well as any correlations between the serum levels of OPG and RANKL and bone mineral density (BMD) , 17 ,-estradiol and free testosterone and the relationship between T-score of BMD and OPG/RANKL ratio. In 50 Thalassaemia Major patients and a control group, the serum values of OPG and RANKL were assayed and correlated with BMD, as well as with the sex hormones values. All the thalassemic patients had reduced BMD and 36% presented osteoporosis. The thalassemic patients had significantly higher serum levels of OPG than the controls, while their higher RANKL levels, were at the threshold of significance. The OPG/RANKL ratio showed higher level respect to the controls. No statistically significant correlation was observed between the T-score and

RANKL neither between the T-score and OPG nor between T-score and OPG/RANKL ratio. Instead, a statistically significant correlation was found between the T-score and free testosterone and between the T-score and 17 β-estradiol. There was no correlation between the sex hormones and OPG and RANKL. The increased OPG values in thalassemic patients could be considered to compensate the increased bone turnover. We confirm hypogonadism as a primary etiopathogenetic factor in the reduced BMD observed in Thalassaemia Major patients.

019
SEVERE INFECTIONS IN THALASSEMIC PATIENTS: ANALYSIS OF LIFE THREATENING EVENTS

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Infections are major complications and constitute the second most common cause of mortality and a main cause of morbidity in patients with thalassaemia. Besides the high risk of blood-borne infections associated with multiple transfusions, the increased susceptibility of these patients to infectious diseases has been attributed to an iron overload, iron chelation therapy, splenectomy and a range of immune abnormalities. The clinical spectrum of severe infections were osserved in our thalassaemic patients. There were 3 patients with episodes of severe infection that included life threatening events. The first case regards a 32 years old male affected by Thalassaemia Intermedia, splenectomized, who was urgently hospitalized for hyperpyrexia accompanied by suspect cholangitis and icterus. The clinical status was complicated by a shock hypovolemic, renal acute failure and respiratory insufficiency, therefore the patient was successfully treated with intensive supportive care and haemodialysis followed by plasma exchange. The second case regards an 18 years old female affected by Thalassemia Mayor, who was hospitalized for septic fever accompanied by spleen pain , dyspnoea and respiratory insufficiency. Blood chemistry results demonstrated an Epstein Barr Virus infection therefore she was successfully treated with antiviral therapy. The third case regards a 40 years old female with Thalassaemia Mayor who was hospitalized for miocarditis and sacrum-iliac inflammation followed by a Escherichia Coli sepsis and spondylodiscitis (D5-D6 and L4-L5). She was successfully treated with antibiotic therapy and bed immobilizing followed by a gradual physical therapy. She was discharged six months after admission with success of conservative management.

020
RITUXIMAB FOR WARM-TYPE IDIOPATHIC AUTOIMMUNE HEMOLYTIC ANEMIA: A RETROSPECTIVE STUDY OF 11 ADULT PATIENTS

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Warm-type idiopathic autoimmune hemolytic anemia (AIHA) is a relatively common hematologic disorder resulting from autoantibody production against red blood cells. Steroids represent the first-line therapy option and immunosuppressive agents as well as splenectomy are used for refractory cases. Recently, the anti-CD20 monoclonal antibody rituximab has shown to control autoimmune hemolysis in patients with refractory chronic disease. We report results from a retrospective analysis of 11 adult patients receiving rituximab for steroid refractory AIHA of the warm type at a mean age of 55 years (range 23 - 81 years). All patients were given methyl-prednisolone as first-line treatment and some of them also received azathioprine and intravenous high-dose immunoglobulins. One patient underwent splenectomy. All patients were considered refractory to steroids and/or immunosuppressive drugs and all were then given weekly rituximab (375 mg per meter square) for 4 consecutive weeks. An increase in hemoglobin (Hgb) levels in response to rituximab, with a mean increment of 3.3 g/dL (95% CI 2.1 – 4.4), was observed in all cases. Four patients required packed red cell transfusions before starting rituximab and all became transfusion-free. At a mean follow-up of 604 days (range 30 – 2,884 days) since the treatment of AIHA with rituximab all patients are alive, 8 (73%) of them in complete remission (CR) and 3 (27%) in partial remission (PR). A moderate hemolysis still persisted in 6 (54%) patients. In conclusion, our experience clearly demonstrate that anti-CD20 monoclonal antibody rituximab is as an effective as safe alternative treatment option for idiopathic AIHA, in particular for steroid-refractory disease. Warm-type idiopathic autoimmune hemolytic anemia (AIHA) is a relatively common hematologic disorder resulting from autoantibody production against red blood cells. Steroids represent the first-line therapy option and immunosuppressive agents as well as splenectomy are used for refractory cases. Recently, the anti-CD20 monoclonal antibody rituximab has shown to control autoimmune hemolysis in patients with refractory chronic disease. We report results from a retrospective

analysis of 11 adult patients receiving rituximab for steroid refractory AIHA of the warm type at a mean age of 55 years (range 23 - 81 years). All patients were given methyl-prednisolone as first-line treatment and some of them also received azathioprine and intravenous high-dose immunoglobulins. One patient underwent splenectomy. All patients were considered refractory to steroids and/or immunosuppressive drugs and all were then given weekly rituximab (375 mg per meter square) for 4 consecutive weeks. An increase in hemoglobin (Hgb) levels in response to rituximab, with a mean increment of 3.3 g/dL (95% CI 2.1 – 4.4), was observed in all cases. Four patients required packed red cell transfusions before starting rituximab and all became transfusion-free. At a mean follow-up of 604 days (range 30 – 2,884 days) since the treatment of AIHA with rituximab all patients are alive, 8 (73%) of them in complete remission (CR) and 3 (27%) in partial remission (PR). A moderate hemolysis still persisted in 6 (54%) patients. In conclusion, our experience clearly demonstrate that anti-CD20 monoclonal antibody rituximab is as an effective as safe alternative treatment option for idiopathic AIHA, in particular for steroid-refractory disease.

021
JAK2 MUTATION IN PH NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS: DATA FROM A SINGLE CENTRE EXPERIENCE

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Chronic myeloproliferative diseases (CMDs) are heterogeneuos disorders defined as expansion of a single neoplastic stem cell. The molecular basis of most CMDs is unknown. In analogy with CML, we may surmise that constitutive activation of a tyrosine kinase might be involved even in other CMDs. Recent data have confirmed that a somatic mutation at position 617(V617F) of the Janus kinase 2 (*JAK2*) gene is found in a majority of PV and in a sizeable proportion of ET and IMF. These findings can lead to new perspective for diagnosis and classification of CMD patients. We screened for V617F bone marrow samples of 57 ET and 23 PV patients, banked at CEINGE-Naples. The V617F *JAK2* mutation was detected in 82.6% PV and in 42.1% ET patients. Many authors have shown that patients with V617F positive CMD have a longer median disease duration and were more likely to develop complication such as secondary myelofibrosis, bleeding or thrombosis than those without the mutation. Moving from these data we looked for correlation among clinical features (spleen volume, LDH and marrow fibrosis grading) and *JAK2* genotype [wild-type(wt) or mutant(m)] in PV and ET patients. We found that the median level of LDH was significantly higher in V617F positive cases. We found that the median value of spleen volume in mutant patients was higher than the wild-type group, although without statistically significant difference. In the mutant group, 70% of patients had fibrosis grade 0-1 and 30% grade 2; in the wt group fibrosis was always 0-1 and never grade 2. Wider studies are needed to confirm these correlation; different approachs are needed to identify markers in the group of patients without JAK2 mutation.

022
MOLECULAR CYTOGENETIC STUDY OF DERIVATIVE CHROMOSOME 9 DELETIONS IN CHRONIC MYELOID LEUKEMIA

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Large deletions adjacent to the t(9;22) breakpoint on the derivative 9 chromosome have now been found, which result in genomic loss at both sides of the translocation breakpoint. We report an update of our FISH study on Chronic myeloid leukemia (CML) cases bearing deletions on der(9) chromosome. FISH analysis with BAC/PAC clones specific for ABL1 and BCR genes was performed on bone marrow cells of 334 CML patients at diagnosis. We have detected der(9) deletions in 60 (18%) CML cases. Deletions of chromosome 9 sequences on the der(9) were found in 50 (83%) cases; they were present in all Ph+ metaphases and ranged from 350 Kb to 41.6 Mb. A tumor suppressor gene (TSG) called prostaglandin E synthase (PTGES) was lost in 39 (78%) cases. Chromosome 22 deletions on der(9) were found in 52 (87%) of the analysed cases; the deleted chromosome 22 sequences were shorter than the deleted chromosome 9 sequences (ranging from 400 Kb to 12.7 Mb). Two TSGs mapping inside the deleted sequences of chromosome 22, SMARCB1 and GSTT1, were found deleted in 37 (71%) cases. Thirty-two (10%) CML patients showed a variant 9/22 rearrangement. Thirtheen (41%) of them were deleted on der(9) chromosome; moreover, in 9 out of 13 cases genomic loss were detected on the third chromosome involved in the variant t(9;22) translocation. The miRBase consulting revealed that 16 (27%) patients had the loss of miRNAs mapping on chromosome 9 whereas on the deleted genomic sequences belonging to the chromosome 22 known miRNAs were not mapped. The observation that deletions on der(9) are associated with the loss of TSGs suggests their possible involvement in the CML outcome, mediated by a haplo-insufficiency mechanism; moreover, further studies are needed to clarify the miRNA role in the CML pathogenesis.

023
"HOME-BREW" FISH ASSAY SHOWS HIGHER EFFICIENCY THAN "BCR-ABL DUAL COLOR, DUAL FUSION" PROBE IN DETECTING GENOMIC MICRODELETIONS AND COMPLEX REARRANGEMENTS ASSOCIATED WITH t(9;22) IN CHRONIC MYELOID LEUKEMIA

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Microdeletions on the der(9) chromosome next to the t(9;22) breakpoint have recently been reported in CML cases by several groups and are associated with a poor prognosis in patients administered alpha-interferon therapy. Genomic microdeletions on der(9) have a variable extension and may involve sequences on both chromosomes 9 and 22, located proximally to the *ABL* and distally to the *BCR* gene. The use of *BCR-ABL* probes in FISH experiments is the method commonly employed for the detection of microdeletions on der(9) in patients with CML. We carried out FISH experiments on 18 Ph+ chronic myeloid leukemia (CML) cases with chromosome 22 genomic deletions with the Vysis *BCR-ABL dual-color/dual-fusion* probe (*BCR-ABL* DC/DF) to compare the hybridization patterns obtained with this approach as compared to our *home-brew* BAC/PAC system. Our results are the following: 1) chromosome 22 microdeletions < 400 Kb were not detected by the *BCR* DC/DF probe; 2) FISH analysis with the *BCR* DC/DF probe in cases bearing chromosome 22 microdeletions ranging from 400 to 700 Kb produced a faint signal on der(9); 3) the *BCR-ABL* DC/DF FISH pattern was comparable to the one obtained by the *home-brew* probe in presence of a 900 Kb chromosome 22 microdeletion. In conclusion, this study showed that our *home-brew* probe system was more successful in investigating genomic microdeletions on der(9) in CML and in revealing the involvement of a third chromosome in variant t(9;22) rearrangements. The reason for this is two fold: firstly, the commercial probes cover large genomic segments, which is a necessity for a reliable and robust diagnostic tool and secondly, there is ~ 300 Kb gap in the BCR probe, which houses the deletions. Therefore our results suggest that the best tool for screening microdeletions on der(9) in CML is represented by the *home-brew* probe system reported in this study.

024
*JAK2*V617F MUTATION, PRV-1 OVEREXPRESSION AND EECS GROWTH IN PATIENTS WITH BCR/ABL-NEGATIVE MYELOPROLIFERATIVE DISORDERS

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Bcr/abl-negative myeloproliferative disorders (MPDs) are an heterogeneous group of clonal stem cell diseases that includes polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (IMF). Even though the pathogenesis of these diseases is not clear, some molecular and functional alterations such as JAK2V617F, PRV-1 overexpression and endogenous erythroid colonies (EECs) growth *in vitro* have been associated. In the attempt to determine the incidence and the reciprocal correlations we analyzed JAK2V617F, PRV-1 overexpression and EEC growth in 100 blood samples from MPD patients (30 PV, 61 ET and 9 IMF). The molecular analyses showed the presence of JAK2V617F in 73.3% PV, 22.9% ET, 33,3% IMF and PRV-1 overexpression in 75% untreated PV and 50% of PV treated with hydroxyurea; 38,5% untreated ET and 38,5% treated ET; 75% untreated IMF and 20% IMF under treatment tested. The functional tests indicated EECs growth in 88,9% PV, 50% ET and 40% IMF tested. Statistical analyses revealed a significant correlation between JAK2V617F and PRV-1 overexpression both in PV (p= 0,01, r=0,4) and in ET (p<0,001, r=0,5); similarly JAK2 correlated with EEC growth both in PV and ET (respectively p= 0,035, r=0,5 and p=0,01 and r=0,4). This study seems to agree with previous reports about the incidence of the molecular alterations found and the absence or the partial inhibition on PRV-1 expression by hydroxyurea. We confirm the correlation between JAK2V617F and PRV-1 overexpression and between JAK2V617F and EEC growth *in vitro* in PV and ET suggesting a role of JAK2V617F in influencing both PRV-1 overexpression and indipendent proliferation of erythroid precursors. Consequently it seems that PRV-1 expression is a secondary factor rather than the cause of abnormal erythropoiesis, therefore its should not be considered alone and could not be useful as single parameter. In conclusion the presence of JAK2V617F might actually be considered the most important molecular indicator that could allow to redefine the diagnostic criteria, the classification and possibly the management of these disorders.

025
THE V617F JAK2 MUTATION INCIDENCE IN BCR/ABL NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS: COMPARISON BETWEEN BONE MARROW AND PERIPHERAL BLOOD

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Based on the knowledge of the pathogenesis of CML, it was speculated that another constitutively activated tyrosine kinase could account for the chronic myeloproliferative disorders (MPDs). Recurrent and activating G to T point mutation resulting in substitution of phenylalanine for valine at position 617 (V617F) in the Janus kinase 2 (Jak2) was reported in bcr/abl-negative cMPD, including polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF). Estimates using different detection methods indicate that JAK2V617F is present in 65%-97% of PV, 23%-57% of ET, and 35%-57% of IMF patients, respectively. However, the distribution pattern among cMPD is changing since more sensitive methods, other than time consuming and not always feasible sequencing technique, were developed. In this study, we used an allele-specific polymerase chain reaction (PCR) assay to detect JAK2V617F. Briefly, a mutation-specific forward primer containing a fluorescent tag (FAM) was used in a PCR reaction with a wild-type sequence reverse primer. Only mutated DNA will be amplified (203bp), if present, and the PCR reaction product was analyzed using capillary electrophoresis in an automated genetic analyzer. All 50 normal samples resulted negative, showing a median signal one log lower than cases belonging to positive PB samples obtained from cMPD. Moreover, BM JAK2V617F mutated cMPD cases sowed a fairly expected two logs higher signal than normal controls. Finally, 32 PV, 38 TE, 25 MIF, 14 cMPD and 26 secondary polyglobulia (secPoly) were analyzed. Out of 135 cases analyzed, 41 were bone marrow cells, 91 resulted JAK2V617F mutated. In particular, none of secPoly cases resulted mutated, while 96.9%, 78.9%, 80% and 71.4% showed JAK2V617F mutation in PV, TE, MIF and cMPD, respectively. The results of this study clearly indicated that it is conceivable that the detection of JAK2 V617F can be used as an initial tool in the diagnosis of chronic hyperleukocytosis, thrombocytosis and erythrocytosis. Clinical and laboratory differences, if any, among MPDs that carry JAK2 V617F with those showing a wild type gene expression should be investigated.

026
PDGFRαFIP1L1-POSITIVE CHRONIC EOSINOPHILIC LEUKEMIA PRESENTING WITH RETRO-ORBITAL LOCALIZATION: EFFICACY OF IMATINIB TREATMENT

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The fusion protein between the platelet-derived growth factor receptor alpha (PDGFRα, P) gene and the Fip1-like1 (FIP1L1, F) may be identified in 14% to 60% of HS and it indicates a clonal hypereosinophilic syndrome called F/P-positive CEL. We herein report a case of F/P-positive CEL with retro-orbital localization, who was successfully treated with imatinib. A 53-year-old male presented an absolute eosinophil count of 25,000/mm^3, anemia (Hb 10,2 g/dL) and a modest increase of the platelet count (571,000/mm^3). A clinical examination revealed left exophthalm, associated with diffuse hhypoesthesia and diplopia. A CT scan of orbits showed a lesion located in the lachrymal fossa of the left orbit with intra and extra-conical extension. Parasitic, allergic, or other known causes of eosinophilia were excluded. Echocardiography as well as ultrasonography of the liver and a total body CT scan excluded any organ involvement. BM histology showed a myeloid hyperplasia with a significant increase of the eosinohilic lineage. Molecular analysis excluded the presence of bcr/abl transcript while a F/P fusion tyrosine kinase signal was documented. Imatinib 100 mg/day was started and, after 7 days of treatment eosinophil count significantly declined (560/mm^3) along with a dramatic reduction of the left exophthalm. Imatinib dosage was increased up to 200 mg/day from the 4th week to 300 mg/day from the 5th week. The drug was well tolerated with an initial modest haematological toxicity (Hb 10.9g/dL). The left exophthalm, as well as hhypoesthesia and diplopia, disappeared after the 4th week of imatinib therapy. MRI showed a further clear reduction of the in intra and extra conical growth process after 9 weeks on imatinib. BM molecular signal of the F/P fusion gene resulted undetectable after 4 weeks of treatment. In conclusion, molecular investigation of this HS let us *i*) to support the diagnosis of F/P-positive CEL; *ii*) to predict response to imatinib, not only in terms of haematological and molecular response, but also for the retro-orbital localization; *iii*) to show a molecular complete response and to avoid surgical treatment.

027
EARLY STAGE IDIOPATHIC MYELOFIBROSIS: SINGLE INSTITUTION EXPERIENCE

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Background. In view of the high platelet count as well as the lack of specific diagnostic clues (i.e. evident splenomegaly; high serum levels of LDH; circulating immature myeloid cells, erythroblasts and dacryocytes) early stages of idiopathic myelofibrosis with associated thrombocytosis (i.e. prefibrotic myelofibrosis and early idiopathic myelofibrosis) are with difficulty distinguished from true essential thrombocytemia. Abnormal megakaryopoiesis offers the possibility of identifying early stage idiopathic myelofibrosis apart from the platelet count, laboratory parameters and clinical symptoms. In essential thrombocytemia megakaryocytes are mostly giant and contain an enlarged nucleus with deep lobulations, but lack the cytological abnormalities; in prefibrotic or early idiopathic myelofibrosis megakaryocytes present bizarre forms with marked nuclear-cytoplasmic anomalies. Early stages of idiopathic myelofibrosis are characterized by poor prognosis and a significant tendency to progress into collagen myelofibrosis and blastic crisis; in fact, life expectancy is relatively normal in true essential thrombocytemia but significantly shortened in prefibrotic myelofibrosis as well as in the various fibrotic stages of myelofibrosis.
Methods and Results. The data of 33 patients with idiopathic myelofibrosis (19 males, 14 females; median age 71 years [range 35-84]) were retrospectively analysed. Evaluation was particularly focused on the discriminating impact of bone marrow and peripheral blood morphology. Eight patients (24%) were affected by early stage idiopathic myelofibrosis (4 prefibrotic myelofibrosis, 4 early idiopathic myelofibrosis). All patients with early stage idiopathic myelofibrosis showed at diagnosis pronounced thrombocythemia (median value $879x10^9$/L [range 427–1.654]) and slight leukocytosis (median value 13 x 10^9/L [range 6–15]), normal serum levels of LDH, uric acid and leukocyte alkaline phosphatase. Physical exam and abdominal echography detected occasionally very small splenomegaly with spleen margin at more than 1 cm from the left costal arc. Cytogenetic analysis revealed a normal kariotype. Peripheral blood smear showed normocromic-normocytic red blood cells, macro-platelets, agranular platelets and small aggregates; in addition, morphological examination of peripheral blood did not show evidence of immature myeloid cells or erythroblasts. The bone marrow biopsies were hypercellular and dominated by atypical immature megakaryocytes, conspicuously large due to an increase of nuclear and cellular size, and always grouped in clusters of four/six elements; reticulin fibrosis was minimal (MF1) or absent (MF0). *Conclusion*. In our Institution the incidence of early stages of idiopathic myelofibrosis is similar to that of international reports. In view of the lack of specific diagnostic clues (i.e. evident splenomegaly; high serum levels of LDH; circulating immature myeloid cells, erythroblasts and dacryocytes) the differential diagnosis between true essential thrombocytemia and prefibrotic or early idiopathic myelofibrosis with associated thrombocytosis is primarily based upon conspicuous differences in the form and size of megakaryocytes in bone marrow aspirate and bone marrow biopsies; therefore, a detailed evaluation of bone marrow morphology is recommended.

028
VALUTAZIONE DELLA CHEMIOSENSIBILITÀ *IN VITRO* COME TEST PREDITTIVO DELLA RISPOSTA CLINICA ALLA FLUDARABINA IN PAZIENTI AFFETTI DA LEUCEMIA LINFATICA CRONICA B

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Una percentuale di pazienti affetti da Leucemia Linfatica Cronica B (LLC-B), variabile a seconda delle casistiche, risulta resistente al trattamento con Fludarabina. Scopo di questo lavoro è stato quello di valutare la chemiosensibilità *in vitro* alla Fludarabina, in pazienti affetti da LLC-B, prima dell' inizio della terapia con Fludarabina e correlare i dati ottenuti con la risposta clinica. *Methods*. I buffy coats periferici (per separazione su ficoll) di 32 pazienti affetti da LLC-B venivano colturati *in vitro* con dose scalari di Fludarabina (20-10-5-2.5-1.25 Ìmoli/mL) e dopo 48 h veniva valutata la percentuale di mortalità con Ioduro di Propidio in citofluorimetria. Tutti i pazienti venivano quindi avviati a trattamento con Fludarabina os (40 mg/mq /die os x 5 gg ogni 4 settimane). Dopo 2 cicli di trattamento veniva effettuata una prima valutazione della risposta terapeutica. I pazienti che avevano ottenuto una risposta terapeutica sulla conta dei WBC > 75 % venivano considerati Responsivi (Resp) e continuavano con la sola Fludarabina per un totale di 4 cicli. Gli altri pazienti vevivano considerati Non Responsivi (N-Resp) ai fini dello studio e modificavano lo schema terapeutico in Fludarabina (40 mg/mq/die x 3 gg os) e Ciclofosfamide (CTX) (300 mg/mq/die x 3 gg os) ripetuto ogni 4 settimane per altri 4 cicli. *Results*. I pazienti Resp mostravano una percentuale di mortalità *in vitro* del 26.4±13.3 % (range 12-50%) vs 4.6±2.7% (range 0-9%) dei pazienti N-Resp ($p<0.00001$). Dei 13/32 pazienti Resp (trattati con sola Fludarabina) 12 (92 %) dei pazienti hanno ottenuto una Remissione Completa ed 1 paziente una Remissione Parziale. Dei 19/32 pazienti definiti N-Resp (trattati con Fludarabina e CTX) 9 hanno ottenuto una Remissione Parziale e 10 sono risultati Resistenti al trattamento. Nessuno dei pazienti definiti N-Resp ha ottenuto una Remissione Completa. *Conclusioni*. I dati riportati suggeriscono che esiste una diretta correlazione tra la valutazione della chemiosensibilità *in vitro* e la risposta clinica al trattamento con Fludarabina. In accordo con i dati della letteratura si conferma che l' aggiunta della CTX al trattamento permette di ottenere un incremento della risposta complessiva, incidendo positivamente sui pazienti che hanno dimostrato resistenza *in vitro* alla fludarabina.

029
SERUM ADIPONECTIN COMPARED WITH ZAP-70, CD38 AND IMMUNOGLOBULIN HEAVY-CHAIN MUTATION STATUS AS A PREDICTOR OF TIME TO FIRST TREATMENT IN EARLY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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We analyzed the correlation between well-established biological parameters of prognostic relevance in B-cell chronic lymphocytic leukemia [CLL] (i.e, mutational status of the immunoglobulin heavy chain variable region [IgV_H], ZAP-70- and CD38-expression) and serum levels of adiponectin by evaluating the impact of these variables on the time to first treatment [TFT] in a series of 69 previously untreated Binet stage A B-cell CLL patients. Higher levels of adiponectin inversely correlated with peripheral blood lymphocytosis (r=-0.254; p=0.03), male gender (p=0.0002), percentage of ZAP-70-positive (r=-0.285; p=0.04) and CD38-positive (r=-0.294; p=0.04) B-CLL cells. After a median follow-up time of 32 months (range, 2-120 months) 28 (40.5%) out of 69 patients experienced a need for chemotherapy. Kaplan-Meier estimates of patients TFT, plotted after searching the best cut-off for adiponectin (i.e., 5.88 ng/mL), demonstrated that low adiponectin concentration was associated with a shorter TFT (median TFT 32 months; p=0.01). The relationship among the various bio-pathological parameters, analyzed by the multiple correspondence analysis (MCA), showed two different clinico-biological profiles. The first, characterized by higher adiponectin serum levels (i.e., > 5.88 ng/mL), higher platelet count (>174x10^9/L), lower β_2-microglobulin [β_2-m] (<2.35 mg/L), presence of mutation in the IgV_H and low percentage of either CD38-positive (< 20%) or ZAP-70-positive (< 20%) B-CLL cells, was associated with a stable pattern of disease generally not requiring therapy. The second, defined by lower adiponectin levels, lower platelet count, high β_2-m concentration, absence of mutation in the IgVH, high percentage of CD38- and ZAP-70 positive B-cells, was associated with a more progressive pattern of disease and a shorter TFT. The univariate Cox proportional hazard model demonstrated that in addition with lower serum levels of adiponectin (Hazard Ratio [HR], 2.936; p=0.01), the absence of IgV_H mutational status (HR, 6.378; p=0.002) and ZAP-70-positivity (HR, 3.314; p=0.02) were associated with a shorter TFT. However, in multivariate analysis only ZAP-70 positivity emerged as predictor of the TFT (HR, 5.187; p=0.008). Our results indicate that in early B-cell CLL clinico-biological profile including among other parameters adiponectin may provide a useful insight into the complex interrelationship of prognostic variables and semplify their interpretation. However, adiponectin may not replace the need for the determination of ZAP-70 and IgVH mutational status.

030
DIAGNOSTIC POTENTIAL OF CD38 COMBINED WITH ZAP-70 EXPRESSION IN PREDICTING MUTATIONAL STATUS OF IMMUNOGLOBULIN HEAVY-CHAIN VARIABLE REGION IN CHRONIC LYMPHOCYTIC LEUKEMIA CASES

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We have conducted here a study on roughly 500 chronic lymphocytic leukaemia (CLL) patients whose neoplastic cells were characterized for ZAP-70 by western blot (cases were classified as ZAP-70strong, ZAP-70weak and ZAP-70neg), CD38 expression and Ig-VH gene status. As a first step, we determined, by ROC curve analysis, 30% as the best cut-off value of CD38 which discriminates between mutated and unmutated cases in CLLs (area under the curve 0.765, p<0.0001). On the basis of standard diagnostic tests, CD38 expression, categorized by 30% cut-off value, had low sensitivity (66%), but relatively high specificity (80%), with a positive and a negative predictive value respectively of 68% and 78% in anticipating VH mutational status. Moreover, Kappa statistic revealed that the agreement between CD38 expression and VH mutational status was low although significant (K=0.46, p<0.001). On the other hand, ZAP-70 (negative and weak ZAP-70 patterns were collectively analysed) showed relatively high sensitivity (76%) and specificity (75%), relatively high negative (89%) but a low positive (54%) predictive value and a low, although significant, K statistic (0.45, p<0.001). Furthermore, we combined the value of CD38 and Zap-70 tests to evaluate whether both variables provided more precise information in estimating VH mutational status compared to that obtained from each single test . In this regard, we obtained the following results: sensitivity, 42%; specificity, 97%; positive predictive value, 90%; negative predictive value, 72%; k statistic 0.43, p<0.001. Moreover, ROC analysis was also performed to detect the optimal percentage of Ig V gene mutations capable of predicting cases with positivity of both CD38 and Zap-70. The best cut-off value was 1.5% (AUC 0.841, p<0.0001). This study demonstrated that neither CD38 nor ZAP-70 by themselves or in combination were able to anticipate VH mutational status, meaning that neither the single marker nor the combined use of CD38 and Zap-70 could surrogate the VH mutational status in CLL.

031
SPONTANEOUS REMISSION OF LYMPHOPROLIFERATIVE DISORDERS AFTER WITHDRAWAL OF IMMUNOSUPPRESSIVE TREATMENT FOR RHEUMATOID ARTHRITIS OR OTHER AUTOIMMUNE DISEASES

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There is a number of intriguing case reports of lymphoproliferative disorders (LPDs) that spontaneously abated shortly after discontinuation of immunosuppressive treatment for underlying autoimmune disease. Such LPDs, completely or partially regressing, occur in the clinical setting of Methotrexate-associated LPDs. We identified 25 literature patients showing a spontaneous complete remission (CR) of their LPD, and 8 others achieving only a partial remission (PR). In addition, a patient of ours with a connective tissue disease experienced the spontaneous PR of her LPD after stopping azathioprine. Most of the literature patients were affected by rheumatoid arthritis, received low-dose and long-term pulsed MTX alone or in combination with other immunosuppressants, and developed a lymphoma. By reviewing the patients achieving CR, it can be drawn the absence of a unique type of LPD, and the occurrence of an increased incidence of diffuse large B cell lymphoma as well as of frequent extranodal involvement and EBV infection. Further, CR occurred mostly within a time interval of 4 weeks after discontinuation of immunosuppressant and appeared to be persistent overtime. Conversely, in the patients experiencing only PR, the time interval between discontinuation of immunosuppressive treatment and clinical response was mostly reported for a longer period than 4 weeks; moreover in many cases, the evidence of LPD or its progression induced to start cytotoxic therapy being the overall outcome of LPDs similar to that observed in general population. However, possible spontaneous remission of MTX-associated LPDs upon reversal of iatrogenic immunosuppression provides a clue to their distinction, and induces a particular clinical approach. At the time of LPD diagnosis in a patient receiving immunosuppressive treatment for autoimmune disease, it is advisable to withdraw the treatment and perform a careful monitoring of the patient for some weeks. We have to be aware that spontaneous remission may occur, and that this favourable outcome cannot be predictable so far.

032
COMBINED ANALYSIS OF ZAP-70 AND CD38 EXPRESSION IDENTIFIES PROGNOSTIC SUBGROUPS OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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B-cell chronic lymphocytic leukemia (B-CLL) has a variable clinical course. Many patients never need treatment, whereas others have a poor outcome requiring intensive treatment early after diagnosis. Several prognostic factors have been evaluated in order to predict an indolent or aggressive disease. The most useful tools indicating aggressive B-CLL are IgV_H mutational status, cytoplasmic ZAP-70 and surface CD38 expressions. The latter two tests were also found to be less expensive and time-consuming, when evaluated by means of flow cytometry. We evaluated the simultaneous expression of ZAP-70 and CD38 in 157 patients (median age 64 years; range 35 – 85 years; 102 males and 55 females) with previously untreated B-CLL. Fifty-seven patients (36%) were positive for ZAP-70 and 46 patients (29%) were positive for CD38. Both ZAP-70 and CD38 expressions were shown to independently predict the clinical course of the disease and were also found highly correlated to each other. Patients were then divided into 3 groups according to the simultaneous evaluation of ZAP-70 and CD38. In 81 patients (52%) there was a negative concordance of both molecules ($ZAP\text{-}70^-/CD38^-$); in 27 patients (17%) there was a positive concordance ($ZAP\text{-}70^+/CD38^+$); in 49 patients (31%) there was a discordant ZAP-70/CD38 expression. A comparison of the clinical and laboratory data of the three groups showed that in $ZAP\text{-}70^+/CD38^+$ patients emphasized that a significantly higher bone marrow and peripheral blood lymphocytosis, lower hemoglobin levels, more advanced Binet clinical stage, and higher number of unmutated IgVH status were found with respect to the other two groups. Furthermore, ZAP-70-/CD38- patients displayed a much shorter treatment-free interval [median 12 months vs 42 months in discordant patients and not reached in $ZAP\text{-}70^-CD38^-$ patients ($p<0.0001$)]. These results were also confirmed when only early stage (A Binet) B-CLL patients [no. 112 (71%)] were separately analyzed. In conclusion, the concomitant evaluation of ZAP-70 and CD38 expression allows the separation of B-CLL patients in three groups with different prognosis, suggesting that a simultaneous assessment of both molecules need to be performed in all B-CLL cases to better define prognosis.

033
REACTIVATION OF CYTOMEGALOVIRUS AND TRANSIENT TRANSPLANT-ASSOCIATED GAMMOPATHY IN MULTIPLE MYELOMA PATIENTS AFTER AUTOLOGOUS HEMOPOIETIC STEM CELL TRANSPLANTATION

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Background. Autologous peripheral blood stem cell transplantation (ASCT) is a well-established therapy for Multiple Myeloma (MM). Cytomegalovirus (CMV) reactivation frequently occurs in transplanted MM patients. *Aim*. We are here describing the appearance in the serum (s) of monoclonal components (MC) with immunochemical type different from the ones of the malignant clone in 5 transplanted MM patients and we correlate it with CMV reactivation. *Patients and methods*. Main clinical and haematological data on 7 patients with MM are showed in the Table 1.

Main clinical and haematological data on 7 MM patients with transient ASCT-associated MC.

Pt. No.	*Age,y./ Gender*	*sMC type pre-ASCT*	*Response status post -ASCT*	*sMC type post-ASCT*
1	54/F	IgAk	PR	IgGk
2	52/F	IgAk	PR	IgGk+λ
3	60/F	IgAk	CR	IgGk+λ
4	51/F	IgAλ	PR	IgGλ
5	51/F	k	CR	IgGk
6	61/F	IgGk	PR	λ
7	57/M	k	PR	IgGλ

All patients were treated with VAD-chemotherapy followed by ASCT. The patients were regularly followed for a period of at least 12 months after transplantation. During monthly check-up examination, they underwent evaluations of serum protein electrophoresis and immunofixation, dosage of serum immunoglobulins and light chains by nephelometry, CMV serology by immunoenzimatic assay and CMV viremia by antigenemia assay. *Results*. According to EBMT/IBMTR response criteria, 2 patients achieved a complete remission (CR), 5 patients a partial remission (PR). After a mean period of 3.5 months (range 2–6), all patients had a sMC with immunologic type different from the one of the malignant clone (IgGk-type in two cases; IgGλ in two cases; IgGk +λ free in two cases; λ free in one case). The 2nd sMC disappeared after treatment of CMV reactivation with ganciclovir. *Discussion and conclusions*. Among immunocompetent adults, CMV has not been considered to be an independent cause of MG. A marked increase in the incidence of MG has been observed after solid organ or allogeneic marrow transplantation. Our results suggest that, in our cases, the appearance of the 2nd MC was caused by some B-cell clones, different from the primitive clone, that may have recovered faster than others after ASCT. Moreover this study support the hypothesis that the decrease of T-cell mediated immune surveillance after ASCT might allow B cell proliferation to escape, particularly if promoted by CMV reactivation, and produce a MC.

034
OUTPATIENT-BASED PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR PATIENTS WITH MULTIPLE MYELOMA

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High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) represents standard practice for newly diagnosed patients with multiple myeloma (MM). Accordingly, there is a growing demand for ASCT resulting in increased pressure on available hospital beds. The main advantages of at-home ASCT are the improvement in the quality of life and the reduction in the number of days during which patients need hospitalization, with a consequent reduction of costs, risk of nosocomial infections and more rational utilization of beds. The aim of this study was to confirm the feasibility and safety of performing ASCT on an outpatient basis, according to an early discharge method. A total of 107 MM patients out of 124 consecutively autografted affected by MM in PR or CR were selected to receive ASCT on an outpatient basis. In particular, after conditioning with high-dose melphalan and stem cell infusion, patients were programmed to go home and to be rehospitalized in the case of febrile neutropenia or other severe toxicities. The remaining 17 patients were excluded from the program because of poor performance status (n=4), BEAM conditioning (n=8), geographical distance and/or lack of caregiver (n=7). All patients accepted the outpatient-based procedure. Out of 107 patients, 83 (78%) did spend the aplastic phase entirely at home following high-dose chemotherapy and stem cell infusion. A second hospital admission was required in 24 patients (22%). Febrile neutropenia and severe mucositis needing total parenteral nutrition were the most frequent causes of hospitalization (45 and 38%, respectively). However, there were no documented infections and either fever or mucositis was easily resolved at the time of hematopoietic recovery in all patients. Of note, percent of second admission was of 43% in the initial 30 patients and 14% in the following y patients (p:0.002). We conclude that ASCT on an outpatient basis is feasible and safe in patients with MM and more than 75% of patients are manageable at home, provided that a caregiver is available. A progressive increase in expertise results in a significant reduction of hospitalization.

035
FACTORS INFLUENCING THE COLLECTION OF PERIPHERAL BLOOD STEM CELLS IN ACUTE MYELOID LEUKEMIA IN FIRST COMPLETE REMISSION

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Several studies have investigated factors influencing peripheral blood stem cell (PBSC) mobilization in patients with non myeloid malignancies; conversely, there are very few reports on low number of patients concerning the efficiency of PBSC mobilization in patients with acute myeloid leukemia (AML). We analyzed the effects of different potentially influential variables on successful mobilization of CD34 positive ($CD34^+$) from a series of 160 consecutive patients with AML in first complete remission (CR1). Data were collected from a cohort of 160 consecutive patients with AML in CR1. The median age was 52 years (14-78). There were 134 patients with *de novo* AML (84%) and 26 patients (26%) with secondary AML (s-AML). According to MRC criteria, cytogenetic findings at diagnosis were classified as favourable in 11%, intermediate in 69% and adverse in 20% of patients. Patients up to 60 years (n=116,72%) received anthracycline based induction and consolidation, while those aged more than 60 (n=44,72%) were treated in induction/consolidation with fludarabine/ARA-C given as continuous sequential infusion. The following variables were analyzed: age > or < 60 years, *de novo* vs. s-AML, anthracycline vs. fludarabine based treatment, cytogenetics at diagnosis, presence of FLT3 mutations (either ITD or D835 mutation), WBC at diagnosis (more or less than $50x10^9$/L), number of courses (i.e. 1 or 2) needed for achievement of CR. The above parameters were correlated to successful mobilization (defined as collection of > $2x10^6$/L). Results: Either univariate or multivariate analysis failed to demonstrate significant influence for any of parameter which were considered into the study. In particular no effect was observed as median number of $CD34^+$ cell collection, median number of apheresis, peak of SC in PB and median number of $CD34^+$ cells collected per single apheresis were concerned. In addition, none of the above parameters was significantly related to hematopoietic reconstitution after autologous stem cell transplantation in terms of WBC and platelet recovery. We conclude that successful mobilization in AML is unpredictable by using the variables analyzed in our study.

036
TARGETED AND NOVEL THERAPIES FOR NON-HODGKIN'S LYMPHOMA: THE ROLE OF RADIOIMMUNOTHERAPY AND HEMOPOIETIC STEM CELL TRANSPLANTATION

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High-dose therapy followed by autologous hematopoietic stem-cell transplantation is a main indication for relapsed or high-risk first remission aggressive non-Hodgkin Lymphoma (nHL) and plays a role for relapsed follicular lymphoma. Preliminary studies suggest that Y-90 ibritumonab tiuxetan in combination with high dose therapy and autologous stem transplantation is associated with high response rates, durable remissions and acceptable toxicity. We evaluated the combination of Y-90 ibritumonab tiuxetan at standard dose (0.4 mCi/Kg) plus high dose regimen (BEAM) in 9 patients with advanced stage nHL who failed to achieve complete remission (CR) after first line chemotherapy. *Methods*. The treatment plan is shown in figure 1. PBSCs were collected after mobilization with DHAP and G-CSF. Patients' characteristics are shown in table 1. *Results*. The median $CD34^+$ cells infused was 7.92 x 10^6/Kg (range 3.2-21.6). The median time to platelet counts higher than $20x10^9$/L were 15 days (range, 9-28 days). The median time to an absolute neutrophil count greater than 0.5 x 10^9/L were 10 days (range, 8-14). Median $CD3^+$, $CD4^+$, $CD8^+$ and $CD56^+$ cells count at day + 30 were 580 (70-2350), 110 (30-540), 350 (40-1630) and 140 (60-180), respectively. No grade IV mucositis was documented. Febrile neutropenia occurred in 88% of cases. Median time onset and the duration of fever were day +2 (range, 1-4) and 4 days (range, 1-12). We observed 1 episode of FUO, 4 pneumonitis and 5 blood stream infections, mainly by Gram+. One patient developed an atrial fibrillation. Median follow-up is 77 days (range, 36-300). The overall response rate is 78% with CR in 56% of the patients. Median time to progression was 92 days (2 cases). One patient died for documented viral encephalites at day +70. Conclusion. The use of RIT plus BEAM provides sufficient results (CR 56%) with sustained engraftment, an acceptable extra-haematological toxicity and a rapid immunological recovery. The power of this program needs to be assessed in a larger series of patients and in a multicenter setting.

Figure 1. Treatment plan

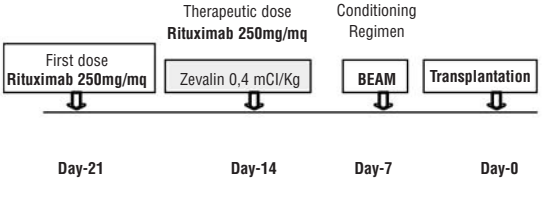

Table 1. Patient's characteristics

Median age, y (range)	53 (25-70)
Histology, no. (%)	
Follicular/Aggressive	3 (33)/6 (67)
III-IV stage at diagnosis, no. (%)	8 (88)
Median number of prior chemotherapy, no. (range)	2 (2-4)
IPI, grade	
0-I/II-III	2 (22)/7 (78)
Bone marrow involvement at diagnosis, no. (%)	5 (56)
Prior rituximab, no. (%)	9 (100)
Status at transplant	
Progression/Rp	2 (22)/7 (78)
Median time to HST, months (range)	13 (8-72)

037
INCREASING INCIDENCE OF CANDIDA GLABRATA IN PATIENTS UNDERGOING BMT FOR HEMATOLOGICAL MALIGNANCIES: CORRELATION TO WIDE SPREAD USE OF AZOLES

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The widespread use of azoles deviates is known to increase breakthrough Candida Krusei colonisation and fungal infections in immunocompromised host. The implication to the selection processes concerning other non-Albicans species is less clear. *Methods*. From March 2005 to September 2006 3415 specimens (swabs, blood cultures, biopsies) from 45 patients were analyzed either as a part of a surveillance program or as clinically indicates in our BMT unit. *Results*. In 575 positive specimen 615 pathogens could be identified. Candida species was documented in 3.75% of these: 60% were C. Albicans, 23.2% C. Glabrata and 16.8% C. Krusei. Compared to a similar cohort of patients hospitalized in Leukemia/Lymphoma wards, the incidence of non-albicans species increased from a median 13.3% to 37.9% of all Candida isolates, the incidence of C. glabrata alone from a median 3.5% to 24.1%. The continuos rising incidence of C. non-Albicans corresponds well to the use of azoles-derivated prophylaxis (fluconazole, itraconazole, and voriconazole) in BMT unit. Eleven patients showed evidence of primary azole resistance. Resistance of C. Glabrata to amphotericin and for caspofungin was not detected. 40% isolates progressed to clinically invasive fungal infection (IFI). Most of these patients showed either or all of the following characteristics: grade II-III GvHD (acute or chronic), steroid therapy, non-sensitive disease, and concomitant use of broad-spectrum antibiotics. Two patients died for IFI. *Conclusion*. The increasing incidence of C. Glabrata may be due to an increased use of azoles and selection pressure on opportunistic yeast. Clinical and therapeutically considerations may be done.

038
MAINTENANCE THERAPY WITH GEMTUZUMAB OZOGAMICIN AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Acute Myeloid Leukemia (AML) is a disease predominantly affecting older adults, with a median age of 65 years. These patients represent a poor risk population with a high chemotherapy-related mortality. Standard of care in the management of elderly patients with AML includes combination therapy with cytarabine plus fludarabine and an anthracycline and can result in complete remission (CR) rate of 40% to 60%. Even if successful, patients relapse quickly. Aggressive postremission therapy does not appear to improve survival. Future directions include therapies targeted at immunomodulation, and, among these newer treatments, Gemtuzumab ozogamicin (GO) has given promising results in relapsed, refractory and untreated $CD33^+$ AML as monotherapy or combination regimens or adjunct to conditioning regimens for Stem Cell Transplantation (SCT). *Patients and methods*. In this study we analyzed the efficacy and the safety of GO as maintenance treatment after Autologous-SCT in three elderly patients (2 males and 1 female; 64, 67 and 69 years, respectively) with $CD33^+$ AML in 1^{st} (2 patients) or in 2^{nd} (1 patient) CR. After a complete hematopoietic engraftment (at least 6 weeks from ASCT), GO was administered alone for 4 doses with 28 days between doses (two patients received 6 mg/mq for the two first doses and 3 mg/mq for the two last doses; the last received 3 mg/mq for four doses). Patients were evaluated for Continue Complete Remission (CCR) and therapy-related adverse events.
Results. All patients are in CCR at +17, +13, +11 months, respectively. Neutropenia (between 500 and 1000 /microl) and thrombocytopenia (between 50.000 and 100.000/microl) were observed in two (when GO was given at a dose of 6 mg/mq) and in three patients. No grade 3 or 4 liver toxicity was observed. *Conclusion*. GO administered in fractionated doses as maintenance treatment after ASCT in older patients with $CD33^+$ AML in first or second CR demonstrated a good efficacy and an acceptable safety profile.

039
PRE-TRANSPLANT LOW DOSE ATG AS PROPHYLAXIS FOR CHRONIC GVHD AFTER PERIPHERAL BLOOD STEM CELL MYELO ABLATIVE SIBLING TRANSPLANTS IN AML: SINGLE CENTER EXPERIENCE

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Chronic graft versus host disease (cGvHD) incidence and severity are generally found to be greater after peripheral blood stem cell transplantation (PBSCT) than after bone marrow transplantation, and some studies increased mortality from cGvHD, particularly the extensive type. *In vivo*, T-cell depletion with antithymocyte globulin (ATG) is an effective strategy for decreasing the incidence of cGvHD but may include side effects such as high risk of infection and relapse. The main utilization of ATG has been as prophylaxis of GvHD in unrelated bone marrow transplantation but few data are available on myeloablative HLA-identical sibling PBSCT. In our study we evaluated the incidence of infections, relapse and cGvHD in 28 patients with acute myeloid leukemia (AML) in first complete remission after a peripheral stem cell myeloablative HLA-identical sibling transplant. Conditioning regimen was oral busulfan (16 mg/kg), cyclophosphamide (120 mg/Kg) and ATG-thymoglobuline (4 mg/kg total dose given on days -2 and -1); the median dose of $CD34^+$ cells and $CD3^+$ cells infused were 4.6 $x10^6$/kg (range 3.1 - 9) and 250 x 10^6/kg (range 84-442), respectively. GvHD prophylaxis was cyclosporin A (2 mg/kg/Die) and short-term methotrexate (15 mg/m^2 on day +1, 10 mg/m^2 on day +3, +6, +11). The median time to neutrophil (>0.5 $x10^9$/L) and platelet (>20 $x10^9$/L) engraftment was 15 (range 13-17) and 18 (range 15-20) days, respectively. Acute GvHD grade I-II was observed in 5/28 or 18%; no patients experienced aGvHD grade III-IV. CMV antigenemia was monitored twice a week for the first 100 days; 15/28 (54%) patients developed CMV reactivation but no CMV disease occurred. As to immunological recovery, the median number of $CD3^+$ and $CD4^+$ after 1, 3 and 6 months after transplantation was 690, 850, 930 and 125, 214, 256 µL, respectively; the median number of CD8 and NK cells after 1, 3 and 6 months was 430, 538, 653 and 216, 156, 230 µL, respectively. At a median observation time of 32 months (range 8-55) the overall cGvHD was 5/28 (18%)(limited 3/5 or 10% and extensive 2/5 or 8%); the relapse rate was 7/28 or 25%. Our data suggest that low dose of ATG is effective in preventing acute and chronic GvHD without increase in relapse; prospective randomized trials are needed to evaluate the role of ATG in AML undergoing allogeneic stem cell transplantation from HLA-identical sibling.

040
SUBSETS OF $CD34^+$ AND ENGRAFTMENT KINETICS IN ALLOGENEIC PERIPHERAL STEM CELL TRANSPLANTATION

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Engraftment kinetics in allogeneic peripheral blood stem cell transplantation (alloPBSCT) depend on the number and efficiency of the stem cells in the graft, the conditioning regimen and GvHD prophylaxis.This study aimed to identify which graft product subset of $CD34^+$ cells might be the most predictive of early hematopoietic recovery following alloPBSCT .The relationships between the number of *mature* subsets of $CD34^+$ cells ($CD34^+/CD33^+$, $CD34^+/CD38^+$, $CD34^+/DR^+$ and $CD34^+/CD133$) and *immature* subsets of $CD34^+$ cells ($CD34^+/CD33^-$, $CD34^+/CD38^-$, $CD34^+/DR^-$ and $CD34^+/CD133^+$) and early neutrophil and platelet engraftment were studied in a homogeneous series (for disease, pre-transplant chemotherapy, conditioning regimen GvHD prophylaxis) of 30 acute myeloid leukemia (AML) patients after alloPBSCT from HLA-identical siblings. All patients received the BU-CY regimen consisting of busulfan 4 mg/kg/day for 4 consecutive days followed by cyclophosphamide 60 mg/kg/day for 2 consecutive days; GvHD prophylaxis included cyclosporin and methotrexate. The $CD34^+$ dose infused ranged from 2.9 to 8.8 x 10^6/Kg (median 4.6); the percentage of immature $CD34^+$ cells was 36% for $CD34^+/CD33^-$, 60% for $CD34^+/CD38^-$, 5% for $CD34^+/DR^-$ and 70% for $CD34^+/CD133^+$. In our experience the total $CD34^+/CD133^+$ cell number was inversely correlated with the days required for recovery of 0.5 x 10^9/L neutrophils ($r = -0.82$, $p=0.02$) and 20 x 10^9/L platelets ($r = -0.60$, $p=0.06$); this correlation was better than the total $CD34^+$ cells dose and neutrophil ($r = -0.70$, $p=0.05$) and platelets engraftment ($r = -0.56$, $p=0.07$). No correlation was found between the other $CD34^+$ subsets and neutrophil and platelets engraftment. We suggest that a high number of $CD34^+/CD133^+$ peripheral blood stem cells may be associated with faster neutrophils and platelets recovery; these findings may help to predict the repopulating capacity of PBSC in patients after allogeneic PBSCT, especially when a relatively low number of $CD34^+$ cells is infused.

041
REDUCED CONDITIONING INTENSITY AND BONE MARROW TRANSPLANTATION IN HIGH RISK MALIGNANCY; A SINGLE CENTER EXPERIENCE

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Background. Many hematological tumors or other malignancies primary resistant or relapsing to two or more lines of therapy lose their chances of cure with conventional therapy. TMO with conventional conditioning regimen could be proposed as alternative salvage therapy in some patients, however the high toxicity due to the pre-

vious therapies and the old age of patients warrants to test the possibility of cure with RIC regimen.
Patients and methods. Twenty patients, 3 AREB-t, 3 MM, 3 HD, 5 NHL, 3 AML, 2 CML in BC and 1 Metastatic Breast Cancer (MBC) represent the basis of the present study. The mean age of patients is 55 years (extremes 36-70 years) and all patients had disease in progression at the time of the procedure. Lymphomas, MM, AML and MBC underwent a minimum of two lines of therapy before RIC-BMT, 3 mean (extremes 2 to 5), AREB-t and CML in BC underwent the procedure as front line therapy (2 pts) or following a failure of induction therapy (4 pts). The conditioning regimen to RIC-TMO was fludarabine 50 mg x 3, 4 days ATG, CTX 300 mg/sm or thiotepa 10 mg/Kg and melphalan 100 mg/sm. *Results*. Engraftment was recorded in all patients. Fifteen patients responded to RIC-TMO, 10 CR and 5 more than 80% reduction of disease. Complete responding patients were 2 MM, 3 AML, 2 HL, 1 NHL and 2 AREB-t. Partial responding patients were 1 HD, 2 NHL, 1 MBC and 1 MM. In lymphomas and myelomas the response was achieved in a period ranging from 1 to 6 months. The two CML in BC, 1 NHL and 1 AREB-t did not responded and early progressed. aGVHD was recorded in 11 patients. One patient with AREB-t died for grade IV aGVHD 2 months from RIC-TMO. The duration of response was 3 to 20 months with 4 patients still in CR. Six patients died for progression of disease, 3 patients died for late aGVHD complications due to CMV activation and one for Gram- sepsis 3 to 6 months following RIC-TMO. *Comment and conclusions*. RIC-TMO is a feasible procedure in high risk patients for age and disease with haematological tumors. Engraftment is the rule. The incidence of aGVHD in almost 50% of patients give a risk of peritransplant mortality of 25% of patients including early and late complications. The efficacy of the procedure seems to be mostly correlated to Graft Versus Tumor expecially in those patients with late resposnse however long lasting observation is still under evaluation. The recognition of CR in lymphomas and myelomas already resistant to many lines of therapy warrants consideration and further studies

042
CORRELAZIONE TRA DUE DIVERSI METODI PER LA ENUMERAZIONE DELLE CELLULE CD34⁺ E LE CFU-GM

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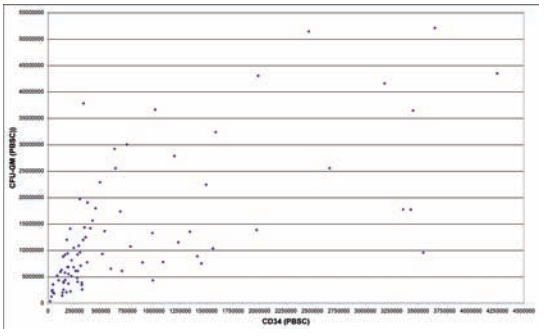
Rationale. La quantificazione delle cellule staminali CD34⁺ costituisce un parametro fondamentale per la validazione del *graft* destinato ad uso trapiantologico sia autologo che allogenico. Numerosi studi indicano che, per la quantificazione delle cellule CD34⁺, è preferibile l'utilizzo della metodologia a singola piattaforma (es: Prot. ISHAGE) anziche' doppia (es: Prot. Milano). *Materiali e metodi*. La quantificazione delle cellule CD34⁺ di 100 prodotti aferetici (PBSC) è stata studiata mediante protocollo ISHAGE (Stem-Kit CD34⁺, Instrumentation Laboratory) e protocollo Milano (CD34PE clone 8G12, CD45 FITC clone 2D1- B.D.). Gli stessi campioni sono stati valutati per il loro potenziale clonogenico (CFU-GM) mediante colture semisolide a breve termine (Methocoult H4534, Stem Cell). *Risultati*. Abbiamo rilevato la correlazione tra il numero di cellule CD34⁺ e il potenziale clonogenico CFU-GM delle PBSC (r= 0,765; $p< 0,01$) (Fig. a).



Confrontando i due diversi protocolli di quantificazione delle cellule staminali, espresse in valore assoluto e percentuale (Prot. ISHAGE e Prot. Milano, rispettivamente), la correlazione tra il numero di cellule CD34⁺ e le colonie CFU-GM (r=0.9; $p<0.01$) veniva confermata. *Conclusioni*. Il nostro studio, evidenzia che la quantificazione citofluorimetrica delle cellule CD34⁺(Prot. ISHAGE, Prot. Milano) correla con le CFU-GM, utilizzate quali indice predittivo del potenziale biologico di attecchimento dell'inoculo.

043
PROLUNGATA SECREZIONE DI STROMAL-DERIVED FACTOR-1 ALPHA ED ESPRESSIONE DI CD62-E/ICAM-1 IN PAZIENTI CON IMA SOTTOSPOSTI A BYPASS CORONARICO

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Razionale. Nell'area necrotica il reclutamento di cellule progenitrici di origine emopoietica e la loro differenziazione in cellule specializzate favorisce sia la neoangiogenesi di vasi collaterali sia la rigenerazione tissutale. L'ischemia miocardica promuove la mobilizzazione delle cellule staminali emopoietiche attraverso la liberazione di mediatori umorali incluso SDF-1alpha. La trans-migrazione delle cellule immuni, in risposta al suddetto fattore di homing, si basa sulla cooperazione tra le molecole di

adesione leucocitarie CD62-E/ICAM 1 e le cellule endoteliali. *Pazienti e Metodi*. In un gruppo di 35 pazienti affetti da infarto acuto del miocardio (IMA), sottoposti ad inserimento di bypass coronarico, è stata valutata la produzione sistemica plasmatica di SDF-1alpha prima (T0) e 24 ore dopo l'intervento chirurgico, e nei successivi +7, +30, +90, +180 e 365 giorni. Con la stessa cinetica di determinazione, mediante analisi citofluorimetrica, sono stati quantificati gli antigeni leucocitari esocellulari: E-Selectina (CD62E)/ICAM 1 (CD11b o integrina). *Risultati*. In tutti i 35 ptz, SDF-1alpha aumentava significativamente, rispetto T0, a +24h fino +180gg, (p<0.001). La concentrazione di SDF-1alpha permaneva significativamente aumentata, rispetto al basale, nei 21 ptz che avevano raggiunto un followup di +365 gg, (p<0.02). Il rientro ai valori basali era osservato entro i due anni, nel gruppo dei primi 7 ptz. La determinazione di CD62E/CD11b dimostrava un rapido e significativo incremento di espressione a +24h (p<0.01). Tale aumento si presentava con una cinetica temporale perfettamente sovrapponibile e correlabile alla curva di secrezione di SDF-1alpha (p<0.0001). *Conclusioni*. La correlazione tra l'aumento stabile nel tempo di SDF-1alpha e l'espressione di CD62E/CD11b, in ptz chirurgici con IMA, evidenzia il significativo ruolo della citochina nel regolare l'homing e la mobilizzazione di cellule progenitrici di origine emopoietica coivolte nel processo rigenerativo.

044
DIAGNOSI DI HCMV: DETERMINAZIONE DEI LIVELLI DI CUTOFF DI INFEZIONE, MEDIANTE PCR REAL-TIME, NEI PAZIENTI GRAVEMENTE IMMUNOCOMPROMESSI

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Razionale. La proteina virale pp65 rappresenta un marker antigenico indiretto di replicazione di HCMV. Tuttavia, è stato dimostrato, che il livello di antigememia aumenta anche durante la terapia antivirale in modo dissociato dai reali valori di viremia. Il DNA virale cell-free (DNAemia) e il DNA virale presente nei leucociti (viral-load), invece, sembrano meglio correlare l'infezione e con le manifestazioni cliniche di malattia citomegalica. *Obiettivo*. Determinare un ottimale valore di cutoff, predittivo positivo/negativo di HCMV infezione, mediante PCR quantitativa (Q-PCR). *Pazienti e Metodi*. 95 campioni seriali di plasma e 95 di sangue periferico di 18 pazienti allotrapiantati e di 13 pazienti onco-ematologici sono stati parallelamente sottoposti ai test di ricerca di HCMV: antigenemia e Q-PCR Il risultato è stato espresso in gEq/mL per i campioni acellulari e gEq/100.000 per i campioni cellulari. *Risultati*. L'infezione da HCMV è stata documentata in 11/33 pazienti (30%), 9 dei allo-trapiantati. Alla prima determinazione 6/11 pz risultavano positivi ad entrambi i test (60%), 3 solo alla pp65, 2 solo all'analisi molecolare. Nel gruppo dei pz con positività molecolare, la DNAemia (range 7200-41.140 gEq/mL) coincideva con la determinazione di viral load (range 1800-13.380 gEq/100.000). Nei 3 ptz pp65+, la positività era limitata ad 1 sola cellula e mai nel follow-up sono state determinate copie di HCMV-DNA. I 2 pz con copie di HCMV-DNA determinabili erano neutropenici, la pp65 veniva successivamente apprezzata dopo 10 e 4 giorni, in coincidenza con l'innalzamento dei dei globuli bianchi. Del gruppo dei 21 ptz negativi al test molecolare, nessuno presentava successiva positività alla pp65. Tutti i casi HCMV+ sono stati sottoposti alla preemptive therapy e si è osservato che, con entrambi i tests , la clearance virale si otteneva mediamente al 12°giorno dall'inizio della terapia. *Conclusioni*. E' verosimile sostenere che i cutoff di HCMV-DNA predittivi di positività d'infezione si collocano intorno a 3.500 copie/100.000 leucociti e 10.000 copie/ml di plasma nei pazienti gravemente immunocompromessi. La condizione di copie virali non determinabili assume significato di predittività negativa d'infezione con specificità pari al 100%.

045
HODGKIN'S LYMPHOMA: THE PESCARA EXPERIENCE DURING THE LAST 7 YEARS

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Beetween 1/1/2000 to 30/9/2006 148 patients were diagnosed with Hodgkin's lymphoma (HL) in the Hematology Unit of Pescara Hospital. The median age was 38 years (range 15-82), male 65, female 83 (M/F ratio: 0,78). The distribution by region provenience was: 86% from Abruzzo, 14% from other regions. The histological subtypes were: NS 76%; MC 10,7%; LP 4%; LD 1,3%; Classical HL unspecified 8%.The clinical staging showed: IA (9), IIA (45), IIB (40), IIIA (15), IIIB (22), IVA (5), IVB (12). Extranodal disease was present in 5% of the stages I-II, Bulky in 10% of the patients.Treatment plan was adapted to patients risk group. There were 54 patients in early stage group (I-IIA) and 94 in advanced stage group (IIB-IV). In early stages treatment consisted of 4-6 courses of chemotherapy (ABVD, VBM) followed by involved field radiotherapy (36Gy), and of 6 courses of chemotherapy (ABVD, COPEBVCAD, BEACOPP, STANFORD V, VEPEMB) in advanced stages. For the 136 evaluable patients, 114 (84%) achieved CR, 1 Cru, 5 PR (4%), 16 (12%) were refractory. Overall, the 7 years OS and EFS rates (median follow-up time: 38 months) were 85% and 79% (early stages: OS 94%, EFS 88%; advanced stages: OS 80%, EFS 74%). As regards the adverse events, one patient died of heart attack, one patient presented carcinoma of the lung and two patients developed a diffuse large B cells lymphoma, respectively 2, 5,16, 28 months after the diagnosis of HL.

046
EARLY RESPONSE TO THE FIRST PART OF CHEMOTHERAPY OVERCOMES THE PROGNOSTIC ROLE OF IPI IN PATIENTS WITH AGGRESSIVE NHL. PRELIMINARY RESULTS OF THE GISL LA05 TRIAL

Pennese E., Mazza P., Brugiatelli M., Stelitano C., Angrilli F., Musso M., Quarta G., Di Renzo N. for GISL

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The achievement of CR after a short course of initial therapy has been considered for predicting survival in patients with aggressive NHL. In April 2000, the GISL started the LA05 trial with the aim of assessing different treatment modalities, defined according to response to 4 initial courses of chemotherapy (CT). Patients younger than 65 years with histologically confirmed diagnosis of DLBCL, MCL, grade III FL and PTCL regardless of stage and IPI score were eligible to the study. After 4 courses of ProMECE-CytaBOM (P-C) patients achieving at least a PR >75% were treated with two additional courses of CT (group 1); those achieving PR <75% were randomized to receive either additional 4 courses of P-C or a modified HDS program followed by ASC support (group 2); patients with SD or PD were off study (group 3). In July 2005, the protocol was amended allowing the use of other CT regimens, including CHOP and MACOP-B, and recommending the addition of Rituximab for B-cell NHL. We present the results of a preliminary analysis of the efficacy of the general strategy of the LA05 trial, in particular focused on the outcome of group 1 patients. As of June 2006, 341 patients were enrolled. Eighty percent of cases had DLBCL, 12% PTCL, 5% GIII FL and 3% MCL. Median age at diagnosis was 49 years (range 18–67). Advanced stage was present in 60% patients, and 38% had a bulky mass. IPI was low (0-1) in 52%, intermediate (2) in 22% and high (3+) in 26% of cases. After 4 courses of CT, 265 patients were evaluable for response; Sixty-four percent of patients achieved at least a PR >75%, 19% achieved a PR <75% and 17% had SD or PD. The probability of achieving at least a PR >75% was 74%, 62% and 42%, for patients with an initial IPI of 0-1, 2 or 3+. At the end of treatment program, a CR was achieved in 57% of cases and a PR in 14%. After a median follow-up of 21 months (range 1-73), the 2-year OS was 69% (IC 95% 62-75), being 85%, 65% and 25% for group 1, 2 and 3 respectively (p<0.001). Two-year OS was 88%, 78% and 78% for patient in group 1 with and IPI of 0-1, 2 and 3+ respectively (p=NS). The achievement of a CR, CRu or PR >75% after the first part of initial CT overcomes the prognostic role of IPI in patients with aggressive NHL.

047
PRIMARY LYMPHOMA OF THE ADRENAL GLANDS: A CASE REPORT AND REVIEW OF THE LITERATURE

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Primary non¬Hodgkin lymphomas of adrenals are rare entities. Only 86 cases have been reported to date in the literature. They usually manifest as huge bilateral adrenal enlargement and may be accompanied by fever and adrenal insufficiency. Usually the adrenal masses are the unique manifestation; if not, extranodal involvement is frequently observed. Given the very few cases reported to date, a standard therapy is not available. We report a 53 year-old male patient with a unique adrenal involvement of non–Hodgkin lymphoma. He presented with weight loss, fever, hyporexia, asthenia, stipsis, dry cough, pain and mass in the left hypocondrium. Laboratory analyses revealed normocitic anemia, leucopenia with lymphocytosis, hypoalbuminemia, increased levels of ferritin, LDH and ACTH; levels of cortisol were normal. Abdominal ultrasonography, CT scan and MRI revealed a well defined 12 cm left adrenal mass. Neck, thorax and pelvis CT scan and ultrasonography of neck, axilla and groin did not show any lymph node enlargement. Whole-body FDG positron emission tomography–computed tomography (FDG PET/CT) showed bilateral adrenal involvement. Ultrasound guided biopsy of the left adrenal gland revealed diffuse, large B-cell lymphoma. Bone marrow trephine biopsy did not show any involvement of the disease. The patient has received four courses of Rituximab plus mega-CHOP-14 chemotherapy. The control abdominal ultrasonography shows a 50% reduction of the mass. Bilateral primary non–Hodgkin lymphomas of adrenal glands are rarely observed. Onset is not always characterized by adrenal insufficiency. Among the imaging modalities used to describe the lesions, FDG PET is indispensabile. Biopsy of the lesion is fundamental for the diagnosis and in this particular site can be ultrasound guided. Rituximab plus intensified CHOP followed by autologous PBSC transplantation could have an important role in the treatment of this disease.

048
ALEMTUZUMAB AND CHOP MODIFIED WITH LYPOSOMAL ADRIAMYCIN, FOLLOWED BY GEMCYTABINE, OXALYPLATIN AND ALEMTUZUMAB IN T-CELL NHL: A NEW PROPOSAL FOR AN UNRESOLVED PROBLEM

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To find a safe, feasible and effective regimen, alternative to CHOP, in T-NHL therapy in patients with comorbidity and advanced stage of disease, is an unresolved problem. This study is a monocentric retrospective study. Eight patients were treated with MC CCOP (Mab Campath® 10

mg sc day 1, Caelyx® 30 mg/sqm day 1, Endoxan 750 mg/sqm day 1, Vincristine 1 mg/sqm day 1, Prednisone 100 mg/day, days 1-5, recycle every 21 days). Five patients received MC-GEMOX (Mab Campath® 10 mg sc day 1,15; Gemcytabine 1000 mg/sqm day1,15; and oxalyplatin 100 mg/sqm day 1,15; for at least 4 cycles), because they were relapsed or partial responder after 6 MC-CCOP. Three patients didn't receive MC-GEMOX because they died during first line treatment for disease progression or complications. Survival at 16 months was calculated using Kaplan-Mayer method. Patients were analyzed by intention to treat. We analyzed 8 patients with T-NHL (7male/1female). Median age was 72 years (R49-76). Histological subtypes were: 4 peripheral T-NHL NOS and 4 Sézary Syndrome. Seven patients on 8 showed comorbidity (myocardiopathy, NIDDM, severe hypertension, COPD). All patients presented III-IV stage disease and 7 of them had B symptoms at onset. MC-CCOP gave 87% of Overall Response Rate, coinciding with parzial remissions. MC-GEMOX gave 100% of Overall Response Rate since first cicle, with a Complete Response Rate of 60% after 3 cycles. In our 8 patients, therapy-related cardiotoxicity was seen in 1 patient (12%), G3 neutropenia in 5 (62%), G3 mucositis in 1(12%) and infectious complications in 4 (50%). Our patients had a median follow up of 8 months (R2-16).
The overall survival at 16 months follow-up was 56% (95%CI 0.17-0.95). In T-NHL patients with advanced stage of disease and comorbidity, MC-CCOP+MC-GEMOX seems to be safe, feasible and effective. Nevertheless these data need confirmation on a larger cohort of patients.

049
RITUXIMAB FOLLOWING CHOP MODIFIED WITH LYPOSOMAL ADRIAMYCIN IN B-CELL NON HODGKIN LYMPHOMA THERAPY: AN ATTEMPT TO REDUCE TOXICITY IN ELDERLY PATIENTS WITH COMORBIDITY

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Combination of Rituximab and CHOP (R-CHOP) is safe and effective in young and elderly NHL. We included lyposomial Adriamycin (Caelyx®) instead of conventional Adriamycin in CHOP regimen in order to reduce myocardic toxicity and enhance doxorubicin uptake in tumour mass in B-NHL of elderly patients with comorbidity, advanced stage and aggressive disease. This study is a monocentric retrospective study. Patients were analyzed by treatment with R-CCOP (Rituximab 375mg/sqm day0, Caelyx® 30 mg/sqm day 1, Endoxan® 750 mg/sqm day 1, Vincristine 1 mg/sqm day 1, Prednisone 100 mg/day, days 1-5, recycle every 21 days). Overall (OS) and progression-free survival (PFS) at 28 months were calculated using Kaplan-Mayer method. We analyzed 20 patients with B-NHL: 13DLBCL, 1 mantle cell lymphoma, 2 SLL and 4 follicular lymphoma. M/F:10/10, median age 76 years (R57-87). 16 patients (80%) showed comorbidity (hepatitis C, NIDDM, severe hypertension, etc).
Ten patients presented stage IV disease(50%). 8 had B symptoms and 5 extranodal disease. 5 patients were treated in I relapse. Median follow-up was 11.5 months (R2-28). Median number of administered cycles was 6 (R2-8). After R-CCOP treatment 4 patients were nonresponders, 15 in complete remission (75%) and 1 in good partial remission. No severe treatment-related toxicities were observed, except G2 WHO neutropenia in 8 patient (40%). Nonresponding patients had extranodal, stage IV disease with B symptoms. Nevertheless, they mantained chemosensitivity to second-line therapy. OS and PFS at 28 monts were respectively 81.5% (95%CI 58-100%) and 62.5% (95%CI 35.5-89.2%). In elderly B-NHL patients with advanced stage of disease and comorbidity, R-CCOP seems to be safe and feasible and effective. The use of R-CCOP seems not impair response to second-line treatment in nonresponding patients. Nevertheless these data need confirmation on a larger cohort of patients. The low PFS probably is due to particularly unfavourable clinical characteristics of treated patients.

050
EFFECTIVE MOBILIZATION BY PEG-FILGRASTIM PLUS ARA-C CONTAINING REGIMEN IN PRETREATED LYMPHOMAS PATIENTS

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Backgroud. Studies performed on mice and healthy human volunteers have shown that a single dose of peg-filgrastim (Peg-GCSF) is effective in stimulating peripheral blood stem cells (PBSC) mobilization. *Aims*. The aim of this study was to evaluate the efficacy of pegfilgrastim, in combination with salvage chemotherapy, in mobilizing $CD34^+$ stem cells into the peripheral blood of pretreated lymphoma patients. *Methods*. We studied 27 pretreated patients (Hodgkin's lymphomas=5; non-Hodgkin's lymphomas=22). The median age was 37 years (range 17-60). The patients received a median of 2 previous chemotherapy regimens. Median time from mobilization to harvest was 11.5 days. The efficacy of the mobilizing procedure was tested in lymphoma patients receiving salvage regimen [DHAP (cisplatin 100 mg/m^2 , cytarabine 2000 mg /m 2 x ^{2}) in 17or MAD (cytarabine 2000 mg /m^2 x 2 x3 day in 10)] plus pegfilgrastim in 11 or filgrastim in 16. Pegfilgrastim was given as single subcutaneous injection (6 mg) on day +5 post chemotherapy. Filgrastim was given daily (10 μg/kg) from day +5. Daily monitoring of circulatory $CD34^+$ cells was started from day 8 after the end of chemotherapy. *Results*. Twenty one/27 patients reached the target cell dose of $2{,}5x10^6$ cells/kg. A median of 2 apheresis (range 1-3) was performed. In pegfilgrastim group, a median of 5.27 $x10^6$ $CD34^{(+)}$ cells/kg (range 1.06-10) was collected. In filgrastim group, a median of 11 $x10^6$ $CD34^{(+)}$ cells/kg (range 0.09-32.84) was collected. No statistical difference (p=0,06) between the two group (pegfilgrastim vs. filgrastim) was found. *Conclusions*.

Our results show that pegfilgrastim as an adjunct to HiDARAC based chemotherapy is an effective mobilization regimen in pretreated lymphoma patients also effective as filgrastim based regimen. This approach is to be confirmed in larger series of patients and probably with increased dose of pegfilgrastim and could open new opportunities in stem cell mobilization for poor or non-mobilizers patients with malignant lymphomas.

051
USE OF LIPOSOMAL DOXORUBICIN IN PATIENTS AFFECTED BY DIFFUSE B-LARGE CELL LYMPHOMA: EXPERIENCE OF TWO HAEMATOLOGY UNITS

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We report the experience of two Haematology Units of the ASL NA1- Naples about the use of liposomal doxorubicin (Myocet) in a cohort of patients affected by Diffuse B-Large Cell Lymphoma. The immunochemotherapy, according to R-CHOP regimen, is today the golden standard treatment for aggressive NHL. However, advanced age and/or presence of comorbidity may represent a limit for the use of R-CHOP regimen due to the haematological and non-haematological toxicity (i.e. cardiotoxicity). We have investigated, in a cohort of *frail* patients (for age and/or comorbidity), the feasibility and effectiveness of a combination therapy (R-COMP) using liposomal doxorubicin (Myocet) instead of doxorubicin in order to reduce toxicity. In the last three years we have treated 30 patients (11 F and 19 M; median age: 68 years, r.: 60-82 years; stage III-IV) with 6 cycles of R-COMP regimen. At diagnosis 20 out of 30 patients showed a cardiopathy (ventricular ejection fraction (VEF) lower than 50%) and 10 out of 30 patients had been previously treated with chemotherapy regimens containing anthracycline for solid tumors. In all patients we have monitored cardiac function before, during and after the treatment. At a clinical re-staging performed after one, three and six months from the end of therapy, 21 patients showed a complete remission and 9 patients showed a partial remission. No VEF reduction was observed in all patients. The overall survival at twenty-four months is 85%. We conclude that R-COMP is highly active therapy in *frail* aggressive-NHL patients. Moreover our results show acceptable toxicity profile of this therapeutic approach.

052
HIGH MOLECULAR REMISSION RATE WITH SEQUENTIAL THERAPY, PURGING *IN VIVO* AND HIGH DOSE THERAPY IN FOLLICULAR LYMPHOMA

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We investigated a novel therapy protocol for management of high risk CD20 Follicular Centre Cell Lymphoma (FCCL) to be implemented as a salvage regime after relapse or in case of resistance or as an up-front regime. The treatment included Rituximab, high dose therapy and PBSTC and consisted of 4 CHOP courses, 2 mobilizations of $CD34^+$ cells with high-dose Cytoxan and Cytarabin + G-CSF, Rituximab as purging agent *in vivo* and PBSCT following BEAM regimen. Clinical, immunephenotypic and molecular parameters were used for evaluation of response. Up to July 2005 42 patients – mean age 56.6 years – completed this management protocol and were analysed in this study. All patients had marrow involvement and were considered at high risk either because of grade 3 histology (14 pts) or IPI score > 2 (17 pts) or progression after previous treatment (11 pts) or B symptoms (8 pts.). After CHOP therapy 17 patients obtained Complete Clinical Response (CCR), all but one had residual disease detected by immunephenotype in the marrow, none obtained a molecular remission. Following high-dose Cytoxan as mobilizing treatment and Rituximab as purging agent, 25 pts (60%) developed CCR, 21 Complete Immunephenotypic Remission (CIR) and 9 (21%) Complete Molecular Remission (CMR). Following high-dose Cytarabine and second course Rituximab, 34 pts (81%) reached a CCR, 29 had a CIR and 19 (47%) a CMR in the marrow sample. At the end of the entire management and following PBSCT 39 pts (94%) reached CCR; one patient died of sepsis during the cytopenic period, 36 pts (88%) resulted in CIR and 30 pts in CMR. At 39 months - mean - follow-up 36 pts (86%) remained in CCR and 29 (69%) still in CMR. Among the 3 patients relapsed, 2 had a residual molecular disease at the end of the treatment. One patient died of brain hemorrhage 5 months after the end of the treatment. In conclusion this study demonstrated that sequential therapy including purging *in vivo* with Rituximab and high-dose therapy is highly effective in inducing durable CR in most of FCCLs.

053
MONITORING OF MINIMAL RESIDUAL DISEASE IN NON HODGKIN'S LYMPHOMA BY CYTOFLUORIMETRY: EXPERIENCE OF AN ISTITUTION

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Flow-cytometry (FC) immunophenotyping is a sensitive approach in hematological malignancies routine diagnostic. To estabilish the efficacy of maintenance treatment with Rituximab (R) in monotherapy on Minimal Residual Disease(MDR) analyzed by FC, we prospectively evaluated 20 patients with B-Non Hodgkin Lymphoma (NHL) in Complete Remission (CR) during R treatment (375 mg/m2 each week for four weeks every six mounths for four times up to two years). Of 20 NHL patients with IV stage for bone marrow involvement, 5 presented a diffuse large B cells lymphoma, 2 splenic marginal lymphoma, 4 follicular lymphoma, 6 lymphocytic lymphoma , 2 lymphoplasmocytic lymphoma and 1 extranodal malt lymphoma. For all patients included in our study (that reached CR after chemioterapy R-CHOP or CHOP like) were assessed bone marrow(BM) and peripheral blood(PB) FC at baseline and only PB FC analysis before each R cycle administration (time 0 , month 6, month 12, month 18, month 24). The antibody panel used in this study were CD19, CD20, CD22, CD23, CD25, k/19, lambda/19, CD19/20, CD5/19.No toxicity were observed during two years of R treatment. The majority of patients 18/20 (90%) showed prolunged negativity of peripheral B antigens in FC since sixty month until end of maintenance and this confirmed a prolunged complete remission demonstrated also with PET-TAC and BM cytology/histology negativity($p< 0.001$). In two patients (10%) the persistence of B peripheral antigens could be predictive of high risk of relapse($p<0.002$), in fact one patient with follicular lymphoma relapsed after 12 months of R-maintenance was treated with H-D chemoterapy followed by allogenic bone marrow trasplantation and the other one with lymphocytic lymphoma completed the maintenance and he showed a extranodal localization of lymphocytic lymphoma. Sistematic evaluations of MRD by FC needs to confirm our previous data for early evaluation of target therapy efficacy and to predicting relapse in LNH patients.

054
USE OF INTRATHECAL LIPOSOMAL CYTARABINE (DEPOCYTE) AS TREATMENT OF LYMPHOMATOUS MENINGITIS

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The presence of lymphomatous cells in the cerebrospinal fluid (CSF) or in a cranial nerve localization along with neurological deterioration, with or without cerebral radiological evidence, is an uncommon event, either as an initial diagnosis and in the course (or as an outcome) of a front line treatment. In the latter cases the Lymphomatous Meningitis (LM) represents an event with a generally poor prognosis and a median survival of few months in untreated patients. Its occurrence is tightly related to the histological type, the response to the therapy and the introduction of a proper prophylaxis in the treatment plan. Currently, the systemic therapy with high doses of Methotrexate (HD-MTX) (3 g/sqm) followed by radiotherapy (WBRT: 50 Gy) represents the elective treatment. The role of the intratecal chemotherapy (IT-CHT) isn't well defined: as a matter of facts, it doesn't seem more effective than the systemic treatment with HD-MTX and it is burdened by a higher neurotoxicity. Furthermore, more frequent intratecal administrations, a proper concentration and a prolonged exposition to the drugs are required. Recently, an important role both in the prophylaxis and in the therapy of LM is played by the intratecal administration of Cytarabine in liposomal formulation (DepoCyte): the slow release from the multivescicular lipidic particles improves the distribution in the liquor, allows a lesser number of intratecal administrations, reduces the undesired effects due to the treatment and increases the compliance of patients. Randomized studies have shown a significant better effectiveness and a reduced toxicity of liposomal formulation treatment with respect to the traditional one. In the present work 9 cases of LM treated with systemic chemotherapy and with DepoCyte (50 mg IT every 15 days x 6 times) are shown. Male/Female ratio is 6/3; average age is 46 years (range 24-78). In all patients neurological symptoms were present; lymphomatous cells were identified in the liquor of 5 patients and Central Nervous System (CNS) localizations were detected by NMR in 7 patients. The 5 CSF-positive cases were heterogeneous, as follows: 1) B-lineage $CD10^+$ ALL meningeal relapse (after two marrow relapses), already undertaken to allogeneic staminal cells transplantation, treated only with DepoCyte; 2) Lymphoblastic T Lymphoma meningeal localization, with mediastinic mass, treated with DepoCyte associated to systemic chemotherapy (LSA2L2 Protocol) and autologous stem cell tranplantation; 3) DLBCL meningeal localization treated with DepoCyte associated to systemic chemotherapy (R-CHOP Protocol); 4) LM at diagnosis in a 78 years old patient with inadequate compliance to systemic therapy, treated with DepoCyte; 5) Mantle Cell Lymphoma meningeal (and systemic) relapse treated with DepoCyte associated to systemic chemotherapy (Codox-M Protocol); In patients 1) to 4) the C.R. was obtained and they are still alive; only the latter patient, namely, case 5), died for disease progression. The remaining 4 CSF-negative cases had been diagnosed as DLBCL (3 at diagnosis and 1 at relapse) CNS solitary localizations. In all cases the systemic therapy with L-VAMP Protocol (modified with HD-MTX) was associated to IT-CHT with DepoCyte. With the only exception of the relapsed patient, the remaining 3 patients had a good response to treatment and they are still alive and in C.R.. Overall, DepoCyte treatment was shown to be mostly effective, well tolerated by all patients and devoid of undesired side effects. The association with various systemic chemothe-

rapy protocols had been demonstrated to be suitable and endowed with synergic effects. Studies with higher number of patients might validate the effectiveness of DepoCyte also in those CSF-negative cases with solitary CNS localization.

055
EFFECTS OF RITUXIMAB ON PERIPHERAL BLOOD STEM CELL MOBILIZATION AND ENGRAFTMENT IN B CELL NON HODGKIN´S LYMPHOMA

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Treatment with rituximab is widely used for B cell non Hodgkin´s lymphomas (B-NHL). However, its effects on peripheral blood stem cell mobilization are not completely known. We retrospectively evaluated 52 consecutive B-NHL (30 follicular and 22 diffuse large cells) patients responding to first-line chemotherapy (CHOP or R-CHOP), but failing to achieve complete remission (CR). In this group we compared the mobilization characteristics and engraftment kinetics of 24 patients receiving and 28 not receiving rituximab six months before PBSC mobilization. Patients mean age was 37 years (range 17-60). 44 patients (84.6%) had stage III-IV disease. 34 patients (65.4%) had bone marrow involvement; systemic B symptoms were present in 30 patients (57.7%). At the time of PBSC mobilization 32 patients (61.5%) were considered to be responsive (complete remission, partial remission or sensitive relapse) and 20 (38.5%) not responsive (refractory relapse or refractory to therapy). Mobilization chemotherapy consisted of a high dose cytarabine containing regimen (DHAP) in all patients. The median $CD34^+$ cells collected was $5.8x10^6$/kg in patients receiving rituximab vs $7.2x10^6$/kg $CD34^+$ cells (p=n.s.) in the non rituximab treated group. Failure to mobilize, defined as failure to reach a circulating $CD34^+$ cell count of 10/mcl, occurred in 2 patients (8.3%) in the rituximab group and 3 (10.7%) in the non rituximab group. All patients were transplanted using myeloablative chemotherapy conditioning regimen (BEAM); Comparison of the two groups showed no statistical significant difference between median days to absolute neutrophil $>0.5x10^9$/L and platelet $>20x10^9$/L counts after autologous stem cell transplantation, and no differences in incidence and severity of infections, days of fever or duration of antibiotic treatment between groups. In conclusion, the use of rituximab six months before PBSC does not affect the ability to collect an adequate number of PBSC for autologous stem cell transplantation in B-NHL. Further studies are warranted in larger populations to determine the impact of rituximab on collection, engraftment and survival.

056
TRATTAMENTO CON BORTEZOMIB IN PAZIENTI CON MIELOMA MULTIPLO REFRATTARIO/RICADUTO

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Il Bortezomib (B), il primo tra i farmaci appartenenti alla classe degli inibitori del proteosoma, è un promettente farmaco approvato per il trattamento del mieloma multiplo. Studi di fase II/ III hanno mostrato una percentuale di risposta del 35% in mieloma refrattario o in recidiva.
La presente comunicazione riguarda un gruppo di 12 pazienti, di cui 5 refrattari e 7 in recidiva, trattati presso il nostro centro dal febbraio 2006 con B ± Desametasone (DMS). I pazienti erano 8 maschi e 4 femmine, tutti pretrattati (mediana 2 precedenti linee, range 1-3);.'età mediana 76,5 anni (51-85). L'esordio della malattia risaliva mediamente a 24 mesi prima (range 8-124) In un caso la malattia era diagnosticata in fase di leucemia plasmacellulare. La dose di B utilizzata nella maggior parte dei casi è stata di 1,3 mg/mq, ridotta nel corso della terapia o fin dall'inizio in alcuni casi per tossicità o per la coesistenza di mielodepressione e/o di insufficienza renale. Il programma prevedeva 6-8 cicli di 21 giorni, con infusione di B al giorno 1, 4, 8 e 11 e di DMS 16 mg nei giorni 1-2, 4-5, 8-9 e 11-12. In 2 casi non rispondenti è stato aggiunto melphalan nei giorni 1-4 di ogni ciclo.
Su 11 pazienti valutabili, 8 pazienti hanno risposto (73%) con 6 remissioni complete (54%), 2 parziali(18%), 1 risposta minore; 2 casi hanno presentato progressione di malattia. La tossicità, valutata su tutti i pazienti è risultata la seguente: gastrointestinale 5 casi (Gr 1-2), neurologica 10 casi di cui 5 di Gr 3, ematologica 3 casi (Gr 1). La dose di B è stata comunque ridotta solo in un caso per neurotossicità. In un caso pesantemente pretrattato la terapia è stata sospesa per decadimento delle condizioni generali dopo 3 cicli in fase di remissione ematologica.
In conclusione, l'ottima efficacia ed il buon profilo di tossicità confermano il ruolo del B nel trattamento del Mieloma Multiplo. Una particolare attenzione deve essere comunque rivolta alla valutazione della neurotossicità.

057
HIGH RATE OF DROP-OUT IN ELDERLY MYELOMA PATIENTS TREATED WITH THALIDOMIDE AT DIAGNOSIS

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We and others have previously demonstrated the efficacy of a combination including thalidomide (200 mg/day, with a starting dose of 100 mg), low dose of cyclophosphamide (100 mg/day) both continuously and pulse dexametasone (40 mg/day for 4 days every month) in relapsed/refractory Multiple Myeloma Patients. We therefore applied this scheme to 16 elderly previously untreated patients (10 females and 6 males). Median age was 75 years (range 60-81), median Hb 11.2 gr/dL, median albu-

min 3.1 g/dL and median β2 microalbumin 4 mg/L. Treatment was scheduled to be continued for 12 months. However, in only two patients the dose of thalidomide was increased to 200 mg and a total of 8 pts (50%) discontinued thalidomide prematurely. Four patients refused thalidomide due to intolerance after 1 month (3 pts) and after 3 months (1 patient) respectively. In 4 pts thalidomide was stopped after 4-6 months due to toxicity.
After a median follow-up of 16 months, 3 pts (19%) achieved a CR (1 IF negative), 8 pts (50%) a PR with a reduction of the monoclonal component (MC) between 52 and 87%, 2 pts a stable disease and only 1 patient progressed while on treatment. Although the sample is too small for a statistical analysis, we observed that a major response (CR + PR > 50%) has been obtained in only 4/8 of pts that discontinue thalidomide vs 7/8 pts treated with thalidomide for the entire course. In this study we have observed a rate of thalidomide discontinuation higher than that we have recorded with the same schema in previously treated myeloma patients. Therefore, after the first 3 pts, we decided to withheld thalidomide for the first two months and to add this drug only at the third month of treatment. However, this measure was not sufficient to reduce the high rate of thalidomide drop-out

058
CYTOPLASMIC LOCALIZATION OF NUCLEAR FACTOR-KAPPA B IN MULTIPLE MYELOMA CELLS

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Nuclear factor-kappa B (NF-KB) is multifunctional transcription factor that regulates different signal transduction pathways such as cell survival and proliferation. A number of tumors display activated NF-KB, which contributes to promote cancer cell growth and resistance to chemotherapeutic drugs. NF-KB has been shown to be constitutively active in multiple myeloma (MM) cells, resulting in increased expression of Bcl-xL and IL-6. Here, we analyzed the nuclear localization of NF-KB in MM cells derived from 9 different patients with MM at presentation and in relapse, as well as in two myeloma cell lines (XG1, RPMI 8226). NF-KB localization was evaluated by either immunohistochemistry or immunofluorescence using a monoclonal mouse anti-human p65 (Rel A) antibody that recognizes the p65 subunit. Surprisingly, nuclear localization of NF-ÎB was (weakly) detected in only one MM sample from a refractory MM patient, while the other samples, including the MM cell lines, exclusively express the cytoplasmic (inactive) form of NF-KB. We next analyzed the sensitivity of MM primary cells to different doses of the proteasome inhibitor Bortezomib (from 1 nanomolar to 10 micromolar), which is known to antagonizes NF-KB activity. We found a consistent dose- and time-dependent antitumor activity against both chemoresistant and chemosensitive myeloma cells in all the samples analyzed, independently of NF-KB localization. These results indicate that Bortezomib is active in MM cells regardless the NF-KB localization and suggest the existence of other molecular targets of proteasome inhibitors in MM.

059
LIPOSOMAL ANTHRACYCLINES IN THE TREATMENT OF MULTIPLE MYELOMA

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Not pegylated liposomal doxorubicine (Myocet) shows a better pharmacokinetic profile and a much lower cardiological toxicity than conventional anthracyclines. In Our Department, from October 2003 to October 2006, we have treated 24 patients affected by Multiple Myeloma (MM), either at a first line approach (n=13) or in resistant and relapsed disease (n=11). At admission diagnosis concerned patients with IgG Myeloma (n=20), IgA Myeloma (n=3) and micro-molecular Myeloma (n=1). All the patients have been treated with one or more cycles according to chemiotherapic scheme: VCR 1mg iv at day 1 + Myocet 25mg/sm iv at day 2 + CTX 100mg/sm iv days 1-4 + PDN 60mg/sm os days 1-4. In all patients Monoclonal Component (MC) and Left Ventricular Ejection Fraction (LVEF) have been assessed in order to evaluate treatment efficacy and cardiotoxicity of liposomal anthracycline administered. *Results*. We have evaluated 24 patients (12 M, 12 F), mean age 68.71±7.96 years (50 min., 82 max.), mean baseline MC 2488.00±1791.74 and mean baseline LVEF 56.04±6.4%. Evaluation of treatment efficacy has been made in 24 patients: MC has been reduced from 2488.00±1791.74 to 1981.30±1240.86 (CR 49%, PR 17%, NR 13%, PD 21%). The possible Myocet cardiotoxicity has been evaluated by assessment of LVEF: before therapy, the mean value was 56.04±6.4% and slightly lowered to 54.08±5.90% (*p=ns*) as a sign of cardiac function maintenance. *Conclusions*. Treatment with liposomal anthracycline (Myocet) lowers MC in patients with MM and leaves cardiac function unaltered.

060
PALIFERMIN REDUCE INTESTINAL TOXICITY AND LENGTH OF HOSPITALIZATION IN MULTIPLE MYELOMA PATIENTS RECEIVING HIGH DOSE MELPHALAN

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Palifermin has been studied after TBI containing regimen and after BEAM schedule while benefits of this agent has not studied after High Dose Melphalan.
We have employed Palifermin in 20 patients affected with Multiple Myeloma that received high Dose chemotherapy with Melphalan (200 mg/m^2) and we compared clinical results obtained using Palifermin with those of a second group of 31 patients affected with MM and treated previously in our Institution using the same High Dose Schedule and the same anti-infectious prophylaxis. The two groups were not different in mean age (p=0.49), sex (p=0.2), WBC in P.B (p=0.3), and number of previous chemotherapy received (p=0.4). All patients were transplanted using PBSC as hematopoietic rescue and CD34$^+$ dose was not different in the two groups (p=0.9), all patients received G-CSF during aplasia post chemotherapy. Oral mucositis grade was assessed using DMS score. Time for hematopoietic reconstitution and transfusion needs were not different in the two groups. We have not detected significant difference in maximum mucositis score in the two groups, neither duration of oral mucositis resulted different, however Palifermin was associated with a reduction of duration of diarrhea (5 days versus 7 days. p=0.06), with a reduction of use of Total Parental Nutrition (p=0.0001) and with a reduction of mean duration of TPN (15 days versus 4.3, p=0.0002). Length of hospitalization was also significantly reduced with Palifermin in respect to patients that did not received KGF (11 days versus 16 days, p=0.0006). FUO was diagnosed in only 16 % of Palifermin patients versus 34 % of group NO-Palifermin (p=0.1).
In conclusion Palifermin in MM patients undergoing Autologous PBSC transplantation determines a reduction of intestinal toxicity and a reduction of hospitalization length. Palifermin could be an attractive drug for building a program of outpatient PBSC transplantation in MM.

061
PLASMOCYTOMA OF SKULL: A CASE REPORT

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Plasmocytoma of skull represents a rare event in the clinical manifestations caused by plasmacellular proliferation. It shows itself prevalently, at the cranial base and in frontal site, sometimes, with a neurological concern, due to cranial nerves compression. There we are describing a case of voluminous frontal plasmocytoma (15 cm. in diameter) with a prevailingly exophytic manifestation, but with a concern of the falx cerebri, too. Such a neoformation was usually associated to an IgA monoclonal component and osteolysis of all bone segments. Monoclonal component disappeared after surgical removal. We have given a therapy according to VACP scheme modified with Doxorubicine liposomal pegylated and monthly inductions with Zoledronic acid. After 12 cycles of therapy we have obtained a reduction of osteolytic lesions, that, persist only at lower and upper limbs.

062
FLOW CYTOMETRIC EVALUATION OF THE BONE MARROW PLASMA CELL CLONE IN PATIENTS WITH MULTIPLE MYELOMA, MONOCLONAL GAMMOPATHY OF UNCERTAIN SIGNIFICANCE AND NORMAL PLASMACYTOSIS

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In this study, BMPC were identified by their characteristic light scatter distribution and by a sequential gating strategy, assessing CD19, CD45, CD38 and CD56 reactivity in 119 BM samples from 55 MM (33 crabMM and 22 sMM), 42 MGUS and 22 polyPL patients. A statistically significantly higher percentage of BMPC expressing CD19 were demonstrated in polyPL cases as compared with MGUS and MM groups (p<0.0001), while the lowest proportion was accounted in the crabMM group which, however, maintained, although at a lower extent, a significant lower proportion of CD19 expression matched up to sMM (p=0.016). In contrast, a progressive increase of CD56 expression was found from polyPL to MM cases through an intermediate value observed in the MGUS group (p=0.001), which, in turn, showed no significant different with crabMM. We determined CD19=6% (AUC=0.8, p<0.0001) and CD56=83% (AUC=0.685, p=0.002) as the best cut-off values which discriminate between MM and MGUS cases, respectively. On the basis of these results, a CD56 expression, categorized by 83% cut-off value, correctly clustered 100% polyP and 83% of MGUS group as 'low CD56' cases, while the 'high CD56' phenotype was detected in 60% and 55% of crabMM and sMM groups, respectively. On the other hand, the phenotype 'high CD19', categorized by 6% cut-off value, correctly predicted 100 polyPC and 81% of MGUS cases, while a 'low CD19'' expression clustered 83% and 55% of crabMM and sMM cases, respectively (Figure 5B). Finally, we categorized cases on the basis of a normal (CD56low/CD19high), malignant (CD56high/CD19low) or intermediate (ether CD56high or CD19low) phenotype. On the basis of this analysis, a malignant phenotype was detected in 0%, 9.5%, 25% and 43% of polyPL, MGUS, sMM and crabMM, respectively. The results of this study clearly indicated a different progressive prevalence of BMPC with a malignant phenotype in patients affected by polyPL MGUS, sMM and

crabMM. Moreover, Whether the amount of BMPC with such a phenotype in either MGUS or sMM may have an impact in predicting a different clinical outcome should be evaluated.

063
SAFETY AND EFFICACY OF BORTEZOMIB IN PLASMA CELL LEUKEMIA

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We conducted a national retrospective survey of patients with plasma cell leukaemia (PCL) who had received bortezomib for the treatment of their disease outside of clinical trials among haematological institutions participating the Italian GISMM, GISL and GIMEMA cooperative groups. Ten patients diagnosed between October, 2002, and May, 2006, were collected (7 males, 3 females; median age 63 years, range 55-74). Seven of them had primary and 3 secondary PCL. Eight patients had previously received 1 to 4 lines of chemotherapy before bortezomib, including autologous transplantation in 5 cases and thalidomide in 4 cases. Two patients were treated at diagnosis. Circulating plasma cells ranged from 2 to 71 x 10^9/L. Median white blood cell (WBC) count was 22x 10^9/L (range 8.1-113). Two patients had a moderate degree of renal failure, 3 had extramedullary disease (jaw, liver, soft tissues). Del 13 was observed in 4 out of 6 patients with available karyotype. Bortezomib was given using the standard schedule of 1.3 mg/sqm days 1, 4, 8, 11, with an interval of 10-12 days between cycles. One patient received thalidomide, 2 dexamethasone and thalidomide, and 5 doxorubicin and dexamethasone (PAD) in combination with bortezomib. A median of 4 cycles was administered (range 2-6). WHO grade 3-4 hematological toxicity occurred in 7 patients, while neurological grade 3 toxicity was observed in a single patient. Infections (grade 3) occurred in 5 patients. Other toxicities (diarrhoea, nausea, skin rash) never reached grade 3. Three patients required red cell and/or platelet support. In 3 subjects a reduction of bortezomib dose was required due to hematological or neurological toxicity. According to International Myeloma Worging Group uniform response criteria, 5 partial- (PR), 3 very good partial- (VGPR) and 2 complete (CR) responses were achieved (100% overall response). Duration of response ranged from 2 to 14 months. Plasma cells disappeared or significantly decreased in peripheral blood in all cases. Response was not influenced by type or number of previous treatments or by other clinical or biological parameters, including chromosome 13 deletion. In 5 patients autologous transplant procedures were started after response induced by bortezomib. Nine patients are currently alive 4-47 months after diagnosis of PCL. One patient interrupted the treatment after 2 cycles due to herpes zoster and died of progressive disease 7 months later.

Only sporadic case reports have been so far published about the use of bortezomib in the setting of PCL, a very aggressive disease characterized by poor prognosis and in which the results of conventional chemotherapy are usually disappointing. Our data, the first obtained in a relatively large number of patients, indicate that bortezomib has acceptable toxicity, is very effective in inducing significant responses and should be considered for the initial treatment in patients with primary or secondary PCL.

064
LIPOSOMAL DOXORUBICIN (MYOCET), THALIDOMIDE, AND DEXAMETHASONE FOR PATIENTS OLDER THAN 65 YEARS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Thalidomide is actually the drug that has been better investigated when compared with new therapeutic agent. The best results were observed when thalidomide was combined with antracyclines although the toxicity was frequent also in younger patients (pts.). For this reason we designed a new specific regimen for patients older than 65 years. Here, we presents the results of a pilot study using a chemotherapy protocol which combines Myocet 40 mg/ 2 on day 1 every 28 days, dexamethasone 40 mg orally on days 1 through 4 and 9 through 12, and thalidomide 100 mg orally at bed time continuously. Supportive therapy included warfarin, zoledronic acid, erythropoietin and ciprofloxacin. Response to treatment was rated according to the EBMT criteria. Between January 2003 and June 2005, 12 pts. (8 males and 4 females, median age of 68 years) were enrolled. Liposomal doxorubicin and dexamethasone were administered for 4 cycles. After complete restaging, patients responding were administered with 2 additional cycles. Patients showing progression were dropped. After 4 cycles of therapy 4 patients achieved CR, 5 VGPR and 2 pts. a minor response (MR), 1 patient showed signs of progressive disease. At the end of therapy, 1 patient achieved CR from VGPR and 1 patient VGPR from MR. After a median 15 months of follow-up 4 pts. relapsed and died. To date 8 pts. are alive and well. Neutropenia of grades 3 and 4 occurred in 6 pts. and 2 pts. respectively. One patient experienced herpetic infection, none died of infections. The effects related to thalidomide were fatigue (3 pts.) and constipation (4 pts.). One patient experienced grade 3 palmar-plantar erythrodysestesia. Venous thromboembolic events occurred in four pts. In conclusion this combination is effective in the treatment of elderly patiernts with multiple myeloma.

065
EVALUATION OF SERUM LEVELS OF OSTEOCALCIN, PARATHORMONE AND ALCALINE PHOSPHATASE DURING THERAPY WITH BORTEZOMIB, ZOLEDRONIC ACID AND DEXAMETHASONE

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Blood and urine parameters of bone marrow metabolism are often altered in patients with multiple myeloma. These parameters tend to normalize after therapy. Increased levels of osteocalcin and PTH have been reported after therapy with bortezomib. In a previous study we observed a reduction of osteocalcin but an increase of parathormone, in the early days following the administration of bortezomib plus dexamethasone and after zoledronic acid administration. The aim of this study is to evaluate if these observations are related only to bortezomib and zoledronic acid or if they are influenced by combination therapy with Dexamethasone. We evaluated five groups of patients: **A**-(15 pts, 35 cycles): bortezomib+Dexa, **B**-(5 pts): Dexa, C-(6 pts): bortezomib (for the first two days of the cycle), **D**-(10 pts): zoledronic acid, **E**-(6 pts): zoledronic acid+dexa. *Results*. After therapy with bortezomib+dexa, Osteocalcin levels reduce to 68% and PTH raises to 145%. After therapy with dexa osteocalcin reduce to 33%, PTH raises to 183%. After administration of bortezomib, osteocalcin levels reduce only in 50% of the patients and PTH tends to reduce, too. After zoledronic acid, osteocalcin reduces to 77% and, after zoledronic acid + dexa, it reduces to 53%. We do not have enough data on PTH and calcitonin. *Conclusions*. Reduction of osteocalcin levels and increase of PTH after combination therapy with bortezomib+dexa are confirmed. We observed the same trends after therapy with Dexamethasone alone. Therapy with zoledronic acid reduces osteocalcin levels, too, while Bortezomib alone does not have omogeneous effects in all the patients. After combination therapy with dexamethasone + bortezomib and/or zoledronic acid we observed a reduction of osteocalcin in almost all patients. We shall continue our study to clarify if the early variations of these parameters can predict response to therapy. Furthermore, we suppose that zoledronic acid effects on osteoblasts (eventually increased by Dexamethasone) correlate with the risk of jaw bone necrosis.

066
EVALUATION OF CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN PATIENTS WITH BISPHOSPHONATE-ASSOCIATED OSTEONECROSIS OF THE JAW

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Osteonecrosis of the jaws (ONJ) associated with the use of bisphosphonates is a newly described entity. To elucidate the mechanism leading to ONJ and to test the hypothesis that in patients with ONJ the bisphosphonates may interfere with endothelial cell proliferation, using flow cytometric analysis we evaluated the number of circulating endothelial progenitor cells (EPCs) and circulating endothelial cells (CECs) in 8 patients with bisphosphonate treatment and osteonecrosis, 8 Multiple Myeloma (MM) patients with bisphosphonates treatment without ONJ and 5 normal subjects.
MM patients and those with ONJ shown an increase of $CD34^+$ cells with respect the control subjects. EPCs and CECs were higher in MM patients compared to controls and ONJ patients. ONJ patients shown a decrease of EPCs compared to control subjects while CECs were similar to the controls group.
Our results seem to confirm the possibility that bisphosphonates could have a suppressive effect on circulating endothelial cells of patients with ONJ.

067
BORTEZOMIB, LIPOSOMAL DOXORUBICIN AND DEXAMETHASONE COMBINATION THERAPY FOR PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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The combination of bortezomib, liposomal doxorubicin and dexamethasone (PAD) had proven effective in phase I-II clinical trials for untreated and previously treated patients with multiple myeloma. Liposomal doxorubicin has a lower cardiac toxicity and comparable efficacy than standard doxorubicin. It can be safely used in elderly patients or in those with prior exposure to anthracyclines. Finally, bortezomib and liposomal doxorubicin have shown, *in vitro*, enhanced antitumor efficacy. In this report we present the preliminary results of the use of PAD combination in relapsed-refractory patients with multiple myeloma. Nineteen patients with refractory or relapsed multiple myeloma, median age 61 years (range 43-76) were enrolled since November 2005. Sixteen among them are evaluable as they have received at least three cycles of chemotherapy. Bortezomib 1.3 mg/mq iv days 1,4,8,11 was administered, liposomal doxorubicin 30 mg/mq on day 1 and dexamethasone 40 mg on days 1-4. This treatment has been repeated every 4 weeks until

the 6 cycles. Among these 16 evaluable patients: 10 present a clinical stage IIIA, 4 IIA, 1 IIIB and 1 IIB. Median time from diagnosis was 33.5 months (range 9-121). Of them 4 were primary refractory patients and 12 were relapsed ones. The prior therapy lines were a median of 2 (range 2-5). Six patients received autotransplant with Melphalan 100 or 200 mg/mq as conditioning regimen, 2 alloBMT, 10 Thalidomide plus dexamethasone and 13 chemotherapy cycles containing anthracyclines. At present 4 patients have completed the treatment, whereas all the others are being treated and four of them have received 5 cycles, one 4 cycles, three 3 cycles and four 2 cycles. Haematological toxicity has grade 2 of neutropenia for one patient and grade 2 thrombocytopenia for two. One patient had shown grade 3 neurological toxicity, two grade 3 gastroenterological toxicity (diarrea) and two grade 3 fatigue. Two patients had HZ infection. Other grade 2 adverse event were: nausea (1), FUO (4), sensitive polineurophaty (3), fatigue (1). For one patient we have performed a dose reduction of Bortezomib because of gastroenterological toxicity and two patients have undergone sospension of Bortezomib for neuropathy. Response was assessed according to the EBMT criteria. Two (12%) achieved nCR, 5 (31%) PR, 4 (25%) MR, resulting in ORR of 68%. Three patients presented progression of disease and 2 patients stable disease. All 11 responsive patients are alive. The four patients who have completed 6 cycles of the treatment were alive in PR at + 4 months from the end of the therapy. Among non-responsive patients: 4 died and one is alive with active disease. The PAD regimen has demonstred an acceptable toxicity, no evidence of cardiac toxicity. The previous use of anthracyclines does not seem influence the response in this setting of patients. Although an adeguate follow-up is needed to draw any conclusions, this regimen appears very effective and we obteined an ORR of 68% in refractory and relapsed patients with multiple myeloma.