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Copenhagen, Denmark, June 12 - 15, 2008

ABSTRACT BOOK

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Affiliated Scientific Societies

SIE (Italian Society of Hematology, *www.siematologia.it*)
AEHH (Spanish Association of Hematology and Hemotherapy, *www.aehh.org*)
SETH (Spanish Society of Thrombosis and Hemostasis, *www.seth.es*)
SIES (Italian Society of Experimental Hematology, *www.sies.ws*)
SISET (Italian Society for Studies on Hemostasis and Thrombosis, *www.siset.org*)
AIEOP (Italian Association of Pediatric Hematology/Oncology, *www.aieop.org*)
EAHP (European Association for Haematopathology, *www.socforheme.org/eahp*)
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European Hematology Association (EHA)

EHA is a scientific society aiming to support research, education and clinical practice in hematology.
Its main objective is to be useful to scientific researchers, clinicians, medical students,
as well as all those working in other fields but who are interested in hematology.

The European Hematology Association was founded in June 1992.
Today, EHA – with over 2700 active members from 95 countries – is a consolidated organization
that pursues a large and growing number of projects and programs.

EHA aims to promote

- Exchange and dissemination of knowledge and scientific information in the field of hematology.
- Education and training in hematology.
- Medical practice in the area of hematology and the position of hematology as medical discipline.
- Scientific research in hematology.
- Exchange of information for all European doctors, scientists and other professionals interested in hematology.
- A unified European training program in hematology in collaboration with European National Societies of Hematology.

In order to achieve these goals, EHA

- Maintains regular contacts and organizes meetings with all European National Societies of Hematology.
- Holds an annual scientific and educational congress in a major European city; European Cooperative Groups and Networks are encouraged to take advantage of this major event to gather.
- Disseminates medical research, both basic and clinic, through the new journal Haematologica/The Hematology Journal.
- Has established a link with European National Societies of Hematology and other organizations such as the European Group for Bone Marrow Transplantation, European Association for Hematopathology, European Society of Medical Oncology, and American Society of Hematology.
- Provides postgraduate education through the annual congresses, seminars, courses, workshops and meetings organized in collaboration with the European School of Haematology.
- Has a Fellowship/Grants Program to promote research in hematology.
- Accredits scientific meetings and provides CME accounts in collaboration with the European National Societies for hematology.

If you recognize the need for a strong European Hematology Association
and would like to take advantage of the various activities of the Association,
you may wish to become a member of the EHA and contribute to its objectives.

Benefits of EHA Membership

- Subscription to Haematologica/ The Hematology Journal, including on-line access
- Reduced registration fee for EHA Annual Congresses
- Eligible to EHA Research Fellowships & Grants
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MAIN BENEFITS OF EHA MEMBERSHIP

- Subscription to Haematologica/ The Hematology Journal (impact factor 5.032)
- Reduction of € 180 on the individual registration fee for the EHA Annual Congress (junior members receive a reduction of € 105).
- Eligible to apply for EHA Research Fellowships & Grants
- Entitled to apply for a scholarship to attend EHA Scientific Workshops
- EHA Newsletter
- Access to the webcast of the EHA Annual Congress
- Access to the EHA membership database

ANNUAL MEMBERSHIP FEE

- € 125 per calendar year *(January to December)*
- € 85 per calendar year *(January to December)* for junior members*

* *hematologists within 15 years of graduation or with less than 10 years of post-doc experience*

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EUROPEAN HEMATOLOGY ASSOCIATION

EHA Fellowship Program

One of EHA's goals is to promote the career development of young scientists involved in basic, clinical and experimental research in hematology. EHA funds research fellowships, including the long-established EHA – José Carreras Foundation Young Investigator Fellowship, for researchers in the field of malignant and non-malignant hematology. Each grant is for a two-year period. The next call for applications will be announced on the EHA website in October 2008. Awards will be made at the 14th Congress of EHA in Berlin and will be payable from January 2010.

EHA Partner Fellowship Program

A partner fellowship program has been established this year to support training in centres of excellence in Western Europe for the career development of young scientists involved in basic and clinical research in hematology in new accession and EU candidate countries. The goal of this program is to build up and create inter-institutional networks of collaboration between hematology institutes in Western Europe focusing on research and clinical hematology and similar institutes in new accession and EU candidate countries. The call for applications is announced on the EHA website and the deadline for the letter of interest is June 30, 2008. Deadline for full application is August 15, 2008 and notification of the award will be November 1, 2008.

EHA – ASH International Fellowship Award

The EHA-ASH International Fellowship Award was established in 2006. This award has been developed as a partnership between the European Hematology Association (EHA) and the American Society of Hematology (ASH) to provide hematologists in training or early in their careers the opportunity to conduct research in another country. The purpose of the program is to give both clinical and laboratory-based researchers an opportunity to establish new collaborations and experience research in a different environment. This program will benefit not only the individual participants, but also each host institution, as it will build stronger ties between the North American and European scientific community. The call for applications is announced on EHA and ASH websites and the deadline for the letter of intent is September 4, 2008. Deadline for full application is November 4, 2008. Awards will be made on July 1, 2009.

Submission: Detailed information on the EHA and EHA/ASH fellowship programs and submission is available in the Fellowships section at the EHA website: www.ehaweb.org or contact fellowships.grants@ehaweb.org

Would you like to gain research experience in another country? Do you need funding to do this?

The EHA-ASH®

International Fellowship Award

can make this possible.

The American Society of Hematology (ASH) and the European Hematology Association (EHA) have partnered to create the EHA-ASH International Fellowship Award to allow hematologists from the **United States and Europe** to conduct research in another country for up to two years. The program is open to ASH or EHA members who have a doctoral degree and are in a post-graduate hematology training program or actively engaged in laboratory or clinical research.

Each awardee will receive $75,000 to cover supplies, travel, housing, and a per diem. In addition, travel stipends to attend the ASH and EHA annual meetings will be offered during the award period.

For more information, visit ***www.hematology.org/education/awards/ifa.cfm.***

Don't miss this opportunity—submit your letter of intent by **September 4, 2008**. Please note: All letters of intent must be submitted in English.

Questions? If you have any questions or need additional information, please contact Courtney Krier, ASH Award Program Coordinator, at +1 202-776-0544 or *ckrier@hematology.org.*

AMERICAN SOCIETY OF HEMATOLOGY 50
Conquering Blood Diseases – From Research to Patient Care

EHA EUROPEAN HEMATOLOGY ASSOCIATION

13TH CONGRESS OF THE
EUROPEAN HEMATOLOGY
ASSOCIATION

COPENHAGEN, DEMNARK,
JUNE 12 - 15, 2008

ABSTRACT BOOK

European Hematology Association
EHA Executive Office
Westblaak 71, 3012 KE, Rotterdam
The Netherlands
Tel.: +31 10 436 1760, Fax: +31 10 436 1817
E-mail: info@ehaweb.org
Website: www.ehaweb.org

13TH CONGRESS
JUNE 12 - 15, 2008
COPENHAGEN

Word of welcome

On behalf of the Scientific and Education Program Committee, we would like to welcome you to Copenhagen for the 13th Congress of the European Hematology Association. We have addressed the challenge of building a scientific program covering the various fields of haematology with a balance between the clinical and scientific topics. We now invite you to participate in the numerous sessions, including Education, Hematology-in-Focus, Science-in-Progress and Plenary sessions as well as the challenging Lunch Debates and Clinical Trial Updates. We also have a number of highly expert 'Meet-the-Expert' sessions and encourage you to register early to grasp the opportunity to exchange information in a very informal and friendly atmosphere. On Friday June 12th, and for the second year, the new Molecular Haematopoiesis Workshop will be take place, where cutting edge science will be presented in a new and exciting way and we hope to particularly attract scientists and young hematologists interested in a scientific career- please come along !

From the high number of abstracts submitted this year, we have also put together an interesting programme of simultaneous oral sessions and poster sessions. The 5 best abstracts have been selected for presentation during the Presidential Symposium on Saturday afternoon. Please take also time to visit the poster sessions when the authors will present their work to you in exchanges animated by expert moderators.

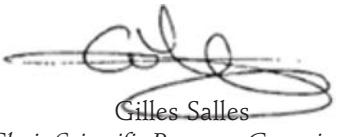
The complex issue of the “Relationship between academia and pharma” will be addressed during this year's joint EHA-ASH Symposium taking place on Saturday, June 14th, with the opportunity to exchange information and opinions from both sides of the Atlantic. The Joint Symposium of the European School of Hematology (ESH) and EHA will again take place on Friday, dedicated to “Communication with transplant patients, the complementary roles of doctors and nurses”. A number of meetings of EHA Scientific Working Groups will be held on Thursday evening, which we hope will help foster direct interactions between physicians and researcher in the various fields of haematology. In addition, 26 Satellite Symposia will run on Super Thursday, covering the State-of-the-Art in experimental and clinical haematology.

We would also like to welcome you to the Opening Ceremony which will take place on Friday, June 12th, directly followed by the presentation of the José Carreras Lecture by Professor John Goldman. In the same session, the winners of the EHA-José Carreras Young Investigator Fellowship and the additional EHA Fellowships and Grants will be presented. For the first time this year EHA has initiated a new award to honour outstanding physicians/scientists for their lifetime contribution to the advancement of hematology. The first EHA Jean Bernard Lifetime Achievement Award will be presented to Professor Dieter Hoelzer on Saturday afternoon.

The congress program is accredited for continuing medical education (CME) by the EHA-CME System, which is the new name for the European Council for Accreditation in Hematology (ECAH). EHA assures the continuity in collaboration with many of the original ECAH partners including the European School of Haematology (ESH). EHA and ESH are dedicated to establishing modern haematology in the European curriculum for medical postgraduate education. The scientific program of the 13th Congress of the EHA has also been reviewed and approved for accreditation by the American Medical Association (AMA).

Copenhagen is a very lively city, with a high tradition of hospitality, and is an excellent venue for the EHA Congress with its focus on exchange of ideas, discussion of results and networking, exchange of scientific principles all in the friendly and safe atmosphere of the city.

On behalf of the EHA Board and the Scientific and Education Committee of the 13th Congress: we are pleased to welcome you to this beautiful city and hope that this top hematology congress in Europe will provide you with fruitful and enjoyable interactions with your peers and induce new creative ideas for your work!



Gilles Salles
Chair Scientific Program Committee

Niels Borregaard
Congress President

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POSTER SESSION I

Acute lymphoblastic leukemia - Clinical

0001

DASATINIB EFFICACY IN PATIENTS WITH IMATINIB-RESISTANT/-INTOLERANT PHILADELPHIA-CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA: 24-MONTH DATA FROM START-L

K. Porkka,[1] G. Martinelli,[2] O.G. Ottman,[3] H. Dombret,[4] F.T. Garzon,[5] C. Zhu,[5] R.A. Larson,[6] B. Simonsson[7]

[1]Helsinki University Central Hospital, HELSINKI, Finland; [2]S.Orsola-Malpighi Hospital, BOLOGNA, Italy; [3]Johann Wolfgang Goethe Universität, FRANKFURT, Germany; [4]Hôpital Saint-Louis, PARIS, France; [5]Bristol-Myers Squibb, WALLINGFORD, USA; [6]University of Chicago, CHICAGO, USA; [7]Uppsala University Hospital, UPPSALA, Sweden

Background. Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL) is characterized by rapid disease progression and transient responses to imatinib. Dasatinib is the most potent inhibitor of BCR-ABL and is 325-fold more potent than imatinib and 16-fold more potent that nilotinib *in vitro*. Dasatinib also inhibits SRC-family kinases, which may contribute to BCR-ABL-independent imatinib resistance in Ph+ALL. Initial findings from START-L demonstrated that dasatinib induces treatment responses in patients with Ph+ALL following imatinib failure. *Aims*. To investigate response durability with dasatinib 70 mg BID in patients with Ph+ALL, 24-month follow-up from the START-L trial has been analyzed. *Methods*. START-L is an open-label, international, multicenter study. Patients with imatinib-resistant or -intolerant Ph+ALL were recruited from January to July 2005. Dose escalation to 100 mg BID was allowed for inadequate response and reduction to 50 or 40 mg BID or interruption was allowed for toxicity. Trial objectives included the evaluation of hematologic and cytogenetic response rates and duration, progression-free survival (PFS) and overall survival duration, and safety/tolerability. *Results*. Among 46 recruited patients, median age was 48 years, median time from initial Ph+ALL diagnosis was 18 months (range 3-163), and 96% of patients were imatinib resistant. During imatinib therapy, 46% had received >600 mg/d, 52% had received imatinib for >12 months, and 50% had achieved a major cytogenetic response (MCyR). Prior to the trial, stem-cell transplantation (SCT) had been performed in 37% of patients and BCR-ABL mutations were present in 78%, including 20% with a T315I mutation. With a minimum follow-up of 24 months, a complete hematologic response (CHR) was achieved with dasatinib treatment in 35% of patients (42% excluding the subgroup with a baseline T315I mutation), a MCyR was achieved in 56% (68% excluding T315I) and a complete cytogenetic response (CCyR) was achieved in 54% (66% excluding T315I). MCyRs were achieved rapidly (median time to response 29 days) and were durable (median 6.3 months). Median PFS was 3.3 months (5.7 months excluding the T315I subgroup), and the 24-month overall survival rate was 31% (34% excluding T315I). In patients with or without baseline BCR-ABL mutations, similar rates of MCyR (55% *vs* 56%) and CCyR (52% *vs* 56%) were observed despite no patient with a T315I mutation achieving a response. Responses were more favorable in patients with prior SCT *vs* no prior SCT (MCyR 76% *vs* 45%; median PFS 7.2 *vs* 2.7 months). Grade 3/4 thrombocytopenia and neutropenia each occurred in 78% of patients. The most common grade 3/4 nonhematologic side-effects were gastrointestinal bleeding (11%), diarrhea (9%), asthenia (7%), and pleural effusion (7%). After 12 and 24 months, five (11%) and one (2%) patients remained on study, with 52% and 57% discontinuing after disease progression, respectively. Discontinuation following study drug toxicity occurred in 9%. *Summary and Conclusions.* In patients with Ph+ALL following imatinib failure, dasatinib 70 mg BID is associated with rapid and durable responses, and long-term survivors are evident. The notable efficacy and well-tolerated safety profile achieved with dasatinib in this very difficult-to-treat population supports the ongoing evaluation of dasatinib in the front-line Ph+ALL setting.

0002

HIGH DOSE METHOTREXATE IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: IMPACT OF THE PHARMACOGENETIC POLYMORPHISMS OF RFC1, MDR1 AND MRP2 ON PLASMA CONCENTRATIONS

M. Fakhoury, T. Adam de Beaumais, Y. Medard, D. Zhang, S. Azougagh, K. Yakouben, E. Jacqz-Aigrain

Robert Debre Hospital, PARIS, France

Background. Methotrexate (MTX) is an antifolate drug administered at high dose (5 g/m^3) (HD-MTX) in the treatment of childhood acute lymphoblastic leukemia (ALL). Its pharmacokinetics is characterized by a wide interindividual variability requiring drug monitoring. *Aims.* We assessed the individual and combined impact of transporters polymorphisms (RFC1, MDR1 and MRP2) on HD-MTX concentrations at H24, H48 and H72. *Methods.* A retrospective pharmacogenetics study was conducted in 86 children (58 boys; mean age: 6.3±4.0) with ALL including in EORTC 58951 protocol at Robert Debre Hospital. Informed consents were obtained. They received 3 or 4 courses of high dose methotrexate during the Interval therapy according to their risk group. For each course, MTX plasma concentrations at 24, 48 and 72 hours from the start of the infusion (H24, H48 and H72) were measured by EMIT®. Allelic variants of RFC1 (SLC19A1: G80A), P-glycoprotein (MDR1: C1236T, G2677T/A, C3435T) and MRP2 (ABCC2: C-24T, G1249A, T3563A, C3972T) were determined by real time polymerase *Results*. Dose-normalized MTX concentrations within each course presented a wide inter-individual variability at each sampling time but there was no inter-course difference. Therefore, MTX concentrations ([MTX]) collected during the first course and standardized by the administered dose, were used for analysis. Mutated RFC1 AA patients (n=31/85, 36.0%) had significantly higher [MTX]/dose at H24 (ratio of 5980 *vs* 3158; p=0.007) and H48 (ratio of 71 *vs* 47; p=0.012) compared to those with a wild-type genotype (WT). When comparing GA or AA patients to WT patients, this association remained significant (p=0.012 at H24 and p=0.023 at H48). The 3 MDR1 mutations were in strong linkage disequilibrium and influenced [MTX] at H48 (p<0.05): for MDR1 C3435T, mutated homozygous patients (n= 16/85, 18.6%) presented a median ratio of 89 for [MTX]/dose *vs* 55 for WT patients. The association remained significant (p=0.045) when comparing carriers of mutated variant to WT patients. Patients carrying a mutated variant for both genes (RFC1 G80A and MDR1 C3435T; n=42) presented higher [MTX] (median ratio [MTX]/dose: 75) than patients with mutated variant for only one gene (median ratio about 55; n=22 for patients RFC1 A80 variant/MDR1 WT and n=14 for RFC1 WT/MDR1 T3435 variant) and than WT patients for the both genes (median ratio: 37, n=4). Polymorphisms of MRP2 had no significant impact on [MTX] variability. *Conclusion*s. Our findings suggest that polymorphisms of RFC1 (G80A) and MDR1 (C1236T, G2677T/A, C3435T) contribute to the interindividual pharmacokinetic variability of high dose MTX. This first report of MDR1 C3435T polymorphism may be a useful tool to optimize MTX therapy. Further pharmacogenetic studies will include additional genes implicated in the metabolism and transport of MTX.

0003

IMPAIRED DEXAMETHASONE-RELATED INCREASE OF ANTICOAGULANTS IS ASSOCIATED WITH THE DEVELOPMENT OF OSTEONECROSIS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

M.L. te Winkel, I.M. Appel, R. Pieters, M.M. van den Heuvel-Eibrink

ErasmusMC-Sophia Children's Hospital, ROTTERDAM, Netherlands

Background. Alterations of coagulation accompanied by impairment of microcirculation may be involved in the pathogenesis of osteonecrosis (ON) in childhood acute lymphoblastic leukemia (ALL). ALL induction treatment includes dexamethasone and asparaginase, which both influence coagulation. *Aims.* The main objective of this study is to investigate whether induction-therapy-related alterations in coagulation are associated with the development of ON in childhood ALL. *Methods*. Retrospectively, we evaluated coagulation parameters of 161 ALL patients: 24 who developed ON (ON-positive, median age: 13.8 (4.0-17.2) years) and 137

who did not (ON-negative, median age: 4.9 (1.0-16.7) years). We studied thrombin generation (prothrombin fragment 1+2, thrombin antithrombin complex), fibrinolysis (α2-antiplasmin, plasminogen, plasmin-α2AP complex, D-dimers), procoagulant factors (fibrinogen, factor II, V, VII, IX, X) and anticoagulant factors (antithrombin , protein C and protein S) at diagnosis and during induction treatment. *Results*. Coagulation parameters of ON-positive and ON-negative patients were similar at diagnosis. After 4 weeks induction treatment including dexamethasone, the anticoagulants antithrombin and protein S were significantly less increased in the ON-positive than in the ON-negative patients. Subsequently, after administration of 4 doses asparaginase and tapering dexamethasone, these coagulation parameters equally decreased in both ON-positive and ON-negative patients. As a result, the nadirs of antithrombin and protein S were significantly lower in ON-positive patients than in ON-negative patients, even reaching levels below lower normal limits in the ON-positive group. No dexamethasone-induced or asparaginase-induced differences of other coagulation parameters were found between both groups. *Conclusions*. We conclude that a reduced dexamethasone-related increase of antithrombin and protein S and subsequent decline of these anticoagulants below normal levels after introduction of asparaginase, may result in a hypercoagulable state. This therapy-induced hypercoagulable state may be a contributing factor to develop symptomatic ON in childhood ALL.

0004

REDUCED INTENSITY *VS* CONVENTIONAL MYELOABLATIVE CONDITIONING (RIC VS. MAC) ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) FOR PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): A SURVEY FROM THE ACUTE LEUKEMIA WORKING PARTY OF EBMT

M. Mohty,[1] M. Labopin,[2] T. Ruutu,[2] H. De Lavallade,[2] A. Gratwohl,[2] G. Socie,[2] J. Esteve,[2] R. Tabrizi,[2] A. Nagler,[2] V. Rocha[2]

[1]CHU de Nantes, NANTES; [2]ALWP of EBMT, PARIS, France

The exact role of RIC allo-SCT for adult patients with ALL is still under considerable debate. While the use of such so-called nonmyeloablative or RIC regimens has emerged as an attractive modality to decrease transplant-related mortality, toxicity might represent only one aspect of the problem, since ALL encompasses a group of chemosensitive diseases, raising concerns that significant reduction of the intensity of the preparative regimen may have a negative impact on long-term leukemic control. In this multicenter retrospective study, the outcomes of 601 adult (age at transplantation >45 y.) patients with ALL who underwent transplantation in complete remission (CR) with an HLA-identical sibling donor, were analyzed according to 2 types of conditioning: RIC in 97 patients, and standard MAC (or high-dose) in 504 patients. Both groups were comparable in terms of gender, CR status (CR1 and CR2), interval from diagnosis to allo-SCT, and recipient/donor CMV serostatus. Patients in the RIC groups were older (median 56 y. *vs* 50y in the MAC group; $p<0.0001$), Most of the patients in the MAC group received high dose TBI (80%), while the majority of the RIC regimens included either low-dose TBI or were ATG+chemotherapy-based regimens. The majority of patients (88%) from the RIC group received a PBSC graft. In the MAC group, the stem cell source consisted of bone marrow in 42% of patients. With a median follow-up of 13 months (range, 1-127), the incidences of grade II-IV and grade III-IV acute GVHD were: 35%, 14%, and 28%, 10% in the MAC and RIC groups respectively (p=NS). The cumulative incidence of non-relapse mortality at 2 years (NRM) was 32% (MAC) *vs* 22% (RIC) (p=0.04). The cumulative incidence of relapse at 2 years was 30% (MAC) *vs* 42% (RIC) (p=0.0007). However, the latter differences did not translate into any significant difference in term of leukemia-free survival (LFS) at 2 years: 38% (MAC) *vs* 37% (RIC) (p=0.42). In multivariate analysis for LFS, the status at transplant was the only factor associated with an improved LFS ($p<0.0001$, RR=0.55, 95%CI, 0.42-0.72). The results of this retrospective registry based study suggest that RIC regimens may reduce NRM rate after allo-SCT for adult ALL when compared to standard MAC regimens, but with a higher risk of disease relapse and no impact on LFS. The latter represent promising findings, since patients who received RIC are likely to have serious comorbidities, which led the transplantation center to choose RIC, and surely most of these patients would not have received a standard allo-SCT in most institutions. Therefore, RIC allo-SCT for adult ALL (>45 y.) may represent a valid therapeutic option when a conventional standard conditioning is not possible, warranting further prospective investigations.

0005

POSTERIOR REVERSIBLE ENCEPHALOPATHY SYNDROME: TWO CASES IN YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

S. Reutenauer, J.F. Albucher, J. Pariente, H. Dumas, O. Milioto, M. Attal, C. Recher, F. Huguet

CHU Toulouse, TOULOUSE, France

The Posterior Reversible Encephalopathy Syndrome (PRES), primarily described by Hinchey *et al.* in 1996, occurs in cases of eclampsia, renal insufficiency, hypertension, or complicates immunosuppressive therapies or chemotherapies. In hematology, this syndrome has been described in pediatric acute leukemias, but not in adults. *Case 1*: A 19-year-old man, treated by GRAALL 2005 protocol for acute lymphoblastic leukemia (ALL), suddenly presents on day 28 of induction chemotherapy a unique tonic-clonic generalized seizure. This neurologic complication occurs in a context of hypertension and acute renal failure. MRI shows signal abnormalities predominating in posterior regions of the brain. With anticonvulsivant therapy, there is no recurrence of seizure. Follow-up MRI 15 days later is normalized, which allows further chemotherapy, without changes of drugs type or dose. *Case 2*: A 22-year-old woman, treated for ALL with the same protocol, suffers on day 23 of induction chemotherapy headaches, decreased alertness, followed by several tonic-clonic non generalized seizures, accompanied by homonymous left hemianopsia. She developed 7 days before an acute renal failure with severe hypertension. Encephalic MRI shows extensive signal abnormalities (Figure 1). With anticonvulsivant medication, and after quasi-normalization of MRI, consolidation course could be initiated 3 weeks later.

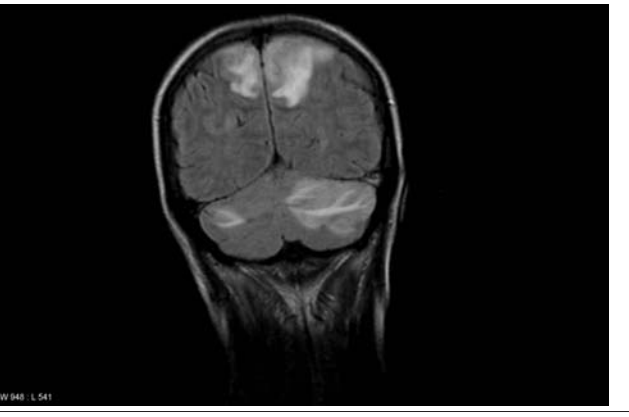

Figure 1.

MRI and clinical presentation of these 2 cases will be detailed. In these 2 cases, the only abnormalities found were neuroradiological, and permitted to diagnose PRES. It is a clinical and radiological entity defined by headaches, altered mental status, seizures and visual abnormalities, as well as characteristic MRI findings of vasogenic oedema in posterior regions of the brain. The main hypothesis for the pathophysiology of this oedema is impairment in cerebrovascular autoregulatory control, and especially in vertebrobasilar territory, due to its sparse sympathetic innervation. It classically occurs in a context of hypertension or hypervolemia, and can be caused by cytotoxic agents. This syndrome of posterior encephalopathy should be better-known by hematologists, because its prevention and its treatment are easily achieved by anticonvulsivant and antihypertensive agents, and can avoid irreversible evolution. Intensive ALL treatments, inspired by pediatric protocols should be added to the growing list of causes of PRES.

0006

EFFICACY AND TOLERABILITY OF INTRATHECAL LIPOSOMAL CYTARABINE FOR TREATMENT OF MENINGEAL RELAPSES IN ACUTE LYMPHOBLASTIC LEUKAEMIA: EXPERIENCE OF A SINGLE PEDIATRIC INSTITUTION

R. Parasole, G. Menna, N. Marra, A. Mangione, S. Buffardi, A. Misuraca, F. Petruzziello, V. Poggi

Santobono-Pausilipon Hospital, NAPOLI, Italy

Background. Treatment of Central Nervous System (CNS) recurrence in acute lymphoblastic leukemia (ALL) remains a problem for clinicians, often difficult to solve. A new liposomal formulation of cytarabine

(DepoCyte®), encapsulated into multivesicular lipid particles, has been used in leukemic meningitis. Liposomal cytarabine is slow released into the cerebrospinal fluid (CSF), resulting in prolonged drug exposure and presumable higher response rate. Recently neurological complications of DepoCyte have been reported in combination with high-dose systemic methotrexate and cytarabine. *Aims.* We studied the efficacy and tolerability of intrathecal administration of DepoCyte in 5 meningeal relapsed ALL children, treated in a single Paediatric Institution. Patients and *Results.* Between May 2005 and October 2007, five children (4 male and 1 female with Down Syndrome; age at diagnosis 2 to 16) with CNS relapsed ALL were treated with intrathecal DepoCyte at variable dose of 25-50 mg/dose. Concurrent dexamethasone therapy, at the dosage of 0,2 mg/kg/dose, twice a day for 5 days, was administered with each dose of i.t. drug. In all patients DepoCyte was associated with systemic chemotherapy and in 4 out of 5 patients to concurrent high dose of cytarabine (2 gr/m^2). Depocyte was started during the first cycle and continued every 15 days, independently from aplasia. Every patient received about 6-7 drug administrations. Liposomal cytarabine was well tolerated in all patients and all patients achieved complete liquoral clearance after the first three intrathecal drug administrations. Four out of them, treated concurrently with high-dose systemic cytarabine, had not showed any additional neurological side effects. Only one patient, a 16 year-old-boy, treated with adult dose (50 mg/dose) for age and weight, presented, after the third administration, grade II headache, rapidly reverted by increasing dexamethasone prophylaxis (0,5 mg/kg/dose). Characteristics of patients are resumed in Table 1. *Conclusions*. In this series, we demonstrate the feasibility and tolerability of intrathecal liposomal cytarabine administration in five children with CSN relapsed ALL. Hence, despite its potential neurotoxicity, DepoCyte remains an interesting formulation, especially in the paediatric setting, for its ability to reduce frequency and total number of intrathecal administrations due to its high detectable intrathecal levels up to 2 weeks. The properties of this formulation, associated with its efficacy, have a favourable impact on children compliance and quality of life; for this reason every effort are necessary to study the best application of DepoCyte in intensive treatment regimen.

Table 1. Clinical characteristics of study patients.

Characteristics	*Patient 1*	*Patient 2*	*Patient 3*	*Patient 4*	*Patient 5*
Age (yr) / Sex Diagnosis	8/male hyperleukocytic ALL-T	2 1/2 / Male hyperleukocytic ALL-T	16 /Male ALL-T	11/Male ALL-Calla+	11/Female ALL-Calla+
Initial CNS involvement	No	No	No	No	No
Front line protocol/ Risk stratification	AIEOP ALL2000/ High risk	AIEOP ALL2000/ High risk	AIEOP ALL2000/ Medium risk	AIEOP ALL9502/ Medium risk	AIEOP ALL2000/ Medium risk
Type of first relapse	isolated CNS relapse	combined	combined	isolated bone marrow relapse	combined
Time from diagnosis	+8 mts	+8 mts	+9 mts	+52 mts	+30 mts
Treatment of relapse	AIEOP ALL REC 2003	AIEOP ALL REC 2003	AIEOP ALL REC 2003	AIEOP ALL REC 98	AIEOP ALL REC 2003 modified
Second relapse/ Time from diagnosis	Yes (combined) / +11 mts	No	No	Yes (CNS isolated) /+ 73 mts	Yes (CNS isolated +44 mots
Cranial RT/TBI	RT (after Depocyte)	TBI (after Depocyte)	TBI (after Depocyte)	RT and TBI (before Depocyte)	No
Starting of Depocyte	Second relapse	First relapse	First relapse	Third combined relapse after SCT	Second relapse
Total N. of administarions	6	6	7	4	7
Dosage (mg)	25-35	25	50(3) -35(4)	35	35
Side Effects	No	No	Mild headache grade II	No	No
CNS Response	CR	CR	CR	CR	CR
Outcome	DOD	Death (TMR post SCT)	RC + 11 mts from SCT	death for sepsis+3 mts from 3h relapse	DOD

SCR, stem cell transplant; RT, radiotherapy; TBI, total body irradiation; CR, complete remission; DOD, dead of disease.

0007

THE PRESENCE OF CD56/CD16 IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA LEADS TO WORSE SURVIVAL AND CORRELATES WITH THE EXPRESSION OF CYTOTOXIC MOLECULES

L.F.F. Dalmazzo, R.H. Jacomo, R.L.G. Cunha, A.F. Marinato, L.L. Figueiredo-Pontes, A.B. Garcia, E.M. Rego, R.P. Falcão

University of Sao Paulo, RIBEIRÃO PRETO - SP, Brazil

Some cases of T-cell Acute Lymphoblastic Leukemia (T-cell ALL) express surface markers commonly found in natural-killer (NK) cells, such as CD56 and CD16. From january/2000 to december/2006, 84 cases of T-cell ALL were diagnosed at our institution. The presence of either CD56 and/or CD16 was identified in 24 of these patients (28.5%), which we designated as T/NK-ALL group. The aim of this study was to compare the clinical and laboratorial features, survival and expression of cytotoxic molecules, such as perforin, granzyme B and T-cell intracellular antigen-1 (TIA-1), in T/NK-ALL and T-ALL patients. Except for the TIA-1, which was analyzed by immunocytochemistry, all the others markers, including CD56, CD16, perforin and granzyme B, were studied by flow cytometry in blasts obtained from the bone marrow. Concerning the clinical characteristics, significant differences between T/NK-ALL and T-ALL groups were observed regarding age (24.9 *vs* 16.4 years, p=0.006) and platelets counts (177×10^9/L *vs* 75×10^9/L, p=0.03), respectively. The immunophenotypic features demonstrated different expression of some markers: CD34, CD45RA and CD33 were most expressed in the T/NK patients, while CD8 and TdT in the T-ALL patients (p<0.05). We also observed that T-cell ALL cases expressing CD56 and/or CD16 had worse prognosis. Kaplan-Meier method estimated that the mean duration of overall survival (863 *vs* 1869 days, p=0.02) and disease-free survival (855 *vs* 2095 days, p=0.002) was shorter in patients expressing CD56/CD16. In order to correlate the presence of CD56/CD16 with cytotoxic characteristics, blasts expression of perforin, granzyme B and TIA-1 was analyzed. These cytotoxic molecules were higher expressed in T/NK-ALL compared to T-ALL cases. Perforin, granzyme B and TIA-1 were detected in 12/17, 4/17 and 7/24 T/NK-ALL and in 1/20, 0/20 and 1/20 T-ALL patients, respectively (p<0.001, p=0.022 and p=0.038). Our results demonstrated that the presence of CD56 and/or CD16 predicts worse outcome and differentiates a specific subtype of T-cell ALL, with some clinical differences and high expression of cytotoxic molecules.

0008

AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: STILL NOT OUT OF FASHION

F. Folber, M. Doubek, Y. Brychtova, M. Krejci, J. Kujickova, I. Palasek, B. Weinbergerova, D. Zackova, Z. Koristek, M. Navratil, Z. Racil, J. Kamelander, D. Dvorakova, J. Mayer

University Hospital Brno, BRNO, Czech Republic

Background. Postremission treatment in adult acute lymphoblastic leukemia (ALL) is still controversial, especially in the point of autologous peripheral blood stem cell transplantation (autoPBSCT). Aims. In this study we show results of autoPBSCT and maintenance therapy with oral 6-mercaptopurin and methotrexate compared to chemotherapy alone in the treatment of adult ALL. Methods. We retrospectively analysed data of 60 consecutive patients treated at our department according to our standard protocol in years 1997 to 2007. There were 35 males and 25 females, with a median age 35.2 years at the time of diagnosis (range: 17.3 to 70.7). Fifty-two (87%) patients had ALL, 7 (12%) lymphoblastic lymphoma (LBL), and 1 (2%) acute biphenotypic leukemia. Seventeen of 52 (28%) patients with ALL showed T lineage and 33 (55%) B lineage. Nine (17%) patients were found Ph positive. Seven (12%) patients were treated with chemotherapy and allogeneic stem cell transplantation (alloSCT). In the cases where there were no donors available, they were treated with chemotherapy alone (35 patients, 58%) or with chemotherapy followed by autoPBSCT and maintenance therapy (18 patients, 30%). Within a median follow-up of 14.7 months (range: 0.1 to 117.8) the data were analysed for achieving complete remission, disease progression, relapse, and causes of death. Results. Out of the total number of 60 patients, 49 (82%) achieved complete remission after the induction chemotherapy (CR1); 44 of 52 (85%) with ALL and 4 of 7 (57%) with LBL. The median PFS was 29.8 months in all patients and 58.1 months in patients in CR1. The median OS was 14.8 months in all patients and 39.6 months in patients in CR1. Patients treated with chemotherapy alone had shorter median PFS than patients who underwent autoPBSCT and maintenance therapy (8.4 *vs* 46.8 months in patients in CR1, p=0.017). Patients treated with chemotherapy alone had also shorter median OS than patients after autoPBSCT or alloSCT (13.0 *vs* 46.8 *vs* 44.3 months in patients in CR1, p=0.046). The differences remained statistically significant even after excluding Ph positive patients. There was no significant difference in PFS and OS in patients with B-ALL and T-ALL. Relapse of the disease was treated by chemotherapy with or without stem cell transplantation in 24 out of 26 cases, but only 7 patients achieved second complete remission. Most common causes of death were infection (41%), disease progression (24%) and bleeding (12%). *Summary and Conclusions*. According to our analysis, autoPBSCT coupled with maintenance chemotherapy is still an option in adult acute lymphoblastic leukemia and provides favourable OS and PFS rates, significantly better than those reached by chemotherapy alone.

0009

RESULTS OF TREATMENT WITH HYPER-CVAD / MTX-ARA-C, A DOSE-INTENSIVE REGIMEN, IN ADULT ACUTE LYMPHOCYTIC LEUKEMIA AND HIGHLY AGGRESSIVE LYMPHOMA IN A SINGLE CENTER IN CHINA

T. Niu, T. Liu, B.X. Xiang, Y-Q. Jia, H.-L. Zhu

West China Hospital,Sichuan University, CHENGDU,SICHUAN, China

Background. Although the safety and efficacy of the Hyper-CVAD/MTX-Ara-C regimen,a dose-intensive regimen, in hematologic malignancies has been well established by several clinical trials developed at the UT M. D. Anderson Cancer Center in the U.S.A, the results of this regimen in patients in China has not been well determined. *Aims*. The objective of this clinical study was to evaluate the efficacy and potential toxicity of this regimen in the adult patients with acute lymphocytic leukemia (ALL) and highly aggressive non-Hodgkin lymphoma (NHL) in our hospital in China. *Methods*. Between June 2004 and June 2007, fifty-six patients with ALL or highly aggressive lymphoma were treated with the Hyper-CVAD/MTX-Ara-C regimen in our institution. The median age of all patients was 26 years (range 13 to 60 years), and 35 patients (62.5%) were male. There were 32 previously untreated patients and 24 refractory/relapsed cases. Among the 41 patients with ALL, B-cell type was present in 82.93%, T-cell type in 17.07%, and Ph chromosome-positive ALL was present in 14.63%, refractory/relapsed disease in 43.90%. Among the 15 patients with highly aggressive NHL, lymphoblastic lymphoma was present in 46.67%, Burkitt's lymphoma was in 53.33% and refractory/relapsed disease in 40.00%. CNS involvement was present in 8% at diagnosis. Treatment consisted of four cycles of Hyper-CVAD alternating with four cycles of high-dose methotrexate (MTX) and cytarabine therapy, together with intrathecal CNS prophylaxis and aggressive supportive care with granulocyte colony-stimulating factor, transfusion and antibiotic prophylaxis therapy. Maintenance therapy based on the cytogenetics and immunophenotypes in most of patients contained 2-year treatment with mercaptopurine, MTX, vincristine, and prednisone (POMP). *Results*. The median follow-up duration was 7 months (range 1+ to 37+ months). Of the previously untreated 31 patients, twenty-nine patients (93.55%) achieved complete remission (CR) and no patients died during induction phase. Of the refractory /relapsed 24 patients, fourteen cases (58.33%) achieved CR. Interestingly, the CR rate of the patients with Burkitt's lymphoma was 75.00% (6/8). The median finished courses during the dose-intensive phase were 4 (range 1 to 8), and the median time to delivery of all eight courses was 10 months. The estimated 3-year overall survival for the untreated and refractory/relapsed patients with ALL was 46.80% and 28.60%, respectively. Meanwhile, the estimated 2-year overall survival for the aggressive NHL patients was 84.00%. Compared with the patients with ALL who did not receive CR and got less than four courses of this regimen, the patients who received CR and got more than four courses of this regimen showed much better overall survival ($p<0.05$). The incidence of CNS relapse was much lower (5%). Myelosuppression-associated complications including documented infections, fever of unknown origin, hemorrhage were the more frequent side effects. Other significant side effects included neurotoxicity, renal and hepatic toxicities, fatigue, mucositis, nausea, vomiting, diarrhea, skin rashes, and G-CSF therapy-associated bone aches. *Conclusions*. The study from our single center in China demonstrated that Hyper-CVAD/MTX-Ara-C, a dose-intensive regimen with much higher CR is superior to our previous regimens, even in highly aggressive lymphomas such as lymphoblastic and Burkitt's lymphoma, and refractory/relapsed ALL/lymphoma patients. Our data also showed that this regimen is less toxic and well tolerated in most of patients in China. Due to the aggressive supportive care, the expense with this regimen is more expensive than previous conventional chemotherapy. Long-term treatment benefits, such as disease-free survival rates and severe side effects need further investigation in a well-designed, multiple-center study in China with more eligible patients entering onto the study.

0010

KINETICS OF CEREBROSPINAL INFILTRATION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA: A FLOW CYTOMETRY STUDY

M. Ramírez,[1] A. Gómez,[1] C. Martínez,[1] A. Lassaletta,[1] C. Moscardó,[2] I. Hernández,[3] J. Sánchez de Toledo,[4] T. Molins,[5] A. Muñoz,[6] M. Baragaño,[7] J.M. Couselo,[2] E. Cela,[8] L. Madero[1]

[1]Hospital Universitario Niño Jesús, MADRID, Spain; [2]Hospital General, ALICANTE; [3]Hospital Son Dureta, PALMA DE MALLORCA; [4]Hospital Vall de Hebron, BARCELONA; [5]Hospital Virgen del Camino, PAMPLONA, Spain; [6]Hospital Miguel Servet, ZARAGOZA; [7]Hospital San Rafael, MADRID, Spain; [8]Hospital Gregorio Marañón, MADRID, Spain

Background. Most children with acute lymphoblastic leukemia (ALL) have subclinical disease in the central nervous system (CNS) at diagnosis. Extramedullary organs such as the CNS may act as sanctuaries for lymphoblasts, preventing the exposure to adequate levels of chemotherapeutic drugs, and serving as a reservoir for protracted relapses. It is of interest to identify those children with ALL at high risk for CNS relapse, since additional or specific therapy could be imparted at earlier times. *Aims*. We are currently studying the role of flow cytometry for identifying ALL blasts in cerebrospinal fluid (CSF) samples of children with ALL, compared to standard cytological methods. In the present abstract we show our initial experience. *Methods*. We have prospectively evaluated 327 CSF samples corresponding to 66 children with ALL in 14 Spanish pediatric oncology units. Samples were obtained at the moment of lumbar puncture at diagnosis and at every day of intrathecal therapy. Leukemic blasts were defined as viable cells (dead cells were excluded by 7-Aminoactinomycin-D expression) that expressed the same cell surface markers than those of the original leukemia. A flow cytometry assay identifying up to 8 parameters in a single tube allowed us to study the kinetics of CSF leukemic blast infiltration during the induction, consolidation and maintenance phase of therapy. *Results*. As expected, we found a higher CFS infiltration at diagnosis among high-risk ALL compared to standard-risk ALL. Patients with high-risk ALL showed a faster clearance of CSF blasts than those with standard-risk ALL, probably due to the more intensive therapy. Flow cytometry was more sensible that cytological methods for detection of ALL. Cytological analyses were negative in all samples, except in a single case of high-risk ALL with extensive CNS infiltration. All patients are under treatment and there has been no relapse so far. *Conclusions*. This preliminary analysis of the ongoing study shows that flow cytometry is a sensible methodology that may help in the detection and follow up of CNS subclinical disease in children with ALL

0011

ALL-TRANS RETINOIC ACID PLAYS AN IMPORTANT ROLE IN EARLY RELAPSES PREVENTION IN INFANTS WITH MLL-REARRANGED LEUKEMIA

L. Fechina,[1] E. Shorikov,[1] L. Vakhonina,[2] G. Tsaur,[2] L. Saveliev,[3] A. Popov,[3] N. Myakova,[4] D. Litvinov,[4] K. Kondratchik,[4] S. Varfolomeeva,[4] T. Zagoskina,[5] O. Aleinikova,[6] A. Karachunsky,[4] A. Roumiantsev[4]

[1]Regional Children's Hospital, EKATERINBURG, Russian Federation; [2]Research Institute of Cells Technologies, EKATERINBURG, Russian Federation; [3]Ural State Medical Academy, EKATERINBURG, Russian Federation; [4]Research Center of Pediatric Hematology, MOSCOW, Russian Federation; [5]Research Institute of Hematology and Transfusion Medicine, KIROV, Russian Federation; [6]Center of Pediatric Oncology/Hematology, MINSK, Belarus, Republic of Belarus

Background. Out of all patients (pts) younger than 1 year suffering from acute lymphoblastic leukemia (ALL) outcome in infants with MLL-rearranged ALL remains most unfavorable. Despite of fast initial tumor burden and high CR rates achieved by current chemotherapy regimens further treatment often fails due to the MRD persistence on the molecular level leading to high proportion of early relapses. New treatment strategy must be developed to overcome the resistance of residual leukemic clone and prevent the relapses. The efficacy of ATRA in daily dose 25 mg/m^2 in combination with conventional chemotherapy in infants with ALL is under investigation. *Aims*. To determine the impact of ATRA consecutive courses included into MLL-Baby protocol in the prevention of early relapses comparing to ALL-MB 2002 - well established regimen in Russia and Belarus, applied in all children's age groups. *Methods*. From September 2003 till July 2007 45 pts with infants ALL were enrolled either on to MLL-Baby or ALL-MB 2002 protocols under the

decision of treating physician. Treatment has been approved by Local and Federal Ethics Committees. Parents' informed consent was obtained in all cases. Out of 22 and 23 pts allocated to MLL-Baby and ALL-MB 2002 respectively, MLL rearrangements were detected in 15 and 10 pts by cytogenetic and PCR methods correspondingly. Both groups were similarly distributed by median age 6 (1-10) and 6 (2-11) months (p=0.72); gender m/f : 7/9 and 2/7 (p=0.44); WBC: 114 (3-450) and 193 (21.5-360) per microliter (p=0.18); initial CNS disease: 4 and 1 pts. (p=0.81); immunophenotype: BI - 11 and 2 pts (p=0.18), BII - 0 and 2 pts (p=0.07), BIII - 4 and 2 pts (p=0.92), biphenotype-1 and 0 (p=0.45). MLL rearrangements in MLL-Baby and ALL-MB 2002 groups were as follows: t(4;11) -7 *vs* 7 pts (p=0.54); t(11;19) -4 *vs* 2 pts (p=0.83) respectively; t(10;11) -2 pts, t(1;11) -2 pts and del 11q23-1 pts have been detected only in MLL-Baby group. MLL-Baby and ALL-MB 2002 chemotherapy regimens are equally intensive and identical by risk-group distribution and design except CNS disease prophylaxis: in MLL-Baby cranial irradiation 12 Gy was performed only for high risk group (HRG) pts with initial CNS involvement and age older than 12 months, while pts allocated on to ALL-MB 2002 were irradiated despite of initial CNS status and risk-group. Cranial irradiation in MLL-Baby group is substituted by 5 additional intrathecal triplets. Pts with t(4;11) within both protocols have been stratified to the HRG arm. *Results*. We did not observe any significant difference in frequency of induction deaths: 1 *vs* 0; CR rates: 94% *vs* 96%; deaths in CR: 1 *vs* 0 but proportion of early relapses differed significantly: 2 *vs* 7 pts. on MLL-Baby and ALL-MB 2002 respectively (p=0.03). Probability of RFS was 0.85 SE 0.09 and 0.22 SE 0.13 (p=0.04) with median of follow-up 18 months (1-56). EFS, OS and DFS were higher on MLL-Baby although did not achieve statistical significance. *Conclusion.* Our preliminary results indicate that ATRA plays an important role in early relapses prevention in MLL-rearranged ALL in infants.

0012

OUTCOME OF 198 ADULTS AFTER RELAPSE OF ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) INCLUDED IN 4 CONSECUTIVE FIRST LINE PETHEMA TRIALS

S. Vives,[1] A. Oriol,[1] M. Tormo,[2] S. Brunet,[2] E. Del Potro,[2] J. Bueno,[2] J. Bergua,[2] J. Sarrá,[2] C. Grande,[2] C. Bethencourt,[2] J.M. Hernandez-Rivas,[2] C. Rivas,[2] J.M. Ribera[1]

[1]ICO Badalona-Hospital Germans Trias i Pujol, BADALONA; [2]PETHEMA Group. Spanish Society of Hematology, BARCELONA, Spain

Background and aims. Most adults with acute lymphoblastic leukemia (ALL) who achieve complete remission (CR) will relapse. We examined the outcome of 198 adults with recurring ALL, representing the 31% of 645 patients previously treated on PETHEMA first line trials (ALL89, ALL93HR and ALL03HR for high-risk ALL and ALL96IR for intermediate risk ALL). *Patients and methods*. All patients included in the prospective PETHEMA trials between January 1989 and January 2006 were eligible if they had experienced a first relapse. Rate of second CR achievement, disease-free survival (DFS) from CR2 to relapse, death from any cause or last follow-up and overall survival (OS) from relapse to death or last follow-up were assessed as outcome measures and the influence of patients baseline and relapse characteristics was analysed. *Results*. Baseline characteristics: 112 males (57%), median age 30 yr (range 15-69), 82 (41%) with WBC count >25×10^9/L, 45 (23%) with poor risk cytogenetics (i.e. t(9;22), t(4;11) or complex karyotype), 127 (64%) of precusor B-cell origin and 55 (28%) slow responders to initial treatment. First-line treatment included daunorubicin, prednisone, vincristine, asparaginase induction and early consolidations were followed by delayed consolidation and 2-yr maintenance (150 pts, 76%) or autologous (20 pts, 10%) or allogeneic (28 pts, 14%) SCT. Median time from CR1 to systemic relapse was 0.9 yr (range 0.1-9). Fifteen patients (8%) were unable to receive intensive treatment, 153 (77%) received standard ALL induction (equal or similar to first line treatment, N=58 or Hyper-CVAD, N=95), 13 (7%) received a fludarabine-idarubicin-based regimen and 17 (9%) underwent SCT without prior induction treatment. The induction death rate was 15% (29 pts), 83 pts (42%) achieved a second CR and 86 (43%) were resistant. Age <30 yr was associated with a higher probability of achieving CR (p=0.003). SCT was performed to 69 patients (35%) either during CR2 (59 pts: 13 auto, 28 allo, 18 unrelated) or active disease (4 auto, 6 allo). At last follow-up 27 patients (15%) remain alive of whom 7 (26%) have undergone an auto-SCT, 12 (44%) some modality of allo-SCT and 8 no SCT. However, 5-yr OS from relapse was 5% (95% CI 2-8) and 5-yr DFS from CR2 was 9% (95%CI 2-16). Young age (median OS 7 months in patients younger than 30 years *vs* 3 months in older patients; p=0.005) and time from CR1 to relapse (OS of 0% in those with a CR1 duration of less than 1 year, 11% for CR1 lasting more than 1 year; p=0.004) were associated to prolonged survival. Duration of CR1 was also associated with a longer CR2 (p=0.012). Young patients with late relapses (>1 year) had the best chance of remaining long-term survivors (5-yr OS 13%). *Conclusion*. Most adults with recurring ALL, whatever their prior treatment, cannot be rescued using currently available therapies. Young patients with late relapses are the only subgroup that may achieve long term survival.

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0013

METHYLENE TETRAHYDROFOLATE REDUCTASE (MTHFR) GENE POLYMORPHISM IN EGYPTIAN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

A.A.G. Tantawy,[1] E.A. El-Bostany,[2] M. Abou El Asrar,[1] A.A.M Adly,[1] E.A. El-Ghouroury[2]

[1]Faculty of Medicine, Ain Shams University, CAIRO; [2]National Research Center, CAIRO, Egypt

Background. Genetic variations in the enzymes responsible for chemotherapy metabolism in cancer patients may play a role in determining relapse and toxicity risks.Methotrexate is a key drug in acute lymphoblastic leukemia (ALL) treatment,it inhibits DNA replication by blocking the conversion of 5,10 methylene tetrahydrofolate to 5- methylene tetrahydrofolate by methylene tetrahydrofolate reductase (MTHFR). MTHFR is central to folate metabolism and has two commom functional polymorphisms (C677T and A1298G). *Aim of study*. To assess the prevalence of MTHFR polymorphisms C677T and A1298G in Egyptian children with ALL and the relation to the frequency of drug induced complications and relapse rate. *Methods.* Forty ALL patients diagnosed and treated in the Pediatric Hematology/Oncology Unit,Children's Hospital,Ain Shams University, Cairo Egypt were included , all were treated according to the protocol of ALL-BFM 1990, and were followed up for 3.1-6.5 years (median 4.5 years). Data on age at diagnosis, sex,incidence as well as severity and duration of hepatic, mucosal and infectious complications during therapy were reported.MTHFR genotyping was done with a PCR-based RFLP assay. *Results*. Among the studied 40 ALL patients, the MTHFR C677T polymorphic allele frequency were 40%, 27.5%, and 32.5% for TT,CT,and CC genotypes respectively. The MTHFR A1298G polymorphic allele frequency were 40%, 35%, and 25% for AA,AC,and CC genotypes respectively. The TT genotype was significantly associated with increased toxicity during methotrexate therapy compared to other genotypes. Diarrhea and oral mucositis developed in 81.3%, 9%, and 7.7% in TT,CT, and CC genotypes respectively (p<0.0001). The oral mucositis index was higher in TT genotype compared to other MTHFR C677T alleles (21.6, 17.5, and 15.9 in TT,CT, and CC genotypes respectively; p<0.05). Elevated liver enzymes (ALT and AST) developed during therapy in 87.5%, 0%, and 7.7% in TT,CT, and CC genotypes respectively; p<0.0001. The TT genotype was significantly associated with relapse in 56.3% compared to 18.2% and 0% in CT and CC genotypes respectively (p=0.001). TT genotype was significantly associated with lower overall 5 years survival (39%,65%, and 92% in TT,CT, and CC genotypes respectively; p=0.02). There was no significant relation between MTHFR A1298G polymorphism and the risks of drug induced complications or relapse rate in the studied ALL patients. *Conclusions*. MTHFR TT genotype is significantly associated with increased mucosal and hepatic toxicity during methotrexate therapy as well as increased relapse rate in childhood ALL. Because of the relatively high prevalence of the TT genotype in Egyptian children with ALL, dosage modification of methotrexate in ALL protocols should be considered.

0014

LONG TERM COGNITIVE STATUS AND MAGNETIC RESONANCE IMAGING FINDINGS OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

S. Aytac Elmas,[1] S. Aytac,[2] I. Saatci,[3] F. Oktem,[4] M. Cetin,[2] S. Yetgin[2]

[1]Hacettepe University Faculty of Medicine, ANKARA; [2]Hacettepe University Faculty of Medicine Pediatric Hematology Department, ANKARA; [3]Hacettepe University Faculty of Medicine Radiology Department, ANKARA; [4]Hacettepe University Faculty of Medicine Pediatric Psychiatry Department, ANKARA, Turkey

Background. Despite improved survival in children with Acute Lymphoblastic Leukemia (ALL), long term complications still remain a big obstacle for those young people with a long life expectations. There were some new approaches to minimalize long term neurocognitive complications as some protocols discontinue giving prophylactic cranial radiotherapy. *Aims.* Our aim was to assess the long term neuropsychological

effect of treatment in children with ALL who were neurologically asemptomatic. *Method*. Between September 2003 and May 2007, ALL patients whose therapy was stopped at least 2 years ago and came to Hacettepe University Pediatric Hematology Department regularly for routine off-therapy control has been invited to this study and 52 of them were accept to be a part of this investigation. Cranial Magnetic Resonance Imaging(MRI) evaluations was done mean 64 months after cessation of therapy and 21 (40%) of them had no identifiable lesions however 22 (42%) had probable therapy associated lesions (cysts, unidentified bright object, cortical-cerebellar signal alterations) and 9(17%) had definitive lesions (atrophy, bleeding focus, gliosis). There were no statistically significant difference for gender, initial ages and treatment procols (Modified St. Jude Total XI and XIII) between those 3 groups of lesions in the MRI (p>0.05). Time interval between the cessation of theray and MRI evaluation show that children with definitive lesions was defined within a long time period (7,5±3 years) than children without any lesions (4,9±3,9 years). Prophlactic cranial radiotherapy was given 28 of 52 patients and there were no significant difference between patients with radiotherapy and without radiotherapy according to initial age and gender (p>0.05). 22 (78%) of 28 patient whose given radiotherapy showed that were probably and/or definitively therapy associated lesions in MRI. Moreover from those 5 patient who had an atrophy and 8 patient who had a bleeding focus in MRI bared that 4 and 7 of them has given radiotherapy in the past, respectively. Neurocognitive evaluation using WISC-R and WAISS show that there were no difference according to the verbal, performance and total IQ levels between patients with radiotherapy (87±19, 97±20 and 92±20) and without radiotherapy (94±20, 102±21 and 98±20), respectively (p>0.05). On the other hand, there were significant difference between patients without lesions, probable and derfinitive lesions according to the verbal IQ (94±18, 95±17 and 73±24, p=0,017), performance IQ (100±19, 105±17 and 85±27, p=0.038) and total IQ (96±18, 100±17 and 79±27, p=0.027), respectively. *Conclusions*. This study suggest that in the long term, most patients with ALL have MRI abnormalities that were significantly correlated with neurocognitive functions and previous radiotherapy. Patients with ALL must be followed up for these complications and detection of abnormalities in MRI tend to increase in a time period. Elimination of prophylactic cranial radiotherapy seems to be future consept in children with ALL therapy.

0015

TREATMENT OF MULTIPLY RELAPSED LEUKEMIC MENINGITIS BY HIGH-DOSE INTRAARTERIAL CHEMOTHERAPY

A. Shuvaev,[1] V. Savello,[1] O. Sarjevsky[2]

[1]Military-Medical Academy, SAINT-PETERSBURG; [2]National Medico-surgical Center n.a. Pirogov, MOSCOW, Russian Federation

Background. With current treatment protocols there is a big part of patients with acute lymphoblastic leukemia (ALL) that will have long-term survival. The central nervous system (CNS) relapse may occur in 2-10% of them. In some part of patients, relapses may occur multiply and have resistance to systemic chemotherapy and CNS irradiation. There are no common standards for prophylaxis, and especially for treatment of neuroleukemia relapse. We have tried to implicate the superselective intraarterial chemotherapy that is used in primary CNS lymphoma to treatment of CNS relapse in adult ALL. *Material*. Patient (man, 26-years old) entered to the hematological department in 10/2005. In results of investigation the diagnosis of Acute lymphoblastic leukemia (CD19+cy CD79+CD10+CD34+cyTDT+HLA-DR+) was made. No CNS involvement was present by cytology. At the beginning, the induction chemotherapy with intrathecal chemoprophylactic was started in according to Hoeltzer protocol. A remission was reached after one course of induction. After late consolidation the patient had developed first isolated CNS relapse. The treatment was consisted of combination (methotrexate, cytarabine, and dexamethasone) intrathecal chemotherapy and whole-brain irradiation. In results of treatment the clearance of cerebrospinal fluid (CSF) was achieved. After the three months of maintenance therapy the second isolated CNS relapse had diagnosed in control lumbar puncture. The treatment by intrathecal chemotherapy and Hyper-CVAD protocol was started that had lead to third remission after one cycle of Hyper-CVAD. But after third cycle, the CNS isolated relapse had developed again. As the patient had received full-dose brain irradiation and CNS disease had resistance to common treatment tactics, we applied the method of intraarterial chemotherapy that used in treatment of primary CNS lymphoma. The treatment protocol consisted of one-month repeated 2-day chemotherapy: methotrexate (2500 mg each day, for a total dose of 5000 mg) administered intraarterially in arteria meningea media at both side, cyclophosphamide (500 mg/m^2 each day, for a total dose of 1000 mg/m^2) and etoposide (150 mg/m^2 each day, for a total dose of 300 mg/m^2) given intravenously; intrathecal administration of methotrexate 15 mg, cytarabine 30 mg and dexamethasone 4 mg in day 0 also used. Granulocyte-colony stimulating factor rescue was given on days 3-9 or until neutrophil recovery. The clearance of CSF was achieved during first course of treatment. There are no neurological toxicities during treatment. In control assessment after the 6 cycles of treatment there are no signs of leukemic lesions in CNS by contrast-enhanced magnetoresonance imaging, cytological or immunophenotyping evaluation of CSF, but there is a minimal residual disease of bone marrow with initial immunophenotype. After the 9th cycle of chemotherapy was completed, the patient had developed severe exacerbation of chronic hepatitis B and treatment was stopped. No signs of ALL relapse were present. *Conclusion*. Today the treatment of CNS relapses in adults ALL is difficult problem in oncohematology. The implication the treatments approaches of primary CNS lymphoma, as superselective intraarterial high-dose chemotherapy may serve as model for future clinical trials in treatment of CNS involvement in adult ALL.

0016

HYPER-CVAD IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: AN ANALYSIS OF THE DOSE-INTENSIVE (INDUCTION-CONSOLIDATION) PHASE

P. Silva-Coelho,[1] A. Carneiro,[2] I. Carvalho,[2] F. Principe,[2] J.E. Guimaraes[2]

[1]Hospital de Sao Joao, PORTO; [2]Hospital de Sao Joao, Department of Clinical Haematology, PORTO, Portugal

Background. Hyper-CVAD is a modified dose-intensive regimen for the treatment of adult ALL, developed by the group at M.D. Anderson (MDACC). *Aims*. To evaluate the efficacy and toxicity of the dose-intensive phase of Hyper-CVAD in adult acute lymphoblastic leukemia. *Methods*. Ten consecutive patients with relapsed ALL, diagnosed between January 1999 and July 2005, and 24 consecutive patients with ALL newly diagnosed between August 2005 and December 2007 were entered into this analysis. Patients with chronic myelogenous leukaemia, lymphoid blast phase were excluded. Immunophenotypic categories were set according to EGIL criteria. Treatment consisted of Hyper-CVAD, according to the schemata published by MDACC. Patients with Ph$^+$ or BCR-ABL$^+$ ALL were treated with Hyper-CVAD + imatinib, from January 2006 onwards. Evaluation of treatment results was restricted to the dose-intensive phase, due to poor maturation of data from maintenance phase. Responses and treatment outcomes were evaluated according to international standardized criteria defined for AML (2003). *Results*. Median age was 31 years (range, 18-76); 22% were at least 50 years old. Thirty-six percent were female. One patient had Down syndrome. Seventy percent had a performance score of 80 or greater (Karnofsky). Fifty-two percent were of B-cell phenotype, 41% of T-cell phenotype and 7% were biphenotypic (3.5% T/myeloid and 3.5% B/myeloid, all predominantly lymphoid according to EGIL score). Median haemoglobin level was 11.3 g/dL, median WBC was 8.8×10^9/L (WBC>30×10^9/L in 34%), and median platelet count was 67×10^9/L. Splenomegaly was present in 38%, hepatomegaly in 21%, lymphadenopathy in 40%, a mediastinal mass in 22% (all of T-cell phenotype in the latter). Forty-four percent of patients were at high-risk for CNS disease. CNS leukaemia was present in 15% of cases at diagnosis. Twelve-percent of cases were Ph-positive, all p190 (21% of B-cell cases), 9% displayed complex karyotypes, 9% were hyperdiploid, 21% were normal and 12% had insufficient metaphases. Of relapse cases included, all were in first relapse, 80% were systemic, 78% had been submitted to SCT (50% allogeneic, 50% autologous), and 3% had been submitted to previous radiotherapy. Ninety-two percent of patients achieved CR (48% after 1 course, median time to CR 40 days, 90.2% maintained CR and 9.8% relapsed during treatment, one each after 4, 5 and 6 cycles), 56% had less than 5% day 14 blasts, 3% had resistant disease, 6% died during induction (bacterial sepsis: 3% pneumonia, 3% peritonitis). Cerebellar toxicity occurred in 6%, mononeuropathy multiplex in 3%, subarachnoid haemorrhage in 3%, aspergillosis in 3%. Eighteen percent abandoned protocol - 3% each due to cerebellar toxicity, diagnosis of concomitant neoplasia and severe myocardial infarction, and 9% due to relapse of disease. Fifteen percent were referenced for allo-SCT. Febrile neutropenia rates ranged from 15% to 74%/cycle. Hospital stay duration medians varied between 17 and 25 days for odd cycles, and 18 and 24 days for even cycles. No significant effect of pretreatment characteristics in CR achievement/maintenance was observed. *Summary and Conclusions*. Hyper-CVAD is an efficacious and safe treatment regimen for remission induction and consolidation in ALL. Further follow-up time is needed to assess long-term results.

Acute myeloid leukemia - Biology I

0017

SELECTIVE SILENCING OF THE NPM1 MUTANT PROTEIN AND APOPTOSIS INDUCTION UPON ATRA *IN VITRO* TREATMENT OF AML CELLS CARRYING NPM1 MUTATIONS

M.P. Martelli,[1] V. Pettirossi,[2] N. Manes,[2] F. Susta,[3] P.L. Orvietani,[3] A. Liso,[4] F. Mezzasoma,[2] F. Cecchetti,[2] M.F. De Marco,[2] B. Bigerna,[2] A. Pucciarini,[2] I. Nicoletti,[5] L. Binaglia,[3] C. Mecucci,[2] M.F. Martelli[2]

[1]Hematology Department, PERUGIA; [2]Hematology, University of Perugia, PERUGIA; [3]Biochemistry, University of Perugia, PERUGIA; [4]Hematology, University of Foggia, FOGGIA; [5]Internal Medicine and Oncology, University of Perugia, PERUGIA, Italy

We previously identified a new AML category carrying NPM1 mutations which lead to aberrant cytoplasmic expression of the nucleolar protein NPM1, hence the term NPMc+ AML [*Falini et al, NEJM 2005*]. This leukemia accounts for about one-third of adult AML and shows distinctive biological and clinical features [*Falini et al, Blood 2007, Review*]. Notably, AML carrying NPM1 mutations in the absence of FLT3-ITD are characterized by a favourable prognosis. However, still a proportion of NPMc+ AML cannot be cured by conventional treatments and new therapeutic strategies need to be explored. We previously identified OCI/AML3 as the only human AML cell line carrying cytoplasmic mutated NPM (type A) in the absence of FLT3-ITD [*Quentmeier et al, Leukemia 2005*]. Because of these features and the ability to engraft in NOD/SCID mice, the OCI-AML3 represents a remarkable tool for the study of NPMc$^+$ AML. Previous findings that ATRA exerts growth inhibitory effects on the OCI/AML3, prompt us to investigate the molecular mechanisms underlying the response to ATRA, with focus on the NPM mutant protein. Interestingly, clinical data recently presented by the group of Dohner at the last ASH meeting [Schlenk *et al.*, Abs # 297, ASH 2007] indicate ATRA might have a role in the therapy of NPMc+ AML patients, in particular when in the absence of FLT3 mutations. As cellular model for our studies, we also used primary leukemia cells originated from a patient with NPMc+ AML (mutation A) bearing FLT3-ITD mutation (MONT1) that have been propagated in NOD/SCID mice since 1999. Whilst NPM1mutation and cytoplasmic delocalization of the mutant protein appeared to be stable, unfortunately FLT3-ITD mutation was lost with time, thus we don't have at the moment a cellular system of NPM1 mutation in the presence of FLT3-ITD mutation. Early cell cycle arrest and pro-apoptotic effects of pharmacological doses of ATRA were confirmed in both cellular models *in vitro*. Morphological signs of differentiation were not evident. Western blot analysis using specific rabbit polyclonal antibodies showed marked downregulation of the leukemic NPM1 mutant protein upon ATRA treatment, preceding apoptosis activation (Figure 1).

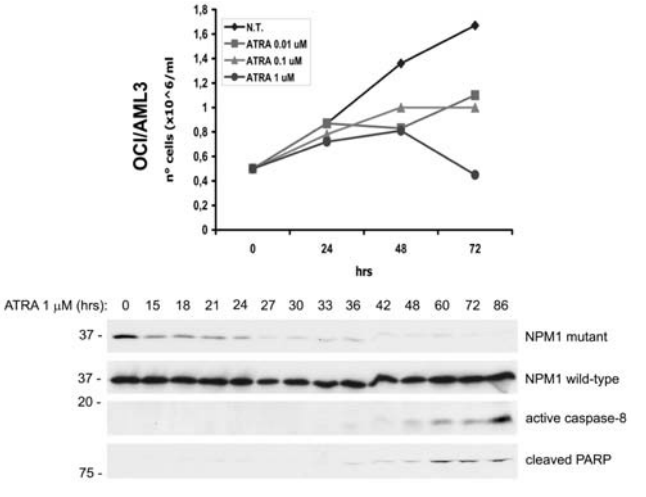

Figure 1.

On the other hand, wild-type *NPM1* protein levels remained unchanged, leading to a condition of *NPM1* haploinsufficiency. Semi-quantitative RT-PCR for NPM mutant A showed no change in mRNA expression following treatment, suggesting a regulation of the NPM mutant protein expression at post-transcriptional level. Indeed, this effect was partly reverted by concomitant treatment with proteasome-inhibitors. Downregulation of NPM mutant protein preceded activation of caspase-8, PARP-cleavage and Bax activation. No NF-kB activation was observed upon ATRA treatment. Importantly, these results were confirmed in the primary NPMc$^+$ AML cells from patient MONT1. Activation of caspase-8 suggests that the response to ATRA in NPMc$^+$ AML cells may be mediated through the death receptor pathway. Although protein levels of TRAIL, TRAIL receptors and TNF-α receptors seem to be unaffected, it might be possible that the *NPM1* mutant protein modulates the signalling through death cell receptors. Analysis of ATRA-induced transcriptome and proteome modifications in NPMc+ AML is ongoing and will be also presented, as well as further pre-clinical studies on patients' primary AML cells. In conclusion, our data suggest that NPM mutant protein might be involved in the *in vitro* response to ATRA in AML cells carrying *NPM1* mutations.

0018

MIRNA EXPRESSION IN ACUTE MYELOID LEUKEMIA CELLS WITH DIFFERENT KARYOTYPES

S. Brundiers,[1] S. Brundiers,[2] K. Battmer,[2] M. Brugman,[1] M. Castoldi,[2] J. Krauter,[1] M. Muckenthaler,[2] A. Ganser,[1] M. Eder,[1] M. Scherr[1]

[1]Hannover Medical School, HANNOVER; [2]University of Heidelberg, HEIDELBERG, Germany

Background. Multiple cyto- and molecular genetic risk factors have been identified in acute myeloid leukemia (AML). microRNAs (miRNAs) are non-coding RNAs in the length of 21-25 nucleotides that are negative regulators of gene expression. For solid tumors and chronic lymphocytic leukemia, it has been shown that miRNA-expression may provide valuable diagnostic and prognostic parameters. *Aims.* Aim of this study was to determine whether miRNA-expression can discriminate (i) between normal progenitor and AML cells and (ii) between cytogenetically defined subgroups of AML and normal CD34$^+$ cells. *Methods*. Total RNA was isolated from purified CD34$^+$ cells from normal bone marrow (BM; CD34>90%, n=4) and peripheral blood (PB; CD34$^+$>95%, n=4), and from primary AML samples at initial diagnosis (CD34$^+$>50%) including samples with normal karyotype (n=5), inv(16) (n=4), -7 (n=4), t(8;21) (n=5), and complex karyotype (n=4), respectively. RNA quality was analyzed by Bioanalyzer, and miRNA-expression profiles were determined by using microarray analysis (miChip, 333 miRNAs) and miRNA-specific quantitative real-time reverse transcriptase-polymerase chain reaction (miR-qRT-PCR). Statistical analysis was performed using Bioconductor and a moderated t-test. Multiple testing corrections were done by calculating the false discovery rate (FDR) according to Benjamini & Hochberg. In addition, differences between miRNA-expression measured by miR-qRT-PCR were analyzed by using the 2-DDCT-method. *Results*. There was no significant difference in miRNA-expression between BM- and PB-derived CD34$^+$ cells. In contrast, we identified a set of thirty-eight out of 216 validated miRNAs differentially expressed between normal PB CD34$^+$ and AML samples based on statistically significant differences in expression levels determined by miChip (p<0.05). Expression levels of twelve miRNAs discriminate AML samples from PB derived CD34$^+$ cells with p<0.01 including miR-223 and miR-10a among others. When compared to miR-qRT-PCR, the expression of six miRNAs was statistically different between AML and PB-derived CD34$^+$ cells in both methods. Next, we analysed miRNA-expression profiles in cytogenetically defined subgroups of AML in comparison to PB derived CD34$^+$ cells. Our analysis identified a subset of nineteen miRNAs with differential expression between AML subgroups by both methods (p<0.05). For example, enhanced expression of miR-181a and reduced expression of miR-99b was found in inv(16) whereas reduced expression of miR-100 and miR-99b was found in t(8;21) as compared to PB derived CD34$^+$ cells. *Summary*. This study identifies a number of specific miRNAs with differential expression in AML and normal CD34$^+$ cells. Furthermore, miRNA expression is heterogeneous within cytogenetically defined subgroups of AML. Prospective studies and correlation to clinical outcome are warranted to confirm the value of miRNA-expression profiling in AML.

0019

AML1/RUNX1 MUTATIONS ARE FREQUENT IN *DE NOVO* AML WITH CERTAIN CYTOGENETICS AND COOPERATING MOLECULAR MUTATIONS DIFFER DEPENDENT ON UNDERLYING CYTOGENETICS

S. Schnittger, F. Dicker, W. Kern, T. Haferlach, C. Haferlach

MLL Munich Leukemia Labortory, MUNICH, Germany

Background. Previous reports have shown that *AML1/RUNX1* mutations are associated with MDS and AML after MDS whereas *de novo* AML were poorly analyzed and cooperating mutations (coop mut) are not analyzed at all. *Aims.* We performed an analysis focused on *de novo* AML and on distinct cytogenetic subgroups. *Methods*. A selected group of 243 AML with normal karyotype (NK) or recurrent chromosomal imbalances were analyzed: NK (n=97); -7 (n=37), +8 (n=30), +13 (n=20), +21 (n=12), others (47). *Results.* In total 79 of 243 patients in these selected cytogenetic subgroups showed at least one RUNX1mut. 67/79 mutations were different. 19 patients (26%) had homozygous mutations and 5 (6.8%) had two different mutations. In NK 20% had *RUNX1mut*, 27% in -7, 33% in +8, 90% in +13, 58% in +21. These results for the first time show that *RUNX1mut* are frequent in *de novo* AML with certain cytogenetics. All cases were also analysed for FLT3-LM, *FLT3-TKD, MLL-PTD, NRAS, NPM1*, *JAK2* and *CEBPA*. NPM1mut and CEBPAmut were found to be mutually exclusive of *RUNX1mut*. The analysis for cooperating mutations (coop mut) was done for subgroups. NK subgroup: 29/79 carried coop mut, 18 x MLL-PTD, 15 x FLT3-LM and 3 x NRAS -7 subgroup: No coop mut was detected in the 10 RUNX1mut cases with -7. + 8 subgroup: In 2 RUNXmut cases 3/10 coop mut were detected. In contrast nearly all RUNXwt with +8 had an FLT3-LM, NPM1mut or NRASmut. +13 subgroup: only 3/20 had coop mut. Instead this specific cytogenetic subgroup shows a 4-fold-elevated FLT3 expression as an alternative cooperating event. Overall, MLL-PTD (62%) was found to be the most frequent coop mut for RUNX1 followed by FLT3-LM (52%) and NRAS (10%). *Summary and Conclusions.* 1) RUNX1mut are frequent in *de novo* AML with normal karyotype or single chromosomal imbalances. 2) Coop mut are frequent in some subgroups (NK and +21), rare (+13) or absent in others (-7, +8). 3) MLL-PTD plays a major role as coop mut in *RUNX1mut* AML.4) some subgroups may exhibit alternative cooperating mechanisms like overexpression of FLT3 in +13.

0020

HIGH INDO (INDOLEAMINE 2,3-DIOXYGENASE) MRNA LEVEL IN BLASTS OF ACUTE MYELOID LEUKEMIC PATIENTS PREDICTS POOR CLINICAL OUTCOME

M.E.D. Chamuleau,[1] A.A. van de Loosdrecht,[1] C.J. Hess,[1] A. Zevenbergen,[1] J.J.W.M. Janssen,[1] R. Delwel,[2] P.J.M. Valk,[2] B. Lowenberg,[1] G.J. Ossenkoppele[1]

[1]VU University Medical Center, AMSTERDAM; [2]Erasmus University Medical Center, ROTTERDAM, Netherlands

Background. Indoleamine 2,3-dioxygenase (IDO) degrades the amino acid tryptophan which is essential for T cells. Tryptophan depletion causes T cell cycle arrest and solid tumors that express high levels of IDO can create immune suppression. Recently, blasts of acute myeloid leukemic (AML) patients were shown to express the IDO protein. *Aims*. We now determined INDO (the encoding gene for IDO) mRNA expression in myeloid leukemic blasts of 285 AML patients by gene expression profiling. Results were validated by quantitative PCR (qPCR) analysis in blasts of an independent cohort of 71 patients. *Results*. Correlation of INDO expression to relevant known prognostic factors and survival identified high INDO expression as a strong negative independent predicting variable for overall and relapse free survival of AML patients. In a Cox regression model, INDO expression level >1.2 was the strongest predictor for survival (p<0.001 odds ratio (OR) 3.2), as compared to WBC (p=0.012, OR 1.006) and age (p=0.012, OR 1.024). As the groups of patients with good and poor cytogenetic risk profile were too small to use in a multiple regression model, the group with an intermediate cytogenetic risk profile was analyzed (n=40). Also in this group INDO expression was a strong predictor for survival (p=0.009, OR 1.146). Our results demonstrate that the INDO mRNA level is a strong independent predicting variable for the outcome of AML patients. As inhibition of IDO by orally available inhibitors like 1-methyl-tryptophan is effective in mice and synergistic with chemotherapy, we now provide more evidence for rapid exploration of the introduction of IDO inhibition in the treatment of AML patients.

0021

FARNESYLTRANSFERASE INHIBITOR SYNERGISTICALLY INCREASES RAPAMYCIN-INDUCED CELL GROWTH INHIBITION IN ACUTE MYELOID LEUKEMIA CELLS

J.-W. Cheong,[1] H.W. Lee,[1] J.I. Eom,[1] J.S. Kim,[1] Y.H. Min[2]

[1]Yonsei University College of Medicine, SEOUL, South-Korea; [2]BrainKorea21 Research Team of Nanobiomaterials for the Cell-Based Implants,YUMC, SEOUL, South-Korea

Proteins regulating the mammalian target of rapamycin (mTOR), as well as some of the targets of the mTOR kinase, are overexpressed or mutated in various human cancers. Accumulating evidences demonstrate that mTOR inhibitor rapamycin suppresses tumor cell growth *in vitro* and *in vivo*. However, it has been shown that acute myeloid leukemia (AML) cells showed substantial resistance to rapamycin-induced cell growth inhibition. Farnesyltransferase inhibitors suppress leukemia cell growth irrespective of Ras mutation. Here we evaluated the interaction between rapamycin and farnesyltransferase inhibitor FTI-277 in inhibiting cell growth in myeloid leukemia cells and analyzed its molecular mechanisms. Flow cytometric evaluation and Western blot analysis demonstrated that phospho(p)-mTOR and mTOR upstream Ras-like GTPase Rheb expression in primary leukemic blasts obtained from AML patients were significantly higher compared to normal bone marrow mononuclear cells (p<0.001 and p<0.001, respectively). We examined the inhibition extent of AML cell growth after treatment with rapamycin (100 μM) in the absence or presence of FTI-277 (10 μM). Cell proliferation was decreased to 87.3±5.3% and 78.7±4.4% of control group in the rapamycin- and FTI-277-treated cells, respectively. However, combined treatment of leukemia cell lines with rapamycin and FTI-277 (Rapa/FTI) led to a synergistic decrease to the level of 22.7±3.4% of control (p<0.001), which was associated with cell cycle arrest at the G2/M phase of cell (p<0.05). The synergistic inhibition of cell growth was associated with prominent downregulation of Rheb, p-mTOR, p-p70S6 kinase, and p-4E-BP1. Interestingly, levels of p-Akt/PKB and p-PTEN protein were markedly decreased in these leukemia cells after co-treatment with FTI-277 and rapamycin. These findings were also observed in the primary leukemia cells obtained from untreated patients with AML. Taken together, these findings indicate that coadministration of farnesyltransferase inhibitor and rapamycin might be a promising therapeutic strategy for AML.

0022

PIN1 IS UPREGULATED IN ACUTE MYELOID LEUKEMIA WITH C/EBPα MUTATIONS AND BLOCKS GRANULOCYTE DIFFERENTIATION VIA C-JUN

J.A. Pulikkan

LZG; AG BEHRE, HALLE, Germany

Transcription factor CCAAT enhancer binding protein alpha (C/EBPα) is one of the master regulators of granulopoiesis. Recent studies suggest that loss of function or expression of C/EBPalpha as one of the major causes for acute myeloid leukemia (AML). C/EBPα is mutated in around 9% of acute myeloid leukemia. The C/EBPα mutations reported are frame shift mutations at N-terminal domain and point mutations at basic region Leucine zipper. The mutant form of C/EBPα (C/EBPα-p30) shows dominant negative function over the wild type protein. Peptidyl-prolyl cis/trans isomerase, PIN1 binds to and isomerizes the peptidyl-prolyl bond in specific phosphorylated Ser/Thr-Pro motifs. *PIN1* has been shown to be overexpressed in many cancers and has significant role in tumorigenesis. We investigated the role of PIN1 in acute myeloid leukemia with C/EBPα mutation. We report C/EBPα-p30 could induce PIN1 transcription as analysed by quantitative Real-Time RT-PCR analysis. Affymetrix mRNA expression analysis show that PIN1 is upregulated in patients with different subtypes acute myeloid leukemia. Silencing of *PIN1* could overcome the dominant negative action of the C/EBPα-p30 over the C/EBPα-p42 transactivation capacity in promoter assay. Silencing PIN1 with inhibitor against PIN1 (PiB), leads to myeloid differentiation in AML blast cells with C/EBPα mutation and Kasumi-6 cells. Western blot analysis shows that PIN1 inhibition by PiB could upregulate wild type C/EBPα protein level. C/EBPα-p30 induces PIN1 promoter activity in association with E2F1 as assessed by luciferase promoter assay for the *PIN1* promoter. Interestingly, wild type C/EBPα interferes with the transactivation of the *PIN1* promoter and downregulates PIN1 mRNA expression. Next, we investigated the mechanism through which the C/EBPα-p30 blocks the wild type protein. c-Jun expression has been shown high in AML patients with C/EBPα muta-

tion and c-Jun could block C/EBPα function by protein-protein interaction. We show that *PIN1* regulate the protein stability of *c-Jun*. In summary, C/EBPα-p30 induces *PIN1* expression and increases the stability of c-Jun and c-Jun blocks the wild type C/EBPα. Our study demonstrates inhibition of PIN1 as a novel way of treating AML patients with C/EBPα mutation.

0023

GENOME-WIDE PROFILING OF DNA COPY NUMBER ABNORMALITIES IN ADULT ACUTE MYELOID LEUKEMIA (AML) USING HIGH DENSITY SINGLE NUCLEOTIDE POLYMORPHISM ARRAY

I. Iacobucci,[1] E. Ottaviani,[1] A. Astolfi,[2] F. Salmi,[1] N. Testoni,[1] S. Luatti,[1] C. Papayannidis,[1] P. Giannoulia,[1] F. De Rosa,[1] S. Paolini,[1] P.P. Piccaluga,[1] D. Cilloni,[3] A. Pession,[2] M. Baccarani,[1] G. Martinelli[1]

[1]Department of Hematology/Oncology Seràgnoli, BOLOGNA; [2]Pediatric Oncology and Hematology L. Seràgnoli, BOLOGNA; [3]Hematology, University of Turin at Orbassano, ORBASSANO, TURIN, Italy

Introduction. Acute myeloid leukemia (AML) is a heterogeneous disease with various chromosomal aberrations. The karyotype at diagnosis provides important prognostic information that influences therapy and outcome of this disease. However, using conventional chromosome banding techniques alone, karyotype abnormalities are detected in only half of all AML cases. Array-based analysis of single nucleotide polymorphism (SNP) takes the advantage to perform a high-throughput, genome wide screening of genomic imbalances. *Aims*. We sought to identify novel genomic regions of interest in normal karyotype AML and to identify novel candidate regions and disease-related genes in patients with complex karyotypes using genome-wide high resolution SNP-array. *Patients and Methods*. 28 AML patients were analyzed until now. Cases included FAB-M0, M1, M2, M4, M5, miscellaneous cytogenetic abnormalities and normal karyotype. 250 ng of genomic DNA were processed on 500K SNP array according to protocols provided by the manufacturer. Copy number state was calculated with respect to a set of 48 Hapmap normal individuals and a set of samples obtained from acute leukaemia cases in remission using Partek® Genomics Suite. *Results*. A wide spectrum of different genetic lesions (gains/losses) involving complete chromosome arms (del 16q, i(13q10), del 3p, del 7p, monosomy 9) or submicroscopic genomic intervals were identified in a substantial proportion of cases without differences in the frequency of losses or gains. Focal genetic alterations were detected at the breakpoints of previously cytogenetically identified chromosomal translocations, such as t(2;3)(p22-23)(q26-27) and t(1;11)(p32;q23). The most frequent genomic gains affected: 9p12 (*ZNF658B, FOXD4L2*), 22q11.1 (*PPYR1*), 5q31-q33 (*CDX1*) and 8p23.2 (*CSMD1*). The most frequent deletions were identified in *ACTBL1* (22q11), NF1 (17q11.2) and often in regions lacking annotated genes. Other recurring genetic lesions were uncommon. Some lesions affected regions with a single gene, such as: *ETAA1, FIGN, STK32B, PRAGMIN, PCM1, GLIS3, MRGPRX1, SESN3, BCL2L14*. Marked differences in the combination of copy number anomalies were identified across the different genetic subtypes of AML. Patients with normal karyotype showed no relevant genetic alterations. *Conclusion*. These data demonstrated that, in contrast to adult acute lymphoblastic leukaemia (ALL), AML is characterized by relatively few recurring copy number alterations, and that spectrum of genetic anomalies is significantly associated with AML disease subtype.

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0024

TREATMENT WITH PAN DEACETYLASE (DAC) INHIBITOR PANOBINOSTAT (LBH589) DEPLETES EZH2 AND DNMT1, DEREPRESSES JUNB AND SENSITIZES ACUTE MYELOID LEUKEMIA CELLS TO DECITABINE

W. Fiskus,[1] Y. Wang,[1] R. Rao,[1] P. Atadja,[2] K.N. Bhalla[1]

[1]Medical College of Georgia Cancer Center, AUGUSTA; [2]Novartis Institutes for Biomedical Research Inc., CAMBRIDGE, MA, USA

Background. The PRC (polycomb repressive complex) 2 is a multi-protein complex, which includes *EZH2*, *SUZ12* and *EED* proteins. The PRC2 complex possesses histone methyltransferase (HMTase) activity mediated by the SET domain of EZH2, which methylates histone H3 on lysine (K)-27. We recently reported that treatment with the pan-DAC inhibitor panobinostat (LBH589, Novartis Pharmaceutical Corp) acetylates and inhibits the ATP binding and chaperone function of heat shock protein (HSP) 90, as well as depletes the levels of EZH2, Suz12 and EED in cultured and primary AML cells (Mol Cancer Ther. 2006;5:3096). Recently, within the PRC2 complex, EZH2 was shown to interact with and modulate the DNA methyltransferases DNMT1 (the target of the MDS drug 5-azacytidine), DNMT3a and DNMT3b, which affects their binding to the EZH2-targeted gene promoters. *Aims*. In the present studies we determined the effects of panobinostat on the interaction between the PRC2 proteins EZH2 and EED, and DNMT1, in the cultured and primary acute leukemia cells. *Methods*. In CML blast crisis (CML-BC) K562 and LAMA-84 cells, treatment with ≥50 nM panobinostat disrupted the interaction of DNMT1 with Ezh2, as well as abrogated the chaperone association of DNMT1 and EZH2 with HSP90. *Results*. This resulted in proteasomal degradation of DNMT1 levels, which were restored by co-treatment with the proteasome inhibitor bortezomib. This was also seen following treatment with the HSP90 inhibitor 17-DMAG (250 nM). Additionally, panobinostat depleted the mRNA expression of DNMT1 in K562 cells. Panobinostat-mediated down regulation of DNMT1 levels was also associated with increase in the levels of JunB mRNA, which is known to be repressed by DNA hyper-methylation in CML-BC cells. Depletion of DNMT1 by shRNA or the DNA methyltransferase inhibitor decitabine treatment also increased JunB mRNA levels. Treatment with panobinostat and/or the decitabine induced JunB mRNA expression in CML cells. While treatment with panobinostat did not, decitabine treatment decreased the JunB, ERα and E-Cadherin promoter DNA methylation. However, utilizing chromatin immunoprecipitation (ChIP) analysis, panobinostat was shown to decrease the binding of DNMT1 to 183 bp region in the promoter upstream to the JunB ORF. Panobinostat also decreased Suv39H1 binding, as well as decreased 3Me K9 H3 and histone H3 acetylation levels in this region. Co-treatment with panobinostat and decitabine, *vs* treatment with either agent alone, caused greater depletion of *DNMT1* and induced more JunB mRNA. Co-treatment with panobinostat and decitabine also caused more loss of clonogenic survival of K562 cells, as well as induced more loss of viability of primary AML cells. *Summary*. In conclusion, treatment with pan-DAC inhibitor panobinostat not only depletes EZH2, but also attenuates DNMT1 levels, thereby targeting two epigenetic regulatory mechanisms. Panobinostat also derepresses JunB and sensitizes acute leukemia cells to decitabine.

0025

DETECTION OF PROTEINASE 3 (PR3) GENE OVEREXPRESSION AND NUCLEAR DELOCALIZATION IN CBF ACUTE MYELOID LEUKEMIAS

D. Cilloni,[1] S. Carturan,[1] I. Defilippi,[1] R. Catalano,[1] E. Messa,[1] A. Rotolo,[1] P. Nicoli,[1] I. Iacobucci,[2] G. Martinelli,[2] E. Bracco,[1] A. Levis,[3] G. Saglio[1]

[1]University of Turin, TURIN; [2]University of Bologna, BOLOGNA; [3]Hematology Institute, ALESSANDRIA, Italy

Background. Proteinase 3 (PR3) gene codes for a serine protease with a broad spectrum of proteolytic activity. PR3 is involved in the control of proliferation of myeloid leukemia cells and it confers factor-independent growth to hematopoietic cells when abnormally expressed. The aim was to investigate the role of PR3 gene in leukemic haematopoiesis. *Methods.* We analyzed the expression levels of PR3 by RQ-PCR in 113 BM samples collected at diagnosis from AML patients. The FAB distribution was as follows: M0=5, M1=12, M2=38, M3=12, M4=37, M5=5, M6=4. 19 patients were characterized by t(8;21) and 16 by inv(16). Moreover we analyzed the expression levels in 57 BM and 42 PB samples from 88 MDS patients (44 RA, 32 RAEB and 12 secondary-AML) and in 15 BM and 40 PB samples from healthy volunteers. PR3 protein amount was analyzed by western blot (WB) and its localization by immunofluorescence assay. The transcriptional activity of CEBP/α, the nuclear factor that negatively regulates PR3 transcription, was investigated in parallel by RQ-PCR, WB and EMSA assay. Gain and loss of function experiments were performed by transfecting COS and 293T cell lines with a plasmid containing the full length PR3 sequence and HL60, Me-1, and Kasumi cell lines with specific shRNA. *Results*. We found that PR3 gene is significantly overexpressed in AML samples. The median value of 2-ΔΔCt is 740, (range 15-5043). Interestingly, patients affected by Core Binding Factor leukemias showed significantly higher PR3 values and lower CEPB/α levels compared to patients with normal karyotypes (NK) (p<0,0002 for t(8;21), p<0,001 for inv16). EMSA assay demonstrated the absence of CEBP/α DNA binding activity in CBF AML cells but not in NK AML. In addition, PR3 overexpression was detected in 60 % of the samples from RA patients (mean value: 10, range 3-268), and in all the cases of RAEB (mean value 201: range:128-803) and secondary AML (mean value 589, range 207-7131). WB demonstrated the correlation between the mRNA and protein amount. Interestingly, immunofluores-

cence demonstrated the de-localization of the protein within the nucleous in CBF AML but it is completely cytoplasmatic in leukemic cells with normal karyotype and in MDS. Transfection experiments with PR3 plasmid demonstrate that overexpression of PR3 results into a significant increasing of the proliferation rate and reduced apoptosis. By contrast transfection with shRNA triggers apoptosis and cell growth inhibition. Conclusion: PR3 gene expression and protein are significantly increased in AML and MDS, particularly in CBF leukemias in which the protein is completed delocalized within the nucleous. PR3 overepression is associated with CEBP/α downmodulation. Ectopic expression of PR3 induces increased proliferation and apoptosis arrest. The mechanism underlying the nuclear localization of PR3 in CBF leukemias and its pathogenetic role remains unexplored.

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CXCR-4 POSITIVE ACUTE MYELOID LEUKEMIAS (AGE < 60 YEARS): IMMUNOPHENOTYPIC, CYTOMORPHOLOGIC, CYTOGENETIC, AND MOLECULAR CHARACTERISTICS

U. Oelschlaegel, B. Mohr, U. Schaekel, F. Kroschinsky, M. Schaich, G. Ehninger, C. Thiede

University Hospital Dresden, DRESDEN, Germany

Background. The interaction of the CXCR-4 receptor with its ligand SDF-1 plays a key role in the process of homing and mobilization of stem cells and might be involved in the trafficking of leukemic blasts, too. *Aims*. The aim of our study was to correlate the CXCR-4 expression with the main diagnostic features of AML. *Methods*. 682 AML patients (other than AML M3) under the age of 60 were included in our investigation. CXCR-4 expression was measured in whole bone marrow or peripheral blood performing multi-color flow cytometry (CD45 FITC/CXCR-4 PE/CD34 PerCP5.5). The cut-off was set at 20% CXCR-4 positive cells. Cytomorphologic, cytogenetic, and molecular analyses have been done in parallel. *Results*. Ninety percent of all patients express CXCR-4 (616/682 patients). These leukemias were significantly more assigned to a monocytic phenotype (33% *vs* 19%, p=0.0228). The percentage of CD34$^+$, HLA DR$^+$, aberrant CD19$^+$, or CD7$^+$ patients was clearly lower in the CXCR-4 positive *vs* negative group (51% *vs* 83%, p<0.0001; 91% *vs* 100%, p=0.0330; 4% *vs* 11%, p=0.0236; 9% *vs* 17%, p=0.0802). Furthermore, in CXCR-4 positive patients low risk cytogenetics was present less frequently (10% *vs* 27%, p=0.0002), whereas patients with high risk cytogenetics were equally distributed in both groups (22% *vs* 27%). There were significantly more NPM1 mutated or FLT3-ITD mutated cases in the CXCR-4 positive group (38% *vs* 8%, p<0.0001; 24% *vs* 6%, p=0.0005). In addition, the median expression level of CXCR-4 was higher on blasts of FLT3-ITD positive *vs* negative patients (81% *vs* 71%, p=0.0115). Interestingly, patients with NPM1 mutations expressed signifcantly more CXCR-4 compared to non mutated cases (87.9% *vs* 62.7%; p<0.0001), independent from the FLT3 status. *Conclusions*. CXCR-4 positive *vs* negative acute myeloid leukemias displayed distinct immunophenotypic and molecular characteristics. Especially the NPM1 mutation status seems to be correlated with CXCR-4 expression.

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FLT3 INTERNAL TANDEM DUPLICATION INVOLVING ITS UBIQUITIN DEPENDENT ENDOCYTOSIS MOTIF SUSPEND MODULATION BY HDM2 AND ARE ASSOCIATED WITH INFERIOR SURVIVAL IN AML

L. Wergeland,[1] E. Oveland,[2] S. Gry,[3] S.L. Bedringaas,[4] R. Hovland,[5] Bruserud,[4] B.T. Gjertsen[6]

[1]University of Bergen, BERGEN; [2]Department of Biomedicine, University of Bergen, BERGEN; [3]Bergen University College, BERGEN; [4]Institute of Medicine, University of Bergen, BERGEN; [5]Center for Medical Genetics, Haukeland University Hospital, BERGEN; [6]Department of Medicine, Haukeland University Hospital, BERGEN, Norway

Background. Internal tandem duplications in the juxtamembrane region of the receptor tyrosine kinase Flt3 (Flt3-ITD) and elevated expression of the oncogenic E3 ubiquitin ligase Hdm2 are frequent features of acute myeloid leukemia (AML). Hdm2 is a well known suppressor of p53, but is also associated with endocytosis of cell surface receptors. Recently we have shown that Hdm2 and Flt3 are reciprocally modulated upon DNA-damage therapy of AML cells *in vitro* and *in vivo*. *Aims*. To elucidate the mechanism behind the Flt3-Hdm2 modulation and the impact of various Flt3-ITDs. Furthermore, determine if the Flt3-Hdm2 modulation may have a clinical impact in AML. *Methods*. Primary AML cells and cell lines (NB4 and MV4-11) with wild type Flt3 (Flt3-wt) or mutated Flt3 (Flt3-ITD) were used with Flt3 ligand (FL), small molecular inhibitors and small interfering RNA (siRNA) to elucidate the relation between Flt3 and Hdm2 on protein level, mRNA expression and modulation of apoptosis. *Results*. Kinase inhibition of Flt3 increase Hdm2 in Flt3-wt cells, but not in Flt3-ITD cells. Modulation of FLt3 and Hdm2 by siRNA indicates that their protein levels are mutually dependent. Cell lines with Flt3-ITD have a more rapid cycling of receptors on the cell surface than cells with Flt3-wt. A sequence alignment of the juxtamembrane region of Flt3 and other related tyrosine kinase receptors lead to the identification of a putative Ubiquitin dependent endocytosis motif (UbE) in the juxtamembrane region of Flt3. Based on transfection studies of mutant Flt3 and Hdm2, internalisation of Flt3 is dependent on both Hdm2 E3 ligase activity and Flt3 UbE domain. A subset of AML patients with Flt3-ITD has a duplication of this domain (Flt3-2xUbE). These patients have a reduced level of surface Flt3 which significantly correlates to an elevated level of Hdm2, and survival data suggest a negative prognostic impact in the presence of Flt3xUbE. *Conclusions*. FLT3 receptor turnover involves Hdm2, and Flt3-ITD results in dysregulated receptor turnover attenuated for Hdm2-induced Flt3 down-regulation. A novel type of Flt3 mutations (Flt3-2xUbE) is associated with the Hdm2-Flt3-signalling pathway and may have prognostic impact within the group of Flt3-ITD positive AML patients.

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MICRORNA PROFILING OF EVI1 DEREGULATED MYELOID LEUKEMIA

A. De Weer,[1] B. Poppe,[1] P. Mestdagh,[1] P. Van Vlierberghe,[1] N. Van Roy,[1] A. De Paepe,[1] B. Verhasselt,[2] J. Vandesompele,[2] F. Speleman[1]

[1]University Hospital, Centre for Medical Genetics, GHENT; [2]University Hospital, Dept of Chemistry, Microbiology and Immunology, GHENT, Belgium

Background. Chromosomal rearrangements involving the *EVI1* gene are a recurrent finding in malignant myeloid disorders. These translocations or inversions contribute to ectopic expression of the *EVI1* gene. *EVI1* transcriptional activation has also been reported in approximately 5 % of acute myeloid leukemia (AML) patients without chromosomal defects affecting the *EVI1* locus. Survival of patients with *EVI1* overexpressing leukemias is poor and new insights into the molecular pathology are needed as a basis for development of targeted therapies. Recently, microRNA deregulation was identified as a major contributor to cancer initiation and progression. Moreover, microRNA genes were shown to be directly regulated by activated proto-oncogenes. *Aims.* The aim of this study was to investigate which microRNA genes are implicated in the transcriptional pathways governed by the *EVI1* oncogene. *Methods*. SiRNA mediated *EVI1* knockdown was performed in *EVI1* overexpressing AML cell lines Kasumi-3 and UCSD-AML1 and validated by qRT-PCR and Western blotting. A total of 384 microRNAs were profiled through automated qRT-PCR using high-throughput quantitative stem-loop RT-PCR (Applied Biosystems). Integrated statistical analysis (SAM-analysis, delta Ct and confidence interval analysis) was performed to identify up- and downregulated microRNAs. *Results*. After siRNA treatment, a reduction of 90% on mRNA level and a reduction of 70% on protein level were observed for both EVI1 overexpressing cell lines Kasumi-3 and UCSD-AML1. Through integrated analysis several statistically significant up- (miR-184 and miR-182#) and downregulated (miR-155, miR-222 and miR-210) microRNAs were identified. *Conclusions*. We identified several *EVI1* regulated microRNAs after siRNA treatment of EVI1 overexpressing cell lines which are currently under further investigation in a large panel of AML patients. Furthermore, microRNA profiling of 3q26 rearranged *EVI1* overexpressing patient samples and non-rearranged *EVI1* overexpressing bone marrow samples is underway. Further studies will also include electroporation of antagomirs or microRNA mimics for *EVI1* regulated microRNAs to asses their contribution to the leukemic phenotype. The discovery of functionally relevant microRNAs may provide new targets for therapeutic intervention in AML.

0029

CLASS II-ASSOCIATED INVARIANT CHAIN PEPTIDE (CLIP) EXPRESSION ON AML BLASTS NEGATIVELY AFFECTS ANTI-LEUKEMIC IMMUNITY BY INTERFERING WITH CD4+ T CELL RECOGNITION

M.M. van Luijn,[1] M.E. Chamuleau,[1] T.M. Westers,[1] J.A. Thompson,[2] S. Ostrand-Rosenberg,[2] A. Zevenbergen,[1] G.J. Ossenkoppele,[1] S.M. Van Ham,[3] A.A. Van de Loosdrecht[1]

[1]VU Medical Center, AMSTERDAM, Netherlands; [2]University of Maryland, BALTIMORE, USA; [3]Sanquin Research and Landsteiner Laboratory, AMSTERDAM, Netherlands

Background. The use of immunotherapeutic strategies during minimal residual disease (MRD) in AML patients emerges as an attractive modality to induce effective immune responses against residual leukemic blasts. As CD4+ T effector cells are indispensable in anti-leukemic immunity, the efficacy of HLA class II-restricted antigen presentation on AML blasts could be crucial. Previously, we showed that expression of the class II-associated invariant chain peptide (CLIP), a small remnant of the Invariant Chain (Ii) involved in the process of HLA class II antigen presentation, on AML blasts predicted a shortened disease-free survival (*Chamuleau et al; Canc Res 2004*). *Aims*. To investigate the contribution of CLIP/HLA-DR expression on AML blasts in anti-leukemic immune escape. *Methods*. Variable CLIP/HLA-DR amounts were created on various AML cell lines as a model to analyse the effect of CLIP on CD4+ T cell recognition. Selected cell lines were retrovirally transduced with specific Ii siRNAs. Differences in CLIP/HLA-DR were determined flow cytometrically by comparing CLIP to HLA-DR MFI ratios. Proliferation of healthy donor CD4+ T cells was measured by [3H]-thymidine incorporation after 5 days of culture. Furthermore, leukemic blasts from an AML patient were sorted for CLIP+ and CLIP- fractions by flow cytometry and co-cultured with PBMCs from the same patient. Cell growth and the TCR-Vβ repertoire of cultured CD4+ T cells were analysed for 28 days. *Results*. Kasumi-1 and THP-1 AML cell lines were selected for our model, as both expressed HLA-DR and CLIP. Both cell lines were silenced for Ii to down-modulate CLIP levels on the cell surface. Indeed, Ii-silenced cells not only showed a decrease of Ii, but also a marked down-modulation of CLIP amount per HLA-DR molecule (fold decline in CLIP/DR ratio of 1.4 for Kasumi-1 and 2.0 for THP-1). Non-modulated (DR+CLIP+) and modulated (DR+CLIP-) cells acted as stimulators for healthy donor CD4+ T cells in functionality assays using different stimulator to responder (S/R) ratios. Modulated DR+CLIP- Kasumi-1 and THP-1 cells induced strong S/R-dependent increases in CD4+ T cell proliferation compared to non-modulated DR+CLIP+ controls, as 2.6- and 3.3-fold increases were respectively found at the highest S/R ratio (means of two experiments performed in triplicate). Blocking of HLA-DR on stimulator cells using the L243 antibody abrogated CD4+ T cell proliferation, thereby confirming HLA-DR restriction of the proliferative response. These results were further supported by co-culture experiments with blasts and PBMCs from an AML patient. In the presence of CLIP- blasts, PBMCs remained viable during 28 days, while no PBMCs survived in co-culture with CLIP+ blasts, indicating an adverse role of CLIP in CD4+ T cell proliferation. Interestingly, TCR-Vβ analysis of CD4+ T cells in co-culture with CLIP- blasts showed a 10-fold increase of a Vβ8 CD4+ T cell clone (~70% of total CD4+ T cells) at day 28, as opposed to negative controls. *Summary and Conclusions*. These data support our hypothesis that CLIP expression on AML blasts abolishes the anti-leukemic CD4+ T cell response, both in an allogeneic and autologous setting. These findings may have important consequences for the design of cellular immunotherapy in AML.

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THE ROLE OF C-FLIP IN ACUTE MYELOID LEUKEMIA

D.P. McLornan,[1] M.F. McMullin,[2] K.I. Mills,[2] P.G. Johnston,[3] D.B. Longley[3]

[1]Queen'S University Belfast, BELFAST; [2]Department of Haematology, CCRCB, Queen's University, BELFAST; [3]Department of Oncology, CCRCB, Queen's University, BELFAST, Northern Ireland

Background. Acute myeloid leukemia (AML) is a heterogeneous disease and overall prognosis is poor. Defects in apoptosis certainly contribute to this aggressive clinical phenotype. The anti-apoptotic protein c-FLIP (cellular FLICE-Inhibitory Protein) is a key inhibitor of processing and activation of Caspase 8 at the Death Inducing Signalling Complexes (DISCs) formed by both the FAS and TRAIL (TNF-related apoptosis-inducing ligand) receptors. Differential splicing gives rise to both a long (c-FLIPL) and short (c-FLIPS) isoform. *Aims*. i) Investigate the role of c-FLIP in mediating drug resistance in AML. ii) Investigate if c-FLIP expression has prognostic significance in AML. *Methods*. Knock down of c-FLIP gene expression was carried out using a siRNA (FT siRNA) targeted against both isoforms in the U937 and K562 cell lines. Transient transfections were performed via standard nucleofection protocols (AMAXA™). The effects of c-FLIP gene silencing were studied alone and then in combination with standard induction agents and recombinant human TRAIL. Cell viability was assessed via Trypan Blue Exclusion Assay and apoptosis assessed via activation of Caspase 8, Caspase 3 and PARP cleavage in addition to the percentage of cells in the sub G0/G1 region as determined via standard Propidium Iodide Flow Cytometry protocols. The expression of FLIP and other antiapoptotic proteins have been assessed at both a protein and mRNA level from bone marrow aspirate samples (n=30) obtained following full ethical consent. The Microarray data was obtained from standard Affymetrix™ chips. *Results*. FT siRNA alone leads to apoptosis in the U937 cell line, but not the K562 cell line, as detected via immunoblotting for Caspase3 processing and PARP cleavage. Silencing c-FLIP also sensitises the U937 cell line, but not the K562 cell line, to the standard induction agents of Cytarabine and Etoposide but not to the topoisomerase II inhibitor Daunorubicin. In addition, FT siRNA sensitises both the U937 and K562 cell lines to recombinant human TRAIL with a dramatic increase in the percentage of apoptotic cells detected via Flow Cytometry and enhanced PARP and Caspase 8 activation. Microarray profiling of a large number of AML samples (n=318) demonstrated heterogeneity of c-FLIP m RNA expression and this appears to be independent of World Health Organisation (WHO) subclassification. Looking at each of the cytogenetic categories (Poor /Standard and Good risk) individually, when RNA expression is divided into < or > mean, then those with > mean FLIP expression persistently have poorer overall survival (Log Rank χ^2 58.284213, p value = 2.74818e-011). In the clinical samples there is wide intra-individual variation in c-FLIP, XIAP and Bcl-2 family member expression at both the protein and gene level and correlations with conventional risk factors and the endpoints of overall survival and recurrence free survival will be made. Further work will be performed to establish if there is correlation of gene expression with protein expression of c-FLIP isoforms. *Conclusions.* These results indicate that c-FLIP may be a key regulator of AML cell survival with potential prognostic significance. Targeting c- FLIP may be a therapeutic strategy for AML.

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IMMUNOGLOBULIN AND T CELL RECEPTOR REARRANGEMENTS IDENTIFICATION REVEALS POTENTIALLY NEW SUBGROUP WITHIN PEDIATRIC ACUTE BIPHENOTYPIC LEUKEMIAS

J. Volejnikova,[1] E. Mejstrikova,[2] E. Fronkova,[2] T. Valova,[2] L. Reznickova,[2] J. Zuna,[2] J. Stary,[2] O. Hrusak,[2] J. Trka[2]

[1]2nd Medical School, Charles University Prague, PRAGUE; [2]2nd Medical School, Charles University, PRAGUE, Czech Republic

Background. Acute biphenotypic leukemia (BAL) is an uncommon type of leukemia in which blasts markedly express antigens of more than one lineage. Whereas coexpression of antigens from different lineage is frequently observed in childhood leukemias, international EGIL classification based on immunophenotype was developed to distinguish cases with significant level of aberrant expression. EGIL provides a precise definition of BAL, although several weak points, eg. no regards to high heterogeneity of BAL, are discussed. Original lineages of cells undergoing malignant transformation and the mechanism of aberrant expression are different among BAL subgroups and are not yet entirely known. Immunoglobulin and T-cell receptor (Ig/TCR) gene rearrangements, arising during hematopoiesis, can indicate commitment to the lymphoid lineage and reflect the maturation stage of leukemic blasts. *Aims.* Our aim was to determine the lineage of BAL origin using Ig/TCR rearrangement analysis in a cohort of BAL cases diagnosed during 10-year period of national-wide childhood acute leukemia (AL) immunophenotyping. *Methods*. In all cases, complete morphological and immunophenotypic examination together with molecular genetic analysis of most frequent childhood AL genetic aberrations (TEL/AML1, BCR/ABL, MLL rearrangements) was performed. We analyzed complete and incomplete immunoglobulin heavy chain (IgH) rearrangements, light chain (IgL) rearrangements to the κ deleting element, complete rearrangements of TCRγ, TCRδ, TCRβ and incomplete rearrangements of TCRδ and TCRβ. *Results*. Between 09/1996 and 08/2006, 38 of 730 childhood AL patients fulfilled the EGIL criteria for BAL: 33 at diagnosis (29 ALL/My+, 4 AML/Ly+), 8 at relapse (6 ALL/My+, 2 AML/Ly+), 4 at both diagnosis and relapse. In cases with B-cell primary lineage, incidence and maturity of Ig/TCR rearrangements generally reflected immunophenotypic assign-

ment of leukemic blasts: In pro-B/My+ BAL, low total number of rearrangements was detected including mostly incomplete or complete IgH rearrangements, with no IgL rearrangements. On the contrary, pre-B/My+ BAL had 1-2 complete IgH rearrangements on one or both alleles and 1-2 IgL rearrangements, suggesting that leukemogenic hit occurred during the light chain recombination. Usage profiles of V and J gene segments and length of hypervariable CDR3 regions did not differ significantly from published non-BAL data, except for more frequent D3.9 segment use in BAL (p=0.004). In TEL/AML1+ leukemias, upregulation of recombinase activation genes caused by TEL/AML1 fusion protein was described. Similarly to non-BAL TEL/AML1+ leukemias, we found multiple Ig/TCR rearrangements in BAL TEL/AML1+ cases. Surprisingly, 4 of 6 pro/pre-T-ALLs with coexpression of myeloid markers had germline configuration of Ig/TCR genes; the same was observed in AMLs with coexpression of T-lymphoid markers (4 of 4 germline). *Summary*. Pattern of Ig/TCR rearrangements in BAL with primary B-cell lineage reflected lineage and developmental stage determined by flow cytometry. In TEL/AML1+ BAL cases, multiple Ig/TCR rearrangements were observed similarly to non-BAL TEL/AML1+ leukemias. We describe a specific subset of T/myeloid leukemias with germline configuration of Ig/TCR genes, resembling AML cases with T-lymphoid marker coexpression. These cases represent potentially a unique newly identified subgroup of childhood AL.

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GENE TARGETS OF 11Q23 AMPLIFICATION AND CONCOMITANT 5Q DELETION IN AML/MDS CELLS

B. Schneider,[1] R. Geffers,[2] H. Quentmeier,[1] M. Kaufmann,[1] S. Nagel,[1] H.G. Drexler,[1] R.A.F. MacLeod[1]

[1]*German Collection of Microorganisms and Cell Cultures, BRAUNSCHWEIG;* [2]*Helmholtz Centre for Infection Research, BRAUNSCHWEIG, Germany*

Some patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) carry genomic amplification of the 11q23-24 region (amp11q), and which is considered a bad prognosis marker. While MLL remains a favored candidate, several reports highlight alternative candidates. Remarkably, amp-11q is invariably accompanied by deletions affecting the long arm of chromosome 5 (del5q) possibly marking disease progression. We addressed two main issues, namely concerning the identity of amp11q gene target(s) in AML/MDS, and whether the obligate partner 5q- gene target(s) match those unaccompanied by amp11q. Studies were performed on amp11q/del5q cell lines established from AML/MDS patients, using control cell lines with del5q alone. Cell lines were cytogenetically analysed by classical cytogenetics and FISH followed by array comparative genomic hybridization (aCGH) using Affymetrix 100K arrays to yield respective commonly deleted/amplified regions (CDR/CAR) on 5q/11q. Global gene expression was studied by transcriptional profiling using Affymetrix U133+2.0 arrays, reverse transcription (RT)-PCR, and realtime-PCR. Gene expression was also measured after treatment with lenalidomide which is believed to reactivate del5q target genes. aCGH analysis showed CAR at 11q22.3-25 at 106-135 Mbp, bimodally peaking at 118 Mbp and 129 Mbp in all del5q/amp11q cell lines. Three 5q CDR were present: at 104-134, 136-140, 162-3 Mbp. Expression analysis at 11q revealed that several peak-CAR genes were differentially overexpressed, showing upregulation, either *de novo* - *CHEK1, PAFAH1B2, KIAA0999, ATP5L*; or intensified - *MLL, NFRKB, H17/FOXRED1*, when compared to del5q alone. Known candidate del5q genes RPS14 and SPARC lay within a minor CDR (140-155 Mbp) present in 2/4 cell lines only. Commonly downregulated genes inside the 5q CDR in del5q/amp11q cells included CXXC5, HINT1, PCYOX1L, BRD8, PAIP; while KIAA04333, PJA2, TNFAIP8, RAPGEF6, HSPA4, SKP1A, were downregulated throughout. Proven MLL downstream targets, MEIS1, HOXA9 and HOXA7 were inconspicuously expressed in amp11q cells, however, where the top10 upregulated genes comprised *C6orf106, DLG1, PIP5KIA, ARFGEF1, EPS15L1, NMD3, NNT, CDK8, KIAA1008, IQCB1*; while *ZA20D3, C21orf56, ODAG, KCNN4, ADAR, P2RX1, GLG1, GMFG, ADD3, FLJ12770* were downregulated. Lenalidomide treatment reactivated the putative 5q target gene SPARC more intensively and progressively in AML cells with an MDS history than in *de novo* AML. Our data downplay the significance of MLL as an amp11q target, unless after upregulation its signalling properties in AML qualitatively differ from translocation cases. We have shortlisted alternative target genes upregulated in amp11q, and their potential downstream partners. Although a handful of 5q genes were differentially regulated in amp11q cells, patterns of 5q downregulation were broadly similar implying a common disease entity. Further work is in progress to determine which additional del5q genes are lenalidomide responsive to serve as potential functional targets. These data validate the first cell line model for amp11q AML with a history of MDS. Signalling pathway analysis of conspicuous target genes will be performed using both NCBI-DAVID and in-house software packages.

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PANOBINOSTAT (LBH589) + DOXORUBICIN: A PRECLINICALLY SYNERGISTIC COMBINATION AGAINST ACUTE MYELOID LEUKEMIA (AML) CELL LINES REPRESENTING A CLINICALLY PROMISING COMBINATION FOR PATIENTS WITH AML

P. Maiso,[1] E.M. Ocio,[1] E. Colado,[2] M. Garayoa,[1] P. Atadja,[3] A. Pandiella,[1] J.F. San Miguel[1]

[1]*Universidad de Salamanca, SALAMANCA, Spain;* [2]*Hospital Universitario de Salamanca, SALAMANCA, Spain;* [3]*Novartis Institutes for Biomedical Research, CAMBRIDGE, MA, USA*

Introduction. The combination of cytarabine with an anthracyclin has been the gold standard for the induction treatment of acute myeloid leukaemia (AML) patients for the last three decades. Nevertheless, 20-50% of patients fail to adequately respond to this scheme and among those that achieve complete response (CR) there is a relapse rate of around 50%. Therefore, novel strategies are needed in order to improve the outcome of these patients. *Methods*. The efficacy of the combination of panobinostat (LBH589; a potent pan-DAC inhibitor) with each one of four other agents (cytarabine, doxorubicin, fludarabine and bortezomib) was analyzed both *in vitro* (in four AML cell lines: HEL, HL-60, KG-1 and MV4.11, by means of MTT) and *ex vivo* (in freshly isolated cells from 6 AML patients by multiparametric flow cytometry). In addition the toxicity in normal hematopoietic cells was analyzed. Mechanistic studies (apoptosis, cell cycle profile, DioC6 staining, Western Blot and GEP analysis by microarrays) were performed in the HEL cell line. *Results*. Panobinostat potentiated the effects of cytarabine and fludarabine, and slightly improved that of bortezomib. The combination of panobinostat with doxorubicin (P+D) showed the highest synergistic effect. Therefore, remaining experiments focused on this combination. Forty-eight hours of culture with P+D showed a very important synergistic effect in the four AML cell lines (HEL, HL-60, KG-1 and MV4.11). This efficacy was confirmed in *ex vivo* experiments in blast cells isolated from six AML patients. In all six cases a potentiation was observed with P+D as compared to the efficacy of the single agents. In four cases this potentiation was in the synergistic range. Interestingly no potentiation was observed in terms of toxicity on non leukemic residual hematopoietic cells from the same patients' samples. Mechanistic experiments showed that P+D activated apoptosis (as assessed by Annexin V staining and PARP- and caspases-cleavage by Western Blot) in a time- and dose-dependent manner at concentrations that did not induce any cytotoxic effect when panobinostat and doxorubicin were used as single agents. P+D activated the intrinsic pathway of apoptosis with loss of mitochondrial membrane potential (by DioC6) and subsequent release of Cytochrome C to the cytoplasm. A decrease in MCL-1 and BCL-X cleavage was also observed with the combination while not with the single agents. P+D also induced cell cycle arrest. To evaluate the effect of these drugs and to determine overlaps between each effect, we compared the gene expression profile (GEP) of HEL cells exposed to each treatment. We identified a total of 841 probe sets significantly deregulated after 12 hours of treatment with P+D, 285 with the same dose of panobinostat for 24 hours and 43 for doxorubicin for 24 hours. Interestingly, 588 probe sets were exclusively deregulated after P+D treatment, indicating that panobinostat and doxorubicin affect different groups of genes and pathways. The classification of these genes revealed a clear effect on DNA damage and c-jun pathways. *Conclusion*. P+D show a marked synergistic activity against AML cells, with unique mechanism of action, and represent an attractive combination for clinical investigation.

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ABT-737 TARGETS THE COOPERATION OF BCL2 AND ONCOGENIC NRAS IN AN *IN VIVO* MULTI-STEP MODEL OF ACUTE MYELOID LEUKAEMIA

S. Beurlet,[1] N. Omidvar,[2] C. Le Pogam,[3] F. Herventin,[4] L. Sarda-Mantel,[4] P. Merlet,[4] A. Janin,[5] C. Leboeuf,[5] M.E. Noguera,[6] A. Soulie,[3] N. Setterblad,[7] M. Pla,[3] M. Konoplova,[8] M. Andreef,[8] C. Chomienne,[3] R.A. Padua[3]

[1]Inserm, PARIS, France; [2]University of Cardiff, School of Biosciences, CARDIFF, UK; [3]Inserm U718, PARIS, France; [4]Hôpital Bichat, PARIS, France; [5]Inserm U728 Hôpital Saint Louis, PARIS, France; [6]Hôpital Saint Louis, PARIS, France; [7]Institut universitaire hématologie Hôpital Saint Louis, PARIS, France; [8]MD Anderson Cancer Center, TEXAS, USA

Myelodysplastic syndromes (MDS) are clonal stem cell hematological disorders, which evolve to acute myeloid leukemia (AML) and thus model multi-step leukemogenesis. The prognosis of this disease is poor and until recently no therapeutic strategies were available. Activating RAS mutations and over-expression of BCL-2 are prognostic features of MDS/AML transformation. Using NRASD12 and BCL-2, we created two distinct models of MDS and AML; expression of hBCL-2 in a primitiv compartment by MMTV-TLR results in a disease resembling human MDS with bone marrow blasts of 15% and increased apoptosis assayed by TUNEL on liver sections, whilst the myeloid MRP8 promoter induces a disease with characteristics of human AML with marrow blasts of up to 90% and liver apoptosis patterns similar to wild type. Expanded leukemic stem cell [Lin-/Sca-1+/c-Kit+(LSK)] populations, increased myeloid colony growth and increased hBCL-2 expression in the RAS-GTP complex are observed in both MDS/AML diseases, which are transplantable. In the MDS mice the majority of the RAS and BCL-2 doubly stain localized to the plasma membrane, where active pro-apoptotic RAS is normally located, whereas in the AML disease RAS and BCL-2 co-localize in the mitochondria, where BCL-2 is normally found. This is concordant with the signaling profile where ERK is increased with decreased AKT in the RAS-mediated MDS mice and where both ERK and AKT are increased in the BCL-2-mediated AML disease. Thus we suggest that this complex can be a specific target for therapy. When hBCL-2 is switched off with doxycycline in the MDS mice, only partial reversal of the phenotype was observed as RAS recruits endogenous mouse (m)BCL-2 to remain active; thus demonstrating the role of the complex in the disease (Omidvar *et al.*, Cancer Research 67:11657, 2007). In order to target both human and mouse BCL2 the efficacy of *in vivo* treatment with the specific BH-3 mimetic inhibitor ABT-737 was determined. This small molecule links BCL2 and prevents the protein from inhibiting the pro-apoptotic complex BAX/BAK. We show that the treatment reduced efficiently the progression of the disease, increased the peripheral blood platelet counts, decreased the bone marrow blast and cleared the tissue invasion in the MDS mice or reduced tissue infiltration of the AML. *In vivo* imaging by SPECT using Tec-99m-labelled Annexin V shows that ABT-737 induces apoptosis of the blasts cells that infiltrate the liver and the spleen of the treated mice, which was confirmed by TUNEL on liver sections. ABT-737 can reduce the myeloid colony growth and the LSK expansion of these mice. This is concordant with the confocal study of these treated mice that show a decrease of co-localization in marrow cells of the AML-like mice and a reduction in RAS activity as both human and mouse BCL2 are targeted. In conclusion ABT 737 diminishes the formation of the RAS/BCL-2 and rescues many disease associated features. This represents the first *in vivo* progression model of MDS/AML dependent on the formation of a BCL-2:RAS-GTP complex.

Acute myeloid leukemia - Clinical I

0035

A DOUBLE BLIND PLACEBO-CONTROLLED RANDOMIZED PHASE III STUDY OF HIGH DOSE CONTINUOUS INFUSION CYTOSINE ARABINOSIDE (ARAC) WITH OR WITHOUT VNP40101M (CLORETAZINE®) IN PATIENTS WITH FIRST RELAPSE OF ACUTE MYELOID LEUKEMIA (AML)

N. Norbert,[1] A. Pigneux,[2] D. Boscovic,[3] J. Kell,[4] T. Robak,[5] P. Staib,[6] B. Johnson,[7] S. O'Brien,[8] F. Giles[9]

[1]Institut Paoli-Calmettes, MARSEILLE, France; [2]Hôpital Haut Lévèque, BORDEUX, France; [3]Clinical Center of Serbia, BELGRADE, Serbia; [4]University Hospital of Wales, CARDIFF, UK; [5]Medical University of Lodz, LODZ, Poland; [6]Klinikum der Universitaet zu Koeln, COLOGNE, Germany; [7]Vion Pharmaceuticals, Inc., NEW HAVEN, USA; [8]MD Anderson Cancer Center, HOUSTON, USA; [9]CTRC University of Texas Health Science Center, SAN ANTONIO, USA

Background. VNP40101M is a novel alkylating agent which preferentially targets the O6 position of guanine resulting in DNA cross-links and has shown anti-leukemic activity in clinical trials. Aims. This abstract provides an update on a multi-center Phase III study that was conducted to compare overall response rate (ORR), defined as CR and CRp, and safety of VNP40101M + araC *vs* placebo + araC, in patients with AML in first relapse. Secondary endpoints were response duration, progression-free and overall survival (OS). Methods. Eligible patients were at least 18 years old, and in first relapse after CR1 of 3-24 months. Patients were randomized using a 2:1 schema to araC 1.5 g/m^2 (d1-3) + VNP40101M 600 mg/m^2 (treatment group), or araC 1.5 g/m^2 (d1-3) + placebo (control group). Patients with at least 20% blast reduction in the bone marrow could receive a second induction. Patients with CR or CRp could be consolidated according to original randomization, but at a lower dose of VNP40101M (400 mg/m^2) or placebo. The study design included a planned interim analysis of 50% patients enrolled (210 patients) for safety and efficacy by a data safety monitoring board (DSMB), which is detailed below. *Results*. Median age for 210 patients was 59 years; 63% patients had CR1 <12 months (median 290 days). ORR for treatment *vs* control group was 37% *vs* 19% (p=0.004); median OS for treatment *vs* control group was 128 *vs* 182 days (p=0.039). An independent safety review revealed on-study mortality (defined as death from any cause ≤30 days, or due to an adverse event ≤60 days from treatment) in 39% (55/140) of treatment group patients, and 8.6% (6/70) of control group patients. 67% (37/55) of treatment group deaths were due to infection, sepsis, or pneumonia. 18% (10/55) of treatment group deaths were due to pulmonary events (8/10 ARDS [4 with a history of prior BMT], 7/10 with intercurrent infection) occurring between days 9-96. Control group deaths were due to sepsis/pneumonia (2), AML (2), or multi-organ failure (2). Grade 3-4 neutropenia was reported in 95% of patients following first induction; however median time to neutrophil recovery was longer in the treatment *vs* control group (32 days *vs* 25 days). *Summary and Conclusions*. VNP40101M + araC is an active regimen in relapsed AML. In this study, higher ORR was achieved with VNP40101M + araC; however, any efficacy advantage could not translate to a survival benefit given the discrepancy in on-study mortality. Further study of VNP40101M in combination with araC in this population will include VNP40101M at a lower dose (400 mg/m^2) in combination with araC for induction, and standardized antimicrobial/antifungal and growth factor support.

0036

A PHASE I STUDY WITH CP-4055 IN PATIENTS WITH HAEMATOLOGIC MALIGNANCIES

F.J. Giles,[1] S.M. O'Brien,[2] N. Vey,[3] D. Rizzieri,[4] J. Sarantopoulos,[1] T. Prebet,[3] F. Ravandi,[5] S. Faderl,[5] A. Charbonnier,[3] T.F. Jacobsen,[6] B.O. Nilsson,[6] K. Staudacher,[6] H.M. Kantarjian[5]

[1]CTRC at the University of Texas Health Science Center San Antonio, SAN ANTONIO, TEXAS, USA; [2]Univ.of Texas MDACC, HOUSTON, USA; [3]Institut Paoli-Calmettes, MARSEILLE, France; [4]Duke University Medical Center, DURHAM, USA; [5]University of Texas M. D. Anderson Cancer Center, HOUSTON, TX, USA; [6]Clavis Pharma ASA, OSLO, Norway

Background. CP-4055 (cytarabine 5'-elaidic acid ester) is a novel cytotoxic nucleoside analogue. Cytarabine is the backbone of therapy in leukaemia. CP-4055 has similar mechanism of action to cytarabine but, unlike cytarabine, it is independent of nucleoside transporters for cellu-

lar uptake. *Aims.* The aim is to determine the maximum tolerated dose (MTD) and the preferred infusion time in patients (pts) with haematologic malignancies. *Methods*. Following informed consent pts received IV CP-4055 over 2-4 hours (hrs) (Arm A) or 24 hrs (CIV, Arm B) in a day (d) 1-5 q3w schedule. The starting dose in Arm A was 300 mg/m^2/d and in Arm B 200 mg/m^2/d, with standard definitions of dose limiting toxicity (DLT) for haematologic malignancies. Dosing increments were by 50% or by 30% in case of grade 2 non-haematologic toxicities. *Results*. In the ongoing study, 77 pts [46 male, median age 60 yrs (range 21-92); ECOG PS 0-1: 59 pts, ECOG PS 2: 18 pts; AML 67 pts, ALL 3 pts, CMML-AML 2 pts, CML-BP 2 pts, BAL 1 pt, MDS 1 pt, CLL 1 pt; mean 3 lines prior chemotherapy (range 0-7)] have been treated at 3 US and 1 European centres. MTD has been determined at 2500 mg/m^2/d for both Arm A and B. Ten pts are ongoing at 2000 mg/m^2/d. *Safety*: nausea, diarrhea, fever, thrombocytopenia, elevation of liver function tests (LFTs), constipation and anemia were the most common possibly related AEs. All DLTs were reversible elevations of LFTs. Efficacy: 2 CR, 1 CRp and 2 PR were reported at doses 875-2500 mg/m^2/d (all AML, age 33-92 yrs). 23 pts with stable disease/mild myelosuppression received > 2 courses of CP-4055. *Summary and Conclusions.* Patients with haematologic malignancies tolerate CP-4055 well. The MTD was determined at 2500 mg/m^2/d for both 2-4 hrs IV and CIV. Clinical activity has been observed with manageable non-haematologic toxicity.

0037

REDUCED INTENSITY CONDITIONING (RIC) ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) FOR PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML): LONG TERM RESULTS OF A *DONOR vs NO DONOR* COMPARISON

M. Mohty,[1] H. De Lavallade,[2] J. El-Cheikh,[2] P. Ladaique,[2] C. Faucher,[2] S. Fürst,[2] N. Vey,[2] D. Coso,[2] A.M. Stoppa,[2] J.A. Gastaut,[2] C. Chabannon,[2] D. Blaise[2]

[1]*CHU de Nantes, NANTES;* [2]*Institut Paoli-Calmettes, MARSEILLE, France*

Standard myeloablative allo-SCT is a well established therapy for adult patients with AML. However, because of the high incidence of procedure-related toxicity, this procedure is often limited to younger patients in good medical condition. RIC regimens have emerged as an attractive modality to decrease transplant-related toxicity. However, the issue of possible higher relapse rates after RIC allo-SCT is still under considerable debate, and no randomized studies between RIC-allo-SCT for AML and chemotherapy alone are yet available. This report describes the long term results of 95 consecutive AML patients, diagnosed between Nov. 1999 and Dec. 2003 in a single institution, and who were considered as potential candidates for RIC-allo-SCT. Using a genetic randomization through a *donor vs no donor* comparison, the aim of this analysis was to assess the real benefit of RIC-allo-SCT for adult AML and its impact on clinical outcome. In this series, 35 patients (37%; *donor* group) had an *identified* HLA-identical sibling donor, while the remaining 60 patients had no HLA-matched related donor (*no donor* group). As per institutional policy, HLA-matched unrelated donors were not considered during the study period. No significant differences in patients or AML features were found between the two groups. In the *donor* group, 25 patients (71%; median age, 51 (range, 26-60)) could actually proceed to the RIC-allo-SCT. The 10 remaining patients with an identified donor did not receive allo-SCT because of early relapse after CR (n=2), patient or donor refusal (n=6), and psychiatric disorders appearing before allo-SCT (n=2). The current median follow-up is 60 months. In an *intention-to-treat* analysis, the KM estimate of leukemia-free survival (LFS) was significantly higher in the *donor* group as compared to the *no donor* group (p=0.003; 60% *vs* 23% at 7 years). When restricting the analysis to patients who could actually receive the RIC-allo-SCT (median follow-up, 40 months from time of allo-SCT), the difference in LFS was also significant between this group of 25 patients (*transplant* group) and the remaining 70 patients (*no transplant* group) who did not receive allo-SCT (p=0.0002; 72% *vs* 24% at 7 years). In the *transplant* group, RIC-allo-SCT was performed at a median of 209 (range, 119-413) days after diagnosis. No major toxicities were encountered during RIC administration (fludarabine, busulfan and ATG), and only 3 patients died from transplant-related toxicity, for a cumulative incidence of TRM of 12% (95%CI, 3-32%) at last follow-up. This relatively low TRM translated towards a significantly higher overall survival (OS) in the *transplant* group as compared to the *no transplant* group (p=0.0003). In the *intention-to-treat* analysis, OS was still significantly higher in the *donor* group as compared to the *no donor* group (p=0.003; Figure 1). After controlling for all relevant factors, in the multivariate analysis, only actual performance of RIC-allo-SCT (p=0.0005; RR=4.1; 95%CI, 1.8-9.1), was significantly predictive of an improved LFS, further confirming the overall benefit of RIC-allo-SCT for adult AML patients. Based on these long term results, and given the low overall TRM rate observed in this high risk population, we conclude that if a matched related donor is identified, RIC-allo-SCT should be proposed since it represents a valid and potentially curative option for AML patients not eligible for standard myeloablative allo-SCT.

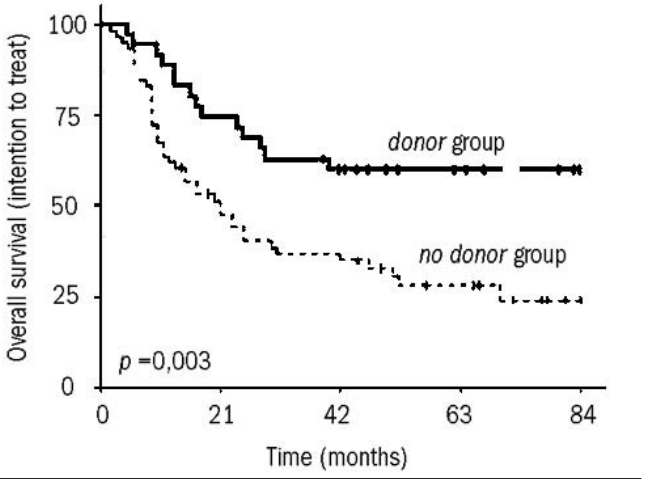


Figure 1.

0038

PHASE I STUDY OF F60008, A TRIPTOLIDE DERIVATIVE, IN PATIENTS WITH REFRACTORY OR RELAPSING ACUTE LEUKEMIAS

J.L. Harousseau,[1] H. Dombret,[2] A. Pigneux,[1] M. Michallet,[3] M. Brandely[4]

[1]*University Hospital, NANTES;* [2]*Hopital Saint Louis, PARIS;* [3]*Hopital Edouard Herriot, LYON;* [4]*Pirre Fabre Oncologie, BOULOGNE BILLANCOURT, France*

F60008 is a semi-synthetic derivative of triptolide. Triptolide was shown to induce apoptosis in a broad range of human tumor cell lines and in various leukemic cell lines as well as in primary acute myeloid leukemia (AML) blasts. Therefore, the first phase I study of F60008 was conducted in adult patients with refractory or relapsing acute leukemias. The primary objective was to establish the maximally tolerated dose (MTD) of F60008 given as one 30 minute infusion for 5 consecutive days every 15 days. Dose escalation was based initially on an accelerated titration design with 50% dose increment and one patient by dose level (DL). Twelve DLs from 0.15 to 13 mg/m^2/day were explored. After occurrence of a fatal acute respiratory distress syndrome concomitant with pulmonary leucostasis at 13 mg/m^2/day, lower DLs of 8.5, 5.7 and 7 mg/m^2/day were studied in cohorts of 3 to 6 patients. A total of 29 patients were treated: 26 AML and 3ALL. There were 18 males and 11 females with a median age of 57 years (range: 30-79). Ten of them (34.5%) had secondary AML. DLT was defined as any non-hematological grade > 3 toxicity or grade 4 pancytopenia >6 weeks. The MTD was the DL at which 2 out of 6 patients developed a DLT during the first cycle. At 8.5 mg/m^2/day, 6 patients were treated and 2 of them experienced grade 4 cerebellar toxicity. This DL met the criteria for MTD and the lower DL of 5.7 mg/m^2/day was given to a cohort of 6 patients without reporting of DLT. An intermediate DL of 7 mg/m^2 was thus tested in 7 patients (6 evaluable for MTD) and was also associated with the occurrence of grade 4 cerebellar toxicity in 2 patients. Overall, the most common drug-related adverse events were grade 1-2 nausea (17.2% of patients), grade 1-2 diarrhoea (13.8%), grade 3-4 neutropenia (10.3%) in addition to cerebellar toxicity (17.2%). The MTD was 7 mg/m^2/day and the recommended dose (RD) was 5.7 mg/m^2/day. One complete remission (CR) and one CR without full platelet recovery were reported at 8.5 mg/m^2/day; and one CR with pancytopenia was seen at 5.7 mg/m^2/day. All responses were in AML patients. In order to study a possible schedule-dependency of F60008 toxicity, the study protocol was amended to test the total daily dose given in 2 equal doses at 12 hours interval for 5 days. At 3 mg/m^2 bid (daily dose of 6 mg/m^2), no DLT was seen in 3 patients but at 4 mg/m^2 bid (daily dose of 8 mg/m^2), 1 of the 3 patients

developed grade 4 cerebellar toxicity which led to stop accrual. In conclusion, F60008 given once a day for 5 consecutive days has activity in refractory or relapsing AML patients at doses >5.7 mg/m^2/day which is the RD of this regimen. The DLT was cerebellar toxicity. Other regimens warrant further exploration.

0039

A NEW PROGNOSTIC MODEL FOR PATIENTS WITH SECONDARY ACUTE MYELOID LEUKEMIA: RESULTS OF THE DSIL AML96 TRIAL

M. Schaich,[1] W.E. Aulitzky,[2] H. Bodenstein,[3] M. Bornhäuser,[2] T. Illmer,[1] U. Schäkel,[1] N. Schmitz,[4] S. Soucek,[1] C. Thiede,[1] H. Wandt,[5] G. Ehninger[1]

[1]Universitätsklinikum C.G. Carus, DRESDEN; [2]Robert-Bosch-Krankenhaus, STUTTGART; [3]Klinikum Minden, MINDEN; [4]Krankenhaus St. Georg, HAMBURG; [5]Klinikum Nord, NÜRNBERG, Germany

Background and Aims. Secondary acute myeloid leukemia (AML) is regarded as an entity with a poor prognosis and patients were normally treated as high-risk AML. However, due to progress in elucidating the impact of cytogenetic and molecular markers for the prognosis and treatment outcome the entity of secondary AML may be as heterogeneous as the *de novo* disease. Thus, scoring systems are needed to subdivide this entity. *Patients and Methods*. Between February 1996 and November 2004 a total of 232 patients with secondary AML were treated within the AML96 trial of the German Study Initiative Leukemia (DSIL). Secondary AML was therapy-related in 80 patients (tAML) and following a myelodysplastic syndrome in 232 patients (mdsAML). All patients received double induction therapy. Consolidation therapy contained high-dose Ara-C and for patients ≤60 years the option of autologous or allogeneic hematopoietic stem cell transplantation (HSCT) according to cytogenetic risk. *Results*. Patients with mdsAML were older, had a higher percentage of CD34$^+$ blast cells, but in a lower extend aberrant karyotypes than patients with tAML. CR rate was 41%, 3-year overall survival (OS) 19% and 3-year relapse-free survival (RFS) 21% for all patients with secondary AML. In the multivariate analysis disease status (mdsAML *vs* tAML) was not an independent prognostic factor for treatment outcome. However, age proofed to be one of the strongest prognostic factors for treatment response and survival. Patients older than 60 years had a CR rate of 21%, a 3-year OS and RFS of 14% and 3% compared to 47%, 26% and 33% of their younger counterparts (p<0.0001, p=0.001 and p<0.0001), respectively. Looking at the group of patients ≤60 years thrombocyte count (p<0.0001) and NPM mutational status (p=0.04) at diagnosis were independent predictors for survival. Using these two at diagnosis parameters a prognostic model for survival was established for patients ≤60 years. The low-risk group had a 3-year overall survival of 71%, the intermediate risk group of 33% and the high-risk group of 5% (p<0.0001). *Conclusions*. The poor prognosis of patients with secondary AML is mostly conferred by an advanced age. For secondary AML patients ≤60 years we provide a new prognostic model for risk stratification using thrombocyte count and NPM mutational status at diagnosis.

0040

ACCURATE PREDICTION OF CYTOGENETIC SUBGROUPS IN PEDIATRIC ACUTE MYELOID LEUKEMIA WITH GENE EXPRESSION PROFILING; A CRITICAL VALIDATION STUDY

B.V. Balgobind,[1] M.M. van den Heuvel-Eibrink,[1] R.X. Menezes,[1] D. Reinhardt,[2] I.H.I.M. Hollink,[1] T.J.C.M. Arentsen-Peters,[1] E. van Wering,[3] G.J.L. Kaspers,[4] J. Cloos,[4] E. de Bont,[5] J. Cayuela,[6] A. Baruchel,[6] J. Trka,[7] J. Stary,[7] R. Pieters,[1] C.M. Zwaan,[1] M.L. den Boer[1]

[1]Erasmus MC - Sophia's Children Hospital, ROTTERDAM, Netherlands; [2]AML-BFM Study Group, HANNOVER, Germany; [3]DCOG, THE HAGUE, Netherlands; [4]VU Medical Center, AMSTERDAM, Netherlands; [5]UMCG-Beatrix Children Hospital, GRONINGEN, Netherlands; [6]St. Louis Hospital, PARIS, France; [7]2nd Medical School, Charles University, PRAGUE, Czech Republic

Background. Pediatric acute myeloid leukemia (AML) is a heterogeneous disease, in which early treatment response and cytogenetic abnormalities are the most important prognostic factors. AML is thought to arise from two different types of genetic aberrations, i.e. type-I (proliferation enhancing) mutations and type-II (differentiation impairing) mutations. Recent studies have focused on the potential to use gene expression profiling to classify AML. *Aims.* To determine whether gene expression signatures generated in a training cohort could correctly predict cytogenetic subgroups in a second and independent group of pediatric AML cases. *Methods*. We used Affymetrix Humane Genome U133 plus 2.0 oligonucleotide microarrays to generate gene expression profiles of 256 children with AML. Probe set intensities were normalized using the variance stabilization (VSN) procedure of R (version 2.2.0). This group was divided into a training cohort (n=170) and a validation cohort (n=86). The training cohort was further subdivided into a group of patients to identify predictive genes and to build a classifier and a 2nd group to test the accuracy of this classifier. Differentially expressed genes between AML subgroups were calculated using a linear model corrected for random effects, and corrected for multiple testing (Bioconductor package: Limma). *Results*. The classifier was built with the top 15 discriminating probe sets of every cytogenetic subgroup. Based on well-known cytogenetic subgroups [including MLL-gene rearrangements, t(8;21), inv(16), t(15;17) and t(7;12)] we identified a classifier that could reliably predict these subgroups. Moreover, when applied to the independent validation cohort of 86 patients, this classifier achieved a mean accuracy and sensitivity of more than 99% to correctly predict all cytogenetic subgroups in this independent validation cohort. *Conclusions*. In conclusion, gene expression profiling can correctly predict the majority of well-known cytogenetic subgroups in pediatric AML, with a high accuracy and high sensitivity, and can therefore be used as a diagnostic tool to assess the main cytogenetic subgroups.

0041

FLOW-CYTOMETRIC MINIMAL RESIDUAL DISEASE DETERMINATION IS A SURROGATE PROGNOSTICATOR IN ADULT AML PATIENTS WITH NORMAL KARYOTYPE

F. Buccisano,[1] L. Maurillo,[2] G. Del Poeta,[2] M.I. Del Principe,[1] D. Fraboni,[1] P. Panetta,[1] M. Irno Consalvo,[1] M. Rizzo,[1] L. Giannì,[1] S. Campagna,[1] S. Faccia,[1] D. Renzi,[1] M. Postorino,[1] M. Cantonetti,[1] F. Lo Coco,[1] S. Amadori,[1] A. Venditti[1]

[1]Tor Vergata University Hospital, ROMA; [2]S. Eugenio Hospital, ROMA, Italy

Background. In a remarkable proportion of adult AML patients (40-50%), no clonal abnormalities are found on standard cytogenetic analysis. In this group of patients, several gene mutations have been described, allowing to discriminate subgroups with distinct clinical outcome. Among these molecular signatures, FLT3, a receptor tyrosine kinase with important roles in hematopoietic stem/progenitor cell survival and proliferation, has been demonstrated to be mutated in about 1/3 of AML cases, identifying a subgroup of patients characterized by disappointing overall cure rates. Therefore, roughly 60% of patients with normal karyotype are not classifiable by this specific mutation. *Aims.* Given the prognostic role, in our experience, of MRD as determined by multiparametric flow-cytometry (MPFC), we investigated a group of patients with normal karyotype to compare the prognostic information coming from this parameter where no specific mutation of *FLT3* have been detected. *Methods*. We analyzed a group of 127 AML cases entered into the *EORTC/GIMEMA* protocols *AML10/AML12* (age<61yrs) or AML13/AML15/AML17 (age >61 yrs). By applying the maximally selected log-rank statistics, the threshold discriminating MRD negative from positive cases was set at 3.5×10^4 residual leukemic cells, a level that selected, at the post-consolidation time-point, two groups of patients with distinct prognosis.

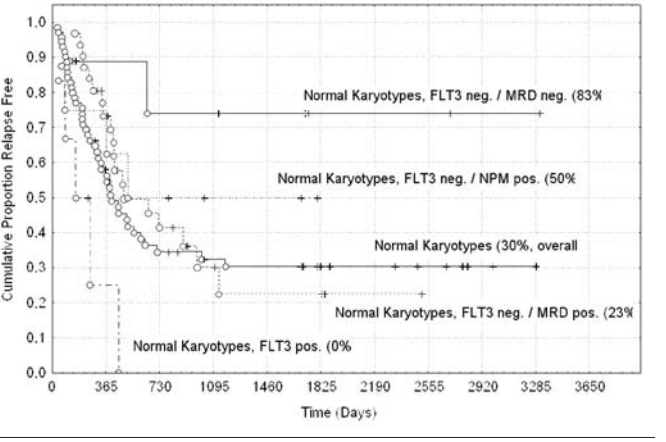


Figure 1.

Results. Seventy-two out of 127 (56%) had a normal karyotype. Among these 72, 53 were studied for FLT3 mutational status; in 13 (25%) a mutated variant of FLT3 was detected. Among the remaining 40 patients with normal karyotype and unmutated FLT3, 39 were evaluable for the MRD status at the post-consolidation time-point: 9 patients were MRD neg. and 30 MRD pos.. These two subsets showed a distinct outcome in terms of 5-years RFS, with FLT3 neg./MRD neg. showing a favorable prognosis as compared to FLT3 neg./MRD pos. (83 *vs* 23%, p=0.031). Interestingly, the latter group shared with those FLT3 positive a similar poor prognosis (Figure 1). When FLT3 status was combined with the mutated status of Nucleophosmin (NPM1) exon-12 gene, the favorable impact of NPM1 mutations on OS and EFS clearly emerged in the group of normal-karyotype AML without FLT3 mutations. In fact, in a subset of 44 patients where both the mutations were investigated, this combined analysis identified 8 out of 44 (18%) patients FLT3 unmutated/NPM1 mutated with a better outcome (5-years OS 50% and RFS 50%, respectively) which was however poorer than that of FLT3 unmutated/MRD neg. cases (Figure 1). *Conclusions*. MRD determination at the post-consolidation check point may help at improving the prognostic assessment of AML patients with normal karyotype, identifying a subgroup of patients with a better outcome as compared to those carrying specific molecular patterns.

0042

IMPACT OF DIFFERENT POSTREMISSION STRATEGIES ON QUALITY OF LIFE ACCORDING TO AGE AND AFFECTED LIFE CYCLE PHASE AT INITIAL DIAGNOSIS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

R.F. Schlenk,[1] K. Döhner,[1] M. Schlosser,[1] S. Groner,[1] D. Späth,[1] R. Hann,[1] D. Messerer,[2] J. Krauter,[3] I. Schäfer,[3] A. Ganser,[3] H. Döhner[1]

[1]University of Ulm, ULM; [2]University of Munich, MUNICH; [3]Hannover Medical School, HANNOVER, Germany

Background. Allogeneic stem cell transplantation (allo-SCT) in patients 16 to 60 of age with acute myeloid leukemia (AML) in first complete remission (CR) seems to have an negative short (Watson *et al.* Eur J Cancer 2004) and long term (Messerer *et al.* Haematologica 2008) impact on quality of life (QoL). In particluar, impaired leisure time activity, difficulties in role functioning, sexuality, and financial affaires as well as a reduced global health status have been reported after allo-SCT. However, the disease and treatment affect different phases of life such as the phase of professional education and family founding, the phase of the full nest with children in house followed by the empty nest phase where children leave home. *Aims.* To investigate the effects of postremission strategy in different age cohorts on QoL. *Methods*. Patients (age 16-60 years) from two multicenter treatment trials (AMLHD93, AMLHD98A) of the German-Austrian AML Study Group (AMLSG), treated between 1993 and 2004, completed a self-report questionnaire issued via the AMLSG Clinical Trials Office. Patients completed the EORTC Quality of Life-Core Questionnaire (QLQ-C30) supplemented by self-assessed concomitant diseases (18 organ complexes), late treatment effects and demographic details including percentage of disability. The QLQ-C30 incorporates 9 multi-item scales with five functional scales (physical, role, cognitive, emotional, social), three symptom scales (fatigue, pain, nausea/vomiting) and a global health and quality-of-life scale. Age groups based on age at diagnosis were defined as follows: i) age group-1: 16-34 years; ii) age group-2, 35-49 years; iii) age-group-3, 50-60 years. *Results*. A total of 1198 patients were registered for the two trials; of 491 patients alive at the time of QoL questionnaire, 394 were in first CR and 245 of these returned the questionnaire (62%). The median time from diagnosis to the QoL assessment was 4.8 years. The distribution of postremission therapy and of age group was n=77 allo-SCT (61 MRD, 16 MUD), n=50 autologous SCT, and n=118 conventional chemotherapy (CCT), as well as n=56 age group-1 (16-34 years), n=116 age group-2 (35-49 years) and n=73 age group-3 (50-60 years). Concomitant diseases were statistically less frequent (p=0.007) in age group-1 compared to age groups 2 and 3. The comparison of the percentage of employed/self-employed patients was restricted to age groups-1 and 2 due to natural retirement in age group-3. In age group-1 the percentage of employed/self-employed patients was 79% compared to 54% in age group-2 (p=0.002). Of note, there was no difference between the treatment strategies in age group-1 which was in contrast to the results in age group-2 where 63% of patients after CCT were employed/self-employed compared to 48% after SCT with no difference between allo-SCT and auto-SCT. Impairments in sexual life were more frequent in the age groups-2 (52%) and 3 (53%) compared to age group-1 (30%) (p=0.005) and, of note, these impairments were significantly more frequent in patients after SCT in age groups-2 and 3 (p=0.008). The general feeling/perception of a positive attitude in life was in trend more frequent in age group-1 (77%) compared to age group-2 (57%) and 3 (63%) (p=0.03) with no difference between postremission strategies. *Conclusions*. There seems to be a differential impact of postremission strategies on QoL according to different age groups with an unfavorable impact of SCT strategies especially in middle aged patients.

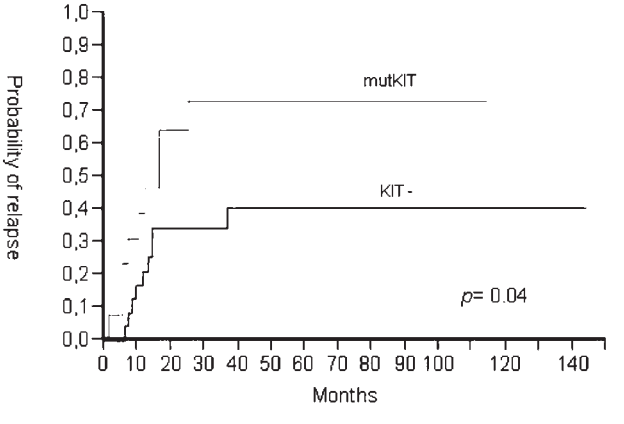
0043

IMPACT OF KIT MUTATIONS IN ACUTE MYELOID LEUKAEMIA WITH INV(16)

R.C. Cairoli,[1] A. Beghini,[2] C.B. Ripamonti,[3] G. Grillo,[3] G. Nadali,[4] E. Di Bona,[5] P. Colapietro,[2] G.B. Bertani,[3] E. Ravelli,[3] G. Nador,[3] C. Castagnola,[6] C Cattaneo,[7] E. Ottaviani,[8] P. Pioltelli,[9] A. Viola,[10] A. Cuneo,[5] F. Ferrara,[10] G. Martinelli,[8] M. Lazzarino,[6] G. Rossi,[7] G. Pizzolo,[4] E. Morra[3]

[1]Niguarda Hospital, MILANO; [2]Dept. of Biology and Genetics for Medical Sciences, MILANO; [3]Dept. of Haematology, Niguarda Hospital, MILANO; [4]Dept.of Clinical and Experimental Medicine, University of Verona, VERONA; [5]Dept. of Haematology, VICENZA; [6]Division of Haematology, University of Pavia, PAVIA; [7]Dept. of Haematology, University of Brescia, BRESCIA; [8]Dept. of Haematology, University of Bologna, BOLOGNA; [9]Division of Haematology, San Gerardo Hospital, MONZA; [10]Division of Haematology and Stem Cell Transplantation Unit, NAPOLI, Italy

Background. Several studies have recently pointed out the adverse impact of KIT mutations (mutKIT) on relapse incidence (RI) and overall survival (OS) in patients with t(8;21) AML. By contrast, the prognostic significance of mutKIT in patients with inv(16) remains unclear. *Aims*. Purpose of this study is to evaluate the prevalence and the effect on outcome of mutKIT in inv(16)(p13q22). *Patients and Methods*. 50 adults with inv(16) AML at diagnosis (median age 46.6 years, range: 17-88; M/F: 30 /20), were centrally analyzed for mutKIT in exon 2, 8, 10, 11 and 17. Mutations were detected using sequencing and other sensitive assays such as ARMS (amplification refractory mutation system) PCR for D816Y and D816H, enzymatic digestion with HINFI for D816V and with Tsp509I for N822K. *Results*. KIT mutations were documented in 17/50 patients (34%). Among the mutKIT cases, we detected mutations in exon 17 (n=12), exon 8 (n= 4) and exon 10 (n=1). We found no significant difference between the mutKIT *vs* the unmutated (KIT-) patients in WBC count at presentation (WBC 13.9x10^9/Liter, range 4.4 to 277.5 *vs* 19.4x10^9/Liter, range 2.5 to 130; Mann-Whitney U test: p=0.649). 42 patients (age <60 years) received intensive chemotherapy; of them, 13 resulted mutKIT upon mutational screening. CR was achieved in 13 of 13 mutKIT patients (100%) *vs* 27 of 29 KIT-patients (93%), (Fisher's exact test: p>0.999). With a median follow-up of 26 months (range: 2 to 147), the Kaplan-Meier plots showed a worse RI among the mutKIT *vs* the KIT- patients (log-rank test: p=0.04; Figure 1). We didn't observe any difference in OS between the two groups of patients (log-rank test: p=0.44).



Conclusions. KIT mutational status seems to influence the incidence of relapse, but not OS, in patients with inv(16) aged 60 years old or younger.

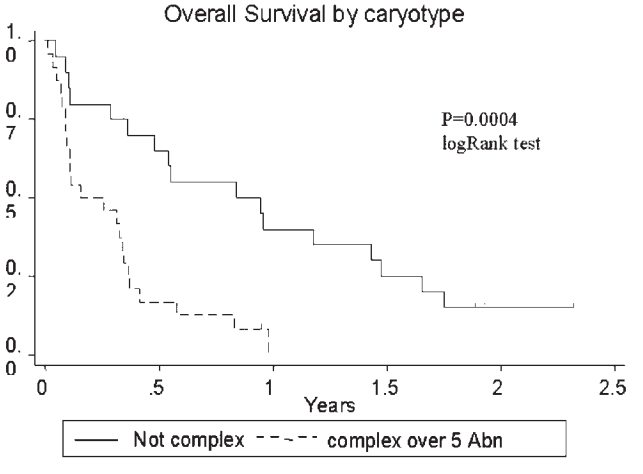
0044

GENTUZUMAB-OZOGAMICIN (GO) PLUS IDARUBICIN (I) AND CYTARABINE (C) AS INDUCTION TREATMENT FOR ELDERLY PATIENTS WITH POOR-RISK CYTOGENETICS ACUTE MYELOID LEUKEMIA (AML),RESULTS OF GOELAMS LAMR04 STUDY

J. Delaunay,[1] C. Recher,[2] N. Vey,[3] F. Witz,[2] B. Lioure,[2] O. Tournilhac,[1] C. Himberlin,[2] D. Bouscary,[2] N. Ifrah,[2] J.L. Harousseau[1]

[1]University Hospital of Nantes, NANTES; [2]University Hospital, TOULOUSE; [3]Institut Paoli Calmette, MARSEILLE, France

Introduction. The prognosis of AML in patients over 60 years of age remains very poor. As in younger patients karyotype is a major prognostic factor. Elderly patients with poor risk cytogenetics have a dismal outcome and complete remission (CR) rates with conventional chemotherapy are usually < 45% (and <25% if complex abnormalities). GO has been used in combination with chemotherapy in relapsed or newly diagnosed AML, but with reduced doses in order to decrease hepatic toxicity. Methods. In this multicentric open-label Phase II study we have evaluated the combination of I (8 mg/m/D x D1-5) + C (100 mg/m/D x D1-7) with GO (6 mg/m on D3) in AML patients aged 60-75 years with poor risk cytogenetics and fit enough to undergo cytotoxic chemotherapy. Other inclusion criteria were: CD33+ AML, PS 2, *de novo* or secondary AML (excluding M3 and Ph1 + AML), no cardiac, renal or hepatic dysfunction. Results. 44 pts aged 60-74 (median 68) were treated in 13 GOELAMS centers. Karyotype results were: complex (>5 abnormalities) in 24/44 (54%), chromosome 5 abnormalities in 24/44 with a significant association with complex Karyotype(p=0.02), chromosome 7 abnormalities in 29/44 and 3q21-21 abnormalities in 7/44. In 13 cases (30%) AML was secondary (chemoinduced 11, prior myeloproliferative or myelodysplastic syndrome 2). Median duration of hospitalisation was 31 D (4-51). In CR patients median duration of neutropenia was 19 D (14-28) and median duration of thrombocytopenia was 15D (5-29). Bacterial or fungal infections were documented in 71% of cases. 7 patients had severe bleeding including 3 deaths, (2 cerebral hemorrhage, 1 intestinal bleeding). Grade 2 hepatic dysfunction was seen in 30% of patients including 3 (7%) pts with veino-occlusive disease (one death). There were 17 (38%) CR (16 CR, 1 CRp), 6 (14%) toxic deaths and 21 (48%) failures. Prognosis factor impacted CR were PS (≥1) with 21% *vs* 66% p=0.003 and complex karyotype (≥5 Abn) 16% *vs* 65% p=0.001.Only 7/17 Pts in CR received a consolidation therapy. OS, EFS and cumulative incidence of relapse at 24 months were respectively 7.5%, 6.8% and 77%. In multivariate analysis, complex karyotype (≥5 abn) and LDH ≥1000 UI/L remain the most significant prognosis factors for OS. Also, Median OS was 3 *vs* 11 months for isolated cytogenetics abnormalities group *vs* complex karyotype group respectively. *Conclusion*. In this population of elderly but relatively fit patients with poor risk cytogenetics AML the addition of GO 6 mg/m^2 to conventional chemotherapy did not appear to increase the CR rate or OS in the whole population . However, CR rate and OS are significantly improved in the isolated cytogenetics abnormalities group indicating that alternative therapy must be defined for elderly patients with complex karyotype.



0045

PHASE II PILOT TRIAL OF GEMTUZUMAB OZOGAMICIN (MYLOTARG) IN COMBINATION WITH FLUDARABINE, CYTARABINE, IDARUBICIN (FLAI) AS INDUCTION THERAPY IN CD33-POSITIVE AML PATIENTS YOUNGER THAN 65 YEARS

A. Candoni,[1] G. Martinelli,[2] M. Tiribelli,[1] E. Simeone,[1] E. Toffoletti,[1] A. Michelutti,[1] A. Chiarvesio,[1] M. Malagola,[3] P.P. Piccaluga,[2] D. Russo,[3] R. Fanin[1]

[1]Division of Hematology, UDINE; [2]Institute of Hematology and Oncology Land A Seragnoli, BOLOGNA; [3]Chair of Hematology and BMT, BRESCIA, Italy

Introduction. The addition of gemtuzumab-ozogamicin (Mylotarg-GO) to an induction regimen including synergistic drugs, such as intermediate dose of cytarabine (Ara-C), idarubicin and fludarabine (FLAI), could reduce treatment failure in AML patients. Nevertheless, the role of this antibody target-therapy in first-line chemotherapy in patients younger than 65 years has not yet been defined. *Patients and Methods*. The primary goal of this prospective phase II pilot study was to evaluate the efficacy and the safety profile of FLAI plus GO as induction regimen. Thirty consecutive AML patients were classified, according to FAB, as follows: M0 (2 pts), M1-M2 (9 pts), and M4-M5 (16 pts). All patients were younger than 65 with a median age of 53 years (range 25-65). The M/F ratio was 16/14, and 21/30 (70%) of patients were poor-risk at diagnosis. CD33 expression exceeded 20% in all cases. The induction regimen (FLAI-GO) included fludarabine (30 mg/m^2) and Ara-C (2 g/m^2) on days 1-5, idarubicin (10 mg/m^2) on days 1, 3, and 5 and GO (3 mg/m^2) on day 6. Hematopoietic stem cell transplant (HSCT) was planned for all patients in first complete remission (CR) after consolidation with intermediate doses of Ara-C and idarubicin (IDAC-IDA). Cytogenetic, multidrug-resistance phenotype, FLT3 mutation, and WT1 expression analyses were performed at diagnosis in all patients. WT1 expression and cytogenetic (in positive cases) analyses were performed after induction to detect and follow MRD. *Results*. Patients were evaluated for response, treatment-related adverse events, overall survival and relapse free survival. After induction with FLAI-GO, CR rate was 90% (26 of 29 evaluable pts); one patient achieved partial remission and two were resistant. There was only one case of death during induction (DDI). After FLAI-GO, the mean value of WT1 dropped from 4200±2777 copies/104ABL to 192±399 copies/104ABL. The toxicity of FLAI-GO was comparable to FLAI alone; 57% of patients experienced transient and reversible GO infusion-related adverse events (especially fever and chills), but no cases of veno-occlusive disease occurred during CHT or after HSCT. After a median follow-up of 13 months (range 1-24), 25/30 (83%) patients are alive (25/25 in CR). The probability of 1-year OS and RFS was 90 and 80%, respectively. Allogeneic and autologus HSCT was performed in 16 (54%) and 4 (13%) patients, respectively. *Conclusions*. These preliminary results suggest that FLAI-GO is a feasible, effective and well tolerated induction regimen for AML patients younger than 65 years, with a high response rate. These results encourage the testing of this new regimen in a multicenter prospective trial. Compared to our previous published experience with FLAI alone, the addition of GO increases the antileukemic efficacy (74 *vs* 90% of CR). The majority of patients that received FLAI-GO obtained an optimal tumour debulking, with a favourable safety profile and low DDI (3%), which allowed a consolidation therapy with HSCT early and in a high proportion of cases (67%).

0046

FACTORS INFLUENCING CLINICAL DECISION MAKING IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML). REPORT ON 340 CONSECUTIVE PATIENTS JUDGED AS UNFIT TO RECEIVE INTENSIVE INDUCTION CHEMOTHERAPY

F. Ferrara,[1] S. Palmieri,[1] G. Gianfaldoni,[2] F. Leoni[2]

[1]Cardarelli Hospital, NAPOLI; [2]Department of Hematology, FIRENZE, Italy

Background. The incidence of AML increases progressively with age. Older patients more often have adverse biologic risk factors and present with clinically relevant comorbidities. As a consequence, a relevant selection is operated as to inclusion into clinical trials based on intensive induction chemotherapy (IC). However, a lack of validated guidelines results in a considerable heterogeneity in the therapeutic attitude between individual clinicians, clinics and geographical districts. *Aims*. In this report we analyzed a series of 340 consecutive patients with AML aged over 60 years, accounting for 48 % of the whole AML patient population observed throughout the period of the study, observed at two hematologic Institutions in Italy, who were considered as not eligible to receive IC. The aim was of defining most relevant reasons of exclusion taken into account in the daily practice. *Methods*. 340 patients with AML, with the exception of APL, aged over 60 years in the period from January 1995 to December 2007 were analyzed. All were judged as not eligible to receive IC aimed at complete remission achievement. *Results*. The median age was 75 years (range 61-94); 80 % of patients were aged over 70 years and 18 % over 80 years. 210 patients (62%) had *de novo* AML, while 130 (38%) had AML secondary to myelodysplatic syndrome. Median performance status (PS) was 3 (range 1-4). In particular, 35 patients (11%) had PS 1, 106 (32%) PS2, 135 (40%) PS 3 and 60 (18 %) PS 4. A concomitant disease (mainly cardiac, hepatic or chronic obstructive pulmonary disease) requiring appropriate treatment was present in 286 patients (82%) Less frequently (11%), severe neurologic disorders were found. 20 % of patients had other neoplasms (prostate cancer, lung cancer, colon cancer, others) and in 50 % of them malignancy was active. Documented infection at diagnosis was demonstrated in 15 % of patients. Of note, only 5 % of patients, judged as not eligible to IIC, were intensively treated after improvement of clinical status following supportive therapy. Specific reasons for exclusion from IC were: age over 80 (%), poor PS (75%), severe concomitant disease requiring specific treatment (60%), active infection (12%), concomitant malignancy (10%), distance from the hematologic institution (10%), lack of caregiver (5%) and patient's refusal (7%). Supportive care was given to all patients, while hydroxyurea was used in the case of leukocytosis (45 % of patients). Median survival was 3 months. Hospitalization was required in 75 % of patients because of infectious (70%) and hemorrhagic complications (34%). Of interest, the rate of inclusion into IC was no different by comparing two distinct decades, i.e. 1995-2000 and 2000-2005 (p=0.34), while age by itself had a major. *Conclusions*. Near half of older patients with AML are excluded from clinical trials aiming at CR achievement. In most cases, selection is operated according to clinical or logistic factors, while relevant biologic features such as cytogenetics at diagnosis are not taken into account. The adoption of well defined comorbidity scales could help for a standardization of elderly AML patient population.

0047

PEGFILGRASTIM IN PATIENTS WITH AML OLDER THAN 60 YEARS. AN ANALYSIS OF THE AML97#38 AND AML2004#69 STUDIES OF THE EAST GERMAN HAEMATOLOGY AND ONCOLOGY STUDY GROUP (OSHO)

H.K. Al-Ali

University of Leipzig, LEIPZIG, Germany

Limited data exist concerning the efficacy of pegfilgrastim in AML. Outcome of patients >60 years after induction-chemotherapy followed-by pegfilgrastim was analysed in the AML2004-study and compared to the outcome of patients who received filgrastim or no G-CSF following the same induction in the AML97-study. Pegfilgrastim serum concentrations and their correlation to WBC recovery are presented. *Methods.* Informed consent was obtained from all patients. 138 patients (77m/61f) received induction-chemotherapy (cytarabine 2 g/m^2 iv day 1,3,5,7 and mitoxantrone 10 mg/m^2 iv day 1,2) followed-by pegfilgrastim 6mg (sc) day 10 in the AML2004 study (group-A). 408 patients (212 m/196f) received the same induction-chemotherapy in the AML97-study followed-by filgrastim 5 µg/kg/day starting day 10 until ANC recovery (>0.5×10^9/L) (n=236,58%) (group-B) or no G-CSF (n=147,36%) (group-C). Patients who received G-CSF later than day 10 were excluded. *Results.* Gender, cytogenetics, and *de novo vs* secondary AML were similar in both studies. Median age in AML2004-, and AML97-study was 68 and 66 years respectively (p=0.001). TRM in the AML2004- and AML97-study was 17% and 13% respectively (p=0.3). Complete remission rate (CR) was 57% in both studies. CR in group-B and group-C were 60% and 51% respectively (p=0.04). There was no statistical difference in CR between group-A and group-B (p=0.6). In multivariate analysis, favourable cytogenetics (p=0.001) and filgrastim-treatment (p=0.04) were associated with higher CR. For group-A, ANC>0.5×10^9/L and WBC>1×10^9/L occurred at a median of 24 and 23 days respectively. In multivariate analysis, age <70 years, and CR were associated with earlier ANC and WBC recovery. Patients <70 years recovered at a median of 22 compared to 28 days for patients >70 years (p=0.002). Patients achieving CR, recovered at a median of 23 compared to 26 days for patients not in CR (p=0.0001). In the AML97-study, WBC>1×10^9/L occurred at a median of 23 days. In multivariate analysis, filgrastim-treatment, and occurrence of CR were associated with an earlier ANC and WBC recovery. Median duration of neutropenia grade 4 was 21 for group-B compared to 25 days for group-C (p<0.0001). For patients achieving CR, pegfilgrastim- and filgrastim-treatment were associated with an earlier ANC and WBC recovery compared to group-C patients in CR (p<0.0001). Median serum concentrations of pegfilgrastim at peak level and at WBC recovery were 87.6(range 8.4-445) and 2.7(range 0.2-40.2) ng/mL respectively. On day 21 of chemotherapy-cycle, median serum pegfilgrastim was 6.8 (range 1.9-64.7) ng/mL. There was no correlation between peak levels and WBC recovery. Therapeutic levels >2 ng/mL were measured for a median of 17(range 9-27) days after a single application. *Conclusions.* Pegfilgrastim had no negative impact on CR, TRM, or duration of neutropenia compared to filgrastim in patients with AML >60 years treated with the same induction-chemotherapy. An in-trial analysis, demonstrated not only the expected faster recovery from grade 4 neutropenia (particularly in patients achieving CR) but also an increase in CR rate with filgrastim-treatment compared to no G-CSF. Whether this is true for pegfilgrastim needs to be evaluated in studies. Some patients had sub-therapeutic pegfilgrastim levels on day 21 of chemotherapy-cycle. Measuring serum pegfilgrastim levels at regular intervals might identify patients who might require additional doses.

Anemia and bone marrow failure

0048

THE OUTCOME OF PATIENTS =60 YEARS WITH ACQUIRED APLASTIC ANEMIA TREATED WITH IMMUNOSUPPRESSIVE THERAPY (IST): NO DIFFERENCE IN RESPONSE RATE AND SURVIVAL BETWEEN STANDARD *vs* ATTENUATED IST

Y. Kao, W. Xu, J.M. Brandwein, J.H. Lipton, H.A. Messner, M.D. Minden, A.C. Schuh, A.D. Schimmer, K. Yee, V. Gupta

Princess Margaret Hospital, TORONTO, Canada

Background. Immunosuppressive therapy consisting of Antithymocyte-globulin (ATG) and cyclosporine (CSA) is the current standard of care for younger patients with acquired aplastic anemia (AA) who are not candidates for bone marrow transplantation. However, the optimal IST strategies for older patients are not well established due to concerns related to intolerability or toxicities associated with standard doses of IST used in younger patients. *Aims*. To evaluate the outcome of IST in an unselected population of older patients (≥60 years) with AA and to identify prognostic factors for survival. *Methods*. Retrospective chart review of all consecutive patients ≥60 years treated at Princess Margaret Hospital (PMH), Toronto, Canada for AA between 1993 and 2007. The diagnosis of AA was established according to the standard criteria (International Agranulocytosis and Aplastic Anemia Study, 1987). *Results*. The study includes 24 consecutive patients treated with IST at PMH. Median age was 70 years (range 61-78) and median follow up of survivors was 48 months (range 8-103). The severity of AA was: very severe (n=9), severe (n=13), and non-severe (n=2). Standard dose treatment was defined as ATGAM (160 mg/kg) or thymoglobuline (18.75 mg/kg) with or without CSA. Attenuated treatment was defined as ≤50% dose of ATGAM/thymoglobuline with CSA or CSA alone. Seven patients received standard dose treatment, and 17 patients received attenuated treatment. The decision regarding treatment intensity was made by the treating physician based on the physician's perception of tolerability of treatment in the individual patient. Six patients (25%) died early within 3 months of starting IST due to sepsis (n=4), bleeding (n=1), or cardiac complications (n=1). Out of 18 patients evaluable for response, 12 achieved CR/ PR according to criteria defined by Camitta (Acta Haematologica, 2000). The 2-year cumulative incidence of response was 42% (95% CI 26%-69%). Of the responders, three patients subsequently had relapse and received a second course of ATG with 2 out of 3 achieving a second response. The 10-year cumulative incidence of evolution to clonal disorders (MDS/AML/symptomatic PNH) was 19% (95% CI 6%-65%). The 3-year probability of overall survival and failure-free survival was 49% (95% CI 27%-68%) and 16% (95% CI 4%-35%), respectively. Moderate or severe co-morbidities (p=0.03) as determined by Charlson Co-morbidity Index and very severe AA (p=0.007) were associated with significantly inferior overall survival. There was no impact of standard *vs* attenuated treatment intensity on response rate (43% *vs* 53%, p=0.4) or 3-year survival (43% *vs* 51%, p=0.6). Early mortality appears higher in the standard treatment group although not statistically significant (43% *vs* 18%, p=0.4). *Summary and Conclusions*. Early mortality is a major problem in the treatment of older patients with AA. Future studies should focus on reducing the early mortality by investigating better supportive care strategies and less toxic IST regimens.

0049

DEVELOPMENT OF A DISEASE SEVERITY SCORING SYSTEM FOR TYPE 1 GAUCHER DISEASE

M.D. Cappellini,[1] T. Cox,[2] E.H. Giannini,[3] G.A. Grabowski,[3] W.L. Hwu,[4] H. Mankin,[5] A.M. Martins,[6] C. Sawyer,[7] S. Vom Dahl,[8] N. Weinreb,[9] M. Yeh,[7] A. Zimran[10]

[1]University of Milan, MILANO, Italy; [2]University of Cambridge, CAMBRIDGE, UK; [3]Cincinnati Children's Hospital Medical Center, CINCINNATI, USA; [4]National Taiwan University Hospital, TAIPEI, Taiwan; [5]Massachusetts General Hospital, BOSTON, USA; [6]Universidade Federal de Sao Paulo, SAO PAULO, Brazil; [7]Genzyme Corporation, CAMBRIDGE, USA; [8]St. Franziskus-Hospital, COLOGNE, Germany; [9]University Research Foundation for Lysosomal Storage Disorders, CORAL SPRINGS, USA; [10]Sha'are Zedek Medical Center, JERUSALEM, Israel

Background. A validated disease severity scoring system (DS3) for type 1 Gaucher disease (GD1) is needed to monitor progression and treatment response in individuals and to compare patient cohorts in clinical studies. *Aims.* To develop and test the reliability and validity of a DS3 to assess and monitor GD1 in routine clinical care and to stratify for disease severity in clinical studies. *Methods*. DS3 domains were established by an expert physician group using nominal group technique (NGT) consensus formation methodology. Items within domains were selected by Delphi survey of 32 international physician experts in GD1. The expert group analyzed survey data (including preliminary screening for reliability, feasibility, and face, content, discriminant, and predictive validity) to determine appropriate measurement techniques for each variable. Measurements were weighted considering how much morbidity and mortality each contributes to GD1. Severity scores for sample patient cases determined by NGT were compared to scores determined by the DS3 and the Zimran SSI, an existing severity scoring index for GD1. *Results*. The DS3 includes bone, hematological, visceral, and physician- and patient-reported domains. Clinical domains were populated with weighted measurements of GD1 signs and symptoms. Patient case scores assigned by the expert group were more highly correlated with DS3 scores (Pearson r=0.98) than those obtained with the existing SSI index (Pearson r=0.88). *Conclusions*. This provisional DS3 provides for accurate assessment of GD1 status. Testing of reliability and validity will continue for ultimate implementation of the DS3 in clinical practice and trials.

0050

DEFICIENCY OF RED CELL BOUND CD55 AND CD59 IN PATIENTS WITH RHEUMATOLOGICAL DISORDERS

A. Sarantopoulos,[1] E. Terpos,[2] S. Masouridi,[3] J. Stavropoulos,[2] G. Karagiannidis,[3] P. Konstantopoulou,[3] C. Antoniadis,[4] G. Vaiopoulos,[3] J. Meletis[3]

[1]Second Blood Transfusion Center, Laikon General Hospital, ATHENS,; [2]Department of Medical Research, 251 General Air Force Hospital, ATHENS; [3]First Dpt of Medicine, University of Athens School of Medicine, Laikon Hospital, ATHENS; [4]Department of Rheumatology, Asklepieion Hospital, ATHENS, Greece

Background. Inappropriate activation or blockage of the inhibition of complement system may be responsible for tissue damage in rheumatological disorders. CD55 and CD59 are complement regulatory proteins that are linked to the cell membrane via a glycosyl-phosphatidylinositol anchor. They are reduced mainly in paroxysmal nocturnal hemoglobinuria (PNH). The aim of this study was to evaluate the presence of CD55 and/or CD59 deficient erythrocytes in patients with rheumatological disorders and explore possible correlations with clinical parameters. *Patients and Methods*. CD55 and CD59 expression was evaluated in erythrocytes surface of 54 patients (10M/44F; median age 62.5 years) with different rheumatological diseases: 23 had rheumatoid arthritis (RA), 16 systemic lupus erythematosus (SEL), 11 Sjogren syndrome (SS), and 4 scleroderma. At the time of evaluation, all but 8 patients were under anti-inflammatory therapy. The detection of CD55- and CD59-deficient red cells was performed using the sephacryl-gel microtyping system (DiaMed-ID MicroTyping System PNH test, Cressier-sur-Morat, Switzerland). The presence of the deficient red cells was blindly scored by two independent observers and expressed semiquantitatively as 100%, 75%, 50%, 25% and 10%. In all samples with CD55- or CD59-negative populations Ham and sucrose lysis tests were also performed. Eight PNH patients and 121 healthy subjects were also studied as control groups. *Results*. Anemia was present in 29 patients (53%); none had positive direct Coombs test. Interestingly, all but one patient (with RA) had erythrocyte populations with CD55 and/or CD59 deficiency. More specifically, deficient red cells for both CD55/CD59 antigens were detected in 18 patients (33%): in 14 patients erythrocytes were deficient for both antigens at a proportion of 10%, while 4 patients had erythrocytes with 25% of CD55 deficiency and 10% of CD59 deficiency. Isolated CD55 negativity was observed in 33 patients (61%): 31 had red cells with 10% CD55 deficiency and only 2 had erythrocytes with 25% CD55 deficiency. Isolated CD59 deficiency was detected in only one SS and one scleroderma patient. There were no clinical or laboratory evidence of hemolysis in our patients. Among 121 normal subjects, two (1.6%) had red cells with double negativity for CD55/CD59, while 3 (2.4%) had erythrocytes with an isolated CD55 or CD59 deficiency; these red cells were counted for not more than 10% of the total. All patients with PNH had a simultaneous CD55/CD59 deficiency. Positive Ham and sucrose tests were found only in PNH patients. There was no correlation between the presence of these defective red cells and the type of rheumatological disease, type or length of anti-inflammatory therapy, presence of anemia, or titers of anti-nuclear or other detected

auto-antibodies. *Summary and Conclusions*. Our study provides evidence supporting the presence of erythrocytes with CD55 and/or CD59 deficiency in patients with rheumatological disorders. A reduction in CD55/CD59 synthesis, blockage or masking of these molecules by auto-antibodies, or proteolytic cleavage in relation to activation of complement on erythrocyte surfaces may be responsible for this acquired phenomenon. Further studies using molecular techniques will be required for clarifying the exact role of this deficiency to the increased susceptibility of these populations in complement lysis.

0051

DETECTION OF CIRCULATING ANTI-ERYTHROPOIETIN ANTIBODIES IN PATIENTS WITH END STAGE RENAL DISEASE ON REGULAR HEMODIALYSIS

M. Attia, M. Eldin, M. Labib, A. Omer

Faculty of Medicine, Ismailia University, ISMAILIA, Egypt

Background. Recombinant human erythropoietin (rHuEPO) has been successfully and safely used to treat anemia in patients with end stage renal disease (ESRD). The safety profile of rHuEPO had been considered to be excellent with possible exception of hypertension and increased risk of dialysis access thrombosis. Recently, antibody-mediated pure red cell aplasia (PRCA) associated with administration of rHuEPO has been identified as a cause of major concern. The use of rHuEPO therapy can stimulate the development of neutralizing anti-EPO antibodies which probably cross react with the patient's endogenous EPO as well and lead to anemia that is more severe than even before the onset of rHuEPO therapy. *Aims*. It was to detect and evaluate the presence of anti-EPO antibodies in patients with ESRD on regular dialysis who are using rHuEPO. *Methods*. Serum anti-EPO antibodies were detected by quantitative ELISA technique in a total of 90 patients who are currently on regular hemodialysis and using rHuEPO alpha subcutaneously for more than 6 months. The dose and type of rHuEPO administrated in all studied patients was insignificantly different. All patients were subjected to full history taking and clinical examination. Complete blood count, reticulocytes count, serum creatinine, blood urea, serum albumin, serum ferritin, and hepatitis markers were done for all. Patients known to have hematological disease that cause bone marrow failure were excluded. *Results.* Our results showed that 35 patients (38.9%) had the anti-EPO antibodies in their blood, while 55 patients (61.1%) didn't have the circulating Abs. The mean hemoglobin (Hb) level was significantly lower in the antibody positive group (8.8 mg±1.35) than in the antibody negative group (9.42 mg±1.32) (p=0.000). The reticulocytes count was also significantly much lower in the patients who had anti-EPO antibodies with mean of 1.99 (±1.14) *vs* 3.15(±0.89) in the antibody negative (p=0.000). The dose of EPO administrated in both studied groups was insignificantly different. *Conclusions*. The incidence of anti-EPO Abs is high in ESRD patients on maintenance hemodialysis. Its presence is associated with increased incidence of anemia possibly due to immune-mediated inhibition of erythropoiesis as evidenced by reticulocytopenia.

0052

FLOW-CYTOMETRIC ANALYSIS OF BONE MARROW GRANULOCYTIC PROGENITOR/PRECURSOR CELLS IS A USEFUL TOOL FOR ESTABLISHING THE DIAGNOSIS OF CHRONIC IDIOPATHIC NEUTROPENIA

H. Koutala, M. Velegraki, M. Ximeri, G. Gvazava, M. Psyllaki, C. Kalpadakis, G. Eliopoulos, H. Papadaki

University of Crete School of Medicine, HERAKLION, CRETE, Greece

Background. Chronic idiopathic neutropenia (CIN) is defined as the persistent, unexplained reduction in the number of peripheral blood neutrophils. The diagnostic criteria of CIN are exclusion criteria: (a) neutropenia more than three months, (b) no evidence for any underlying disease associated with neutropenia, (c) no history of exposure to irradiation, use of chemical compounds or intake of drugs to which neutropenia might be ascribed, (d) normal bone marrow (BM) karyotype, (e) negative serum anti-neutrophil antibodies. *Aims.* To study the immunophenotypic quantitative and qualitative characteristics of BM granulocytic progenitor/precursor cells in patients with CIN compared to normal subjects and investigate the possible utility of flow-cytometry in establishing the diagnosis of CIN. *Methods*. Heparinized BM aspirates obtained from 16 patients with CIN and 28 healthy controls after informed consent, were stained within 12 hours of collection using a whole blood lysis technique and a panel of directly conjugated antibodies. Five-parameter, 3-color flow-cytometry was performed using CD45 *vs* side scatter (SSC) as the basic scattergram to define the granulocytic progenitor/precursor cell gate. The following populations were defined: the $CD34^+/CD33^+$ cells corresponding to the granulocytic progenitors; the DR^+ cells corresponding to myeloblasts; the $CD13^+/CD16^-$ cells comprising mainly to promyelocytes; the $CD11b^+/CD16^-$, $CD11b^+/CD16^{dim}$ and $CD11b^+/CD16^{bright}$ cells comprising mainly the myelocytes, metamyelocytes, and bands plus neutrophils, respectively. CD10 antigen, mainly expressed in bands and neutrophils among cells of the granulocytic lineage, was also studied. The myeloperoxidase (MPO) positive cells and SSC properties (mean SSC value) of total granulocytic cells were also evaluated to assess overall granularity. *Results*. The proportion of the $CD34^+/CD33^+$ granulocytic progenitor cells did not differ significantly between patients and controls. A significant increase was observed in the proportion of DR^+ myeloblasts in CIN patients (8.10±4.23%) compared to controls (4.59±1.24%; p=0.0033) suggesting a shift-to-the left of the granulocytic series. The proportion of $CD13^+/CD16^-$ cells (promyelocytes) did not differ significantly between patients and controls. However, a significant increase was obtained in the proportion of $CD11b^+/CD16^-$ cells (myelocytes) in patients (26.46±7.73%) compared to controls (17.99%±5.09%; p<0.0001) that was associated with a significant decrease in the proportion of $CD11b^+/CD16^{dim}$ (metamyelocytes) and $CD11b^+/CD16^{bright}$ (bands and neutrophils) cells in the patients (26.82±6.29% and 31.16±9.43%, respectively) compared to controls (21.76±5.44% and 49.02±11.88%, respectively; p=0.0056 and p<0.0001, respectively). In accordance with the low proportion of the mature cells of the granulocytic series was the low proportion of the $CD10^+$ cells in the CIN patients (13.48±14.93%) compared to controls (37.15±11.87%, p<0.0001). The proportion of MPO^+ cells and the mean SSC value did not differ significantly between patients and controls suggesting normal granularity of granulocytic precursor cells in CIN. *Conclusion*. Flow-cytometric analysis of BM granulocytic cells in CIN patients indicates a shift to-the-left with increased proportion of myeloblasts and myelocytes and decreased percentages of metamyelocytes, bands, and neutrophils compared to controls. The granular content of cells is normal suggesting the lack of dysplastic features of granulocytic series in CIN. Since diagnosis of CIN is mainly based on exclusion criteria, we suggest flow-cytometric immunophenotyping as an additional, useful tool for establishing diagnosis of CIN.

0053

PRESENCE OF THREE POINT MUTATIONS IN THE ELA2 GENE OF A PATIENT WITH SEVERE CONGENITAL NEUTROPENIA (SCN)

M. Lanciotti, G. Caridi, S. Pigullo, T. Lanza, C. Dufour

G. Gaslini Institute, GENOVA, Italy

Background. Severe congenital neutropenia (SCN) is an inborn disorder of granulopoiesis with a marked propensity to develop acute myeloid leukemia and myelodysplasia. Mutation of the ELA2 gene encoding neutrophil elastase are responsible for most cases of SCN and cyclic neutropenia (CN) a related but milder disorder of granulopoiesis. To date more than 50 distinct mutations of the ELA2 gene have been identified in patients with CN or SCN. With a few exceptions, specific ELA2 mutations are associated with SCN or CN, but not both, suggesting a genotype-phenotype correlation. *Aims*. Here we report the rare phenomenon of the presence of three point mutations in the ELA2 gene of a patient with SCN with a severe phenotype, characterised by long-lasting fungal pneumonia and myelodysplasia evolved to acute myeloid leukemia. The study of this case could give more information on the pathological mechanism of the congenital neutropenia. *Methods*. The ELA2 gene (GenBank Y00477) were amplified from genomic DNA extracted from peripheral blood of the patient and her parents and directly sequenced on ABI PRISM 3100. PCR fragments were also subcloned and individual clones were isolated and sequenced. Paternity and maternity were confirmed by specific microsatellite markers analysis. *Results*. Sequencing of ELA2 in genomic DNA of patient identified three different heterozygous single-base substitutions. One *de novo* mutation (2192G>A) generates a nonconservative amino acid missense substitution (V53M) within exon 3, it is described associated with CN phenotype. The second mutation is a previously undescribed single-base change (2240G>A) within exon 3 leading to amino acid missense substitution (V69M). This mutation was inherited from her unaffected mother. The third was a undescribed *de novo* intronic mutation (IVS3-22G>A). Both the undescribed mutations were neither detected in 80 healthy controls nor in 70 unrelated subjects who had neutropenia without ELA2 mutations, so that a polymorphic change could be excluded. The subcloning and sequencing of PCR amplified segment revealed that all three mutations always occurred together in cis on the maternal allele.

Summary and Conclusions. The presence of more than one mutation in ELA2 gene in a patient with SCN or CN is a very rare phenomena. So far only two cases (1 SCN and 1 CN) with a double mutation have been described. In both cases the presence of two mutations seems to have a positive effect on the diseases giving a clinical mild phenotype. On the contrary in our case the presence of the three mutations seems to have a sinergistic negative effect on the disease giving an extremely clinical severe phenotype. In fact the *de novo* V53M mutation has been reported in a case of CN which is a mild syndrome, with no increased propensity to develop acute myelogenous leukemia or mielodysplasia. The V69M mutation seems to be silent in the case of the unaffected mother, but it could have some synergistic effect on the structure of the patient protein giving rise to a particularly disrupting structure. The peculiarity of this case offers the opportunity to investigate molecular mechanism distinguishing SCN from CN.

0054

QUANTITATIVE EXPRESSION OF CD64 ON NEUTROPHIL GRANULOCYTES AS EARLY MARKER OF SEPSIS OR SEVERE INFECTION

G.B. Lobreglio, P. D'Aversa, L. Leo, S. Scolozzi, G. Fiore

A.O. Card G. Panico, TRICASE, Italy

Background. One of the most important aspects of infectious disease in recent decades has been the increase in cases of sepsis, endocarditis and bacteremia. Relevant studies have indicated that quantitative neutrophil (PMN) CD64 (high-affinity Fc receptor) expression is a worthwhile candidate for evaluation as a more sensitive and specific laboratory indicator of sepsis or the presence of a systemic acute inflammatory response. *Aims*. We prospectively evaluated performance of CD64 expression on PNMs as improved laboratory indicator of severe infection, sepsis/septic shock and compared it to serum procalcitonin (PCT), interleukin 6 (IL-6), other standard laboratory tests (ESR: erythrocyte sedimentation rate, CRP: C-reactive protein) and to the final results of blood cultures. *Methods*. 30 clinical samples, divided into two groups (Gr. 1, Gr. 2) were analysed. Gr. 1: 14 patients admitted in different Emergency Department of the hospital with clinical diagnosis of sepsis; Gr. 2: 16 samples as outpatients. In all samples the levels of leukocyte neutrophil CD64 were measured with Leuko64 kit (Trillium Diagnostics, Maine, USA) on haematology platform, CELL-DIN Sapphire (Abbott Laboratories, Abbott Park, USA); the other parameters mentioned were determined with different immunoassaies. Leuko64 test was considered positive for an index of expression of CD64 > 1,5. *Results*. Among 11 patients with positive blood cultures 10 had CD64 index >1,5; by contrast all the patients without infection had CD64 index <1,5: sensitivity 91,7%; specificity 100%. As shown in Table 1 there is a poor or fair correlation between CD64 index and other biochemical parameters. *Summary and Conclusions*. The rise of neutrophil CD64 index in patients with positive blood cultures suggests that it is an improved laboratory parameter in identifying patients with sepsis; moreover, the test is easy to perform on a routine hematology analyzer and thus may find a wider spread in clinical practice for studying early onset infection.

Table 1. Correlation indexes of the several markers pair and CD64 index against blood cultures result.

Correlation markers	Correlation index (r)
CD64 index - PCT	0,4740
CD64 index – IL- 6	0,4047
CD64 index - CRP	0,5921
CD64 index - ESR	-0,0495
Blood cultures + : 11	CD64 index >1,5: 10
Blood cultures - : 19	CD64 index <1,5: 20

0055

ALTERATIONS OF MESENCHYMAL STROMAL CELLS IN PATIENTS WITH APLASTIC ANEMIA

V. Petrova, N. Nifontova, A. Mikhaylova, J. Drize

National Hematology Research Centre, MOSCOW, Russian Federation

Background. Ineffective hematopoiesis in patients with aplastic anemia (AA) could be caused by hematopoietic stem cells (HSC) failure, disordered interactions between HSC and stromal cells and alterations in stromal microenvironment itself. Mesenchymal stromal cells (MSC) and their early progeny fibroblast colony forming units (CFU-F) are the progenitors of stromal microenvironment. *Aims*. The goal of the study was to investigate the main properties of CFU-F and MSC of AA patients. *Methods*. MSC were isolated by cultivating 2,5-3 ı 106 mononuclear cells from the bone marrow in the 25 cm^2-cultivation flasks in α-MEM with 10% of fetal calf serum. Cells were passaged on average once in 10 days. To induce osteogenic differentiation MSC were cultivated with dexametasone, ascorbate-2-phosphate and NaH2PO4. CFU-F frequency was calculated using standard assay. To characterize alterations in the expression of *FGF2* and *BMP4* in MSC and CFU-F semi-quantitative analysis of RT-PCR products was performed using PhosphoImager Cyclone, Packard Bell (USA) after Southern blot hybridization with appropriate sequences. The expression level of β-actin was used as a normalization factor. *Results*. The ability to establish MSC cultures was the same for donors and patients with AA. There were no CFU-F detected in the bone marrow of 3 out of 20 tested AA patients; 4 out of 20 contained regular-sized colonies, equal to the ones of the donors, and in 13 cases the formed colonies were much bigger than the donors' ones judging by size estimated using the electronic images by means of Scion Image software (28,7±1,3 px/mm^2 *vs* 9,1±0,5 px/mm^2 , p<0.001). The increase in CFU-F frequency and size of the colonies could be a consequence of alterations in the expression level of such genes as FGF2 and BMP4. The expression level of these genes in donor cultures was approximately the same in MSC and CFU-F (*for data see Table*). In MSC the FGF2 expression in donors and AA cultures did not differ, but in CFU-F the expression level of this gene increased gradually and reached maximum in patients with refractory anemia. The expression of BMP4 decreased in cultures from AA patients and increased up to donor's level in cultures from patients with severe AA. There was inverse negative relationship between the ability of AA MSC to perform osteogenic differentiation, estimated as +, 2+ and 3+, and expression of BMP4 (/3± 0.08, /2± 0.29,/± 1.00, /-/ - 1.06).

Table 1.

Group	CFU-F		MSC	Relative expression level			
	Number per 10^6 bm cells	% enlarged colonies	% of osteogenic differenciation	CFU-F		MSC	
				FGF2	BMP4	FGF2	BMP4
Donors	30.98 ± 10.4	0	100	0.54 ± 0.11	0.87 ± 0.32	0.65 ± 0.09	0.92 ± 0.27
AA	50.68 ± 19.91	57.14	80	0.77 ± 0.32	0.19 ± 0.07	0.83 ± 0.23	0.34 ± 0.12
Severe AA	50.47 ± 11.48	90	57.2	1.0 ± 0.15	1,24 ± 0.24	0.82 ± 0.26	1.28 ± 0.28
Ref. A	99.0 ± 53.6	25	25	1.5 ± 0.09	0.81 ± 0.26		

Conclusions. The data suggest that the ability of MSC to osteogenic differentiation reduced in AA patients accompanying the alterations in *BMP4* expression. The number and the size of CFU-F increased in patients correspondingly the deterioration of the disease. Simultaneously the expression level of FGF2 significantly increases in the CFU-F. It seems that alterations in the number of stromal precursor cells and changes of gene expression profile indicate the *attempts of the organism* to enlarge the hematopoietic territory for improvement of hematopoiesis.

0056

MULTIFOCAL LANGERHANS CELL HISTIOCYTOSIS IN ADULTS. SINGLE CENTER EXPERIENCE

D. Bogdanovic,[1] M. Cemerikic Martinovic,[1] D. Jovanovic Perunicic,[1] N. Bogunovic,[1] B. Milenkovic,[2] M. Pavlovic,[3] L. Medenica,[4] P. Kendereski,[5] R. Colovic,[6] P. Mandaric,[2] D. Colovic[1]

[1]Institute of Hematology CCS, BELGRADE; [2]Institut of lung diseases and TBC, BELGRADE; [3]Institute of infectious and tropic diseases, BELGRADE; [4]Clinics for dermatovenerology CCS, BELGRADE; [5]Institute of endocrinology CCS, BELGRADE; [6]Institute for digestive surgery CCS, BELGRADE, Serbia

Langerhans cell histiocytosis (LCH) is rare, clonal disease characterized by histocyte proliferation. Langerhans cells have specific immunophenotype and specific Bürbeck granule on electron microscopy. This disease is very rare in adults and most data are based on appearance of LCH in children. In adults, most of cases are single eosinohillic granuloma and multifocal disease is uncommon. Patients. In period of last 12 years, in a single center, we have evaluated and treated 6 patients with multifocal LCH. Patients were confirmed by specific immunohistochemistry profile, HLA-DR$^+$, S-100$^+$, CD1a$^+$, CD68$^+$ (Dako, Danmark) with also had positive Histiocyte X Ag$^+$ (Immunotech, France). We had predominance of male gender (5 males and one female), mean age of 54 years at presentation. The occurence of LCH was very different. Patients had the following types of the LCH 1) fever with bone marrow infiltration and infiltration of spleen; 2) multiple infiltrates in lungs with diabetes insipidus; 3) skin infiltration with erythrodermia, pulmonary lessions and cervical and axillar lymph node infiltrates; 4) multiple recidivant changes in lungs with affection of mediastinal lymph nodes; 5) multiple changes affecting spleen, liver, bone marrow and CNS; 6) multiple lymph node infiltration in neck. *Treatment*. From our 6 patients, in one we had noted spontaneous dissappearance of the disease after elective splenectomy, and we found previous intermittent course (fibrous scars in spleen and liver). In two patients lung surgery was necessary followed by short term corticoids. Two patients were treated with chemotherapy. Patient No 3 with eryhtrodermia was treated by LCH-1A protocol adjusted for adults, but patient No 5 was treated with LCH-2 protocol after limitted surgery and afterwards by other chemotherapy (*CHOP, MTX*) and by intratecal chemotherapy due to progression of the LCH in CNS. One patient was treated only by involved field radiotherapy. Besides the patient with CNS disease, who subsequently died due to progression, other five patients, all males, are in good condition, and all are in remission of the LCH lasting several years after treatment. *Conclusions*. Multifocal Langerhans cell histocytosis in adults is much rare disease than in childhood. It runs as chronic, intermittent disease, and do not need prompt chemotherapy except in cases with disease progression (scoring system of the International Histiocyte Society). Elective surgery is efficient in patients with lung affection and also radiotherapy has its place in selected cases. The establishment of specific LCH-A1 protocol will provide better treatment strategy in future therapy of adults with LCH.

0057

HIGH PROPORTION OF MIXED CHIMERISM WITH T-CELL DEPLETED PERIPHERAL STEM CELL TRANSPLANTS FOLLOWED BY T-CELL ADD-BACK IN PATIENTS WITH APLASTIC ANEMIA

F. Verholen, E. Levrat, E. Roosnek, M. Terrettaz, C. Helg, J.R. Passweg, C. Chalandon

Service d'hématologie, GENÈVE, Switzerland

Stable mixed chimerism has been described as being beneficial in patients with aplastic anemia after stem cell transplantation, as these patients were protected from severe graft *vs* host disease and from rejection, the major drivers of mortality in these patients. Patients with marrow failure syndrome do not need full donor chimerism as they do not benefit from a graft-versus-malignancy effect. Here we describe a protocol resulting in high rate of stable mixed chimerism in patients conditioned with cyclophosphamide + ATG and transplanted with peripheral stem cells depleted by Campath with add-back of T- lymphocytes post-transplant at different dose levels, according to donor-recipient relationship. We included 10 patients in this protocol, median age was 35,5 (15-58) years, 5 were male, donors were identical siblings in 7, mismatched related in 1 matched unrelated in 1 and mismatched unrelated in 1, respectively. Stem cell dose was 11.5 (5.4-40.2) CD34×10^6/kg. Time to neutrophil engraftment was 15 (8-17) days and to platelet engraftment 14 (8-24) days. Median follow-up of surviving patients is 627 days (13-2533). Acute grade II GvHD was observed in 1 patient (with the mismatched unrelated donor), this patient died subsequently, while all other patients remain alive. There is mild chronic GvHD in 1, none of the patients lost the graft. Chimerism analysis showed full donor chimerism in 2, transient mixed chimerism (defined as mixed chimerism developing into full donor chimerism) in 1, and stable mixed chimerism in 6, limited to mononuclear cells in 1 and observed in the granulocyte and mononuclear compartment in 5. Mixed chimerism remained stable for up to 4 years. A protocol of conditioning with cyclophosphamide and ATG and GvHD prophylaxis with T-cell depletion using Campath and T-cell addback may induce stable mixed chimerism in a large proportion of patients with aplastic anemia with low GvHD and graft failure risks.

0058

IDENTIFICATION OF NEW RPS19 DELETIONS IN DIAMOND-BLACKFAN ANEMIA USING THE MLPA TECHNIQUE

P. Quarello,[1] E. Garelli,[1] A. Carando,[1] M.F. Campagnoli,[1] A. Brusco,[2] P. Pappi,[2] M. Barberis,[2] I. Dianzani,[3] U. Ramenghi[1]

[1]Hematology Unit, Pediatric Department, University of Turin, TURIN, Italy; [2]Department of Genetics, Biology and Biochemistry, University of Turin, TURIN, Italy; [3]Department of Medical Sciences, University of Eastern Piedmont, NOVARA, Italy

Background. Diamond-Blackfan anemia (DBA, MIM105650) is a rare congenital pure red cell aplasia due to an intrinsic defect in erythropoietic progenitors. Defects in the RPS19 gene are the main known cause of DBA and account for 25% of patients. In literature four complete deletions of RPS19 gene found by FISH and microsatellite analysis were described (*Gustavsson et al 1997, Gustavsson et al 1998, Campagnoli et al 2004*). Other 4 partial gene deletions (exon 3, 4 and 5) were reported by a haplotype analysis approach (*Orfali et al, 2004*) or by sequencing (*Draptchinskaia et al 1999, Proust et al 2003*). The usual PCR-based methodology used for conventional mutation detection fails to detect heterozygous deletions. The aim of this study is to establish the actual contribution of unidentified deletions to the overall detection rate of RPS19 mutations. *Patients and Methods*. DBA Italian patients (pts) that did not carry RPS19 mutations but were apparently homozygous (55 pts) for the intragenic RPS19 SNPs (c.1-450T>C; c.71+80_71+81insC; c.71+89C>G; c.356+14A>G; c.357-90C>T; c.412-175C>T) were included in this study. The analysis was made using Multiplex ligation-dependent probe amplification technique (MLPA; MRC-Holland, Amsterdam). The MLPA kit is designed to detect deletions/duplications of one or more exons of the RPS19 gene. Deletions of probe recognition sequences were identified by a 35-50% reduced relative peak area of the amplification product. *Results*. We identified three heterozygous deletions that spanned the whole gene. Neither RPS19 intragenic deletions nor duplications were found. No deletions were discovered in 8p23.3-8p22, a region linked to DBA (*Gazda et al, 2001*). Conclusions. Our study shows that large deletions in the RPS19 gene are more frequent than expected, representing around 12% of all mutations detected in our cohort (*vs* an average of 6% reported in literature). RPS19 is involved in at least 27% of Italian DBA patients.

0059

ASYMPTOMATIC CEREBRAL MICROBLEEDS IN PATIENTS WITH APLASTIC ANEMIA

S. Sharma, P. Malhotra, V. Lal, P. Singh, N. Varma, S. Varma

Post Graduate Institute of Medical Education and Research, (PGIMER), CHANDIGARH, India

Background. Severe thrombocytopenia is an important risk factor for the development of intra cerebral hemorrhage (ICH). The present guidelines recommends prophylactic platelet transfusion in patients whose platelet count is below 10,000/μL. However, sometimes serious episodes of hemorrhage do occur at relatively high platelet counts emphasizing the importance of factors other than platelet count in the cause of bleed. Recent studies suggest that cerebral microbleeds (CMB) seen on T2* - weighted gradient echo magnetic resonance imaging (GE-MRI) sequences as rounded areas of signal loss, generally less than 1cm are an indicator of imminent ICH. *Aims.* The aim of the present study was to find out the prevalence of CMB in patients of Aplastic anemia (AA) who are neurologically asymptomatic and relate with future development of ICH in them. *Methods*. Twenty-six patients of AA (diagnosed by standard criterias) below 40 years of age underwent GE-MRI brain for evaluation of CMB. All patients underwent evaluation for AA in the form

of complete blood count, bone marrow aspiration and trephine biopsy, serum chemistries, infectious disease markers. Patients received different types of treatment (Allogeneic stem cell transplant, antithymocyte globulin and cyclosporine, danazol and supportive treatment) based on their financial affordability as they did not have health insurance. All patients were followed up for at least 6 months for development of overt ICH. *Results*. The median age of the patients was 20.5 years (range 12-40 years). The median values of complete blood counts were hemoglobin 5.7 gm/dL (range 2.5-9.2 gm/dL), white cell count 2320 (800-7000/μL), platelet count 11500 (700-45000) at the time of GE-MRI of brain. Three patients (11.5%) had CMB at baseline. Additionally two patients had asymptomatic cerebral macrobleeds (>1 cm). During follow-up period of 6 months, four patients developed spontaneous ICH (two documented on imaging and two presumed on the basis of symptoms). Out of these 4 patients, only one patient had documented CMB at diagnosis while other three patients did not have CMB at the time of diagnosis. *Conclusions*. Asymptomatic CMB were found in 11.5% of patients with AA below 40 years of age. The overall prevalence of asymptomatic ICH (CMB & macrobleeds) was 19.2%. The present study could not demonstrate association between presence of CMB at the time of diagnosis of AA and future development of spontaneous ICH.

0060

ENERCA2, THE EUROPEAN NETWORK AIMED AT IMPROVING THE DIAGNOSIS, EPIDEMIOLOGICAL KNOWLEDGE AND MEDICAL EDUCATION ON RARE AND CONGENITAL ANAEMIAS

P. Aguilar-Martinez,[1] on behalf of the ENERCA2[2]

[1]CHRU de Montpellier, MONTPELLIER, France; [2]Hospital Clinic Provincial, BARCELONA, Spain

Background and Aims. ENERCA is an European project co-financed by the EC Rare Disease Programme (DG SANCO) and co-ordinated from the Hospital Clínic i Provincial of Barcelona. Its primary goal is to contribute to the better knowledge, diagnosis, treatment and prevention of rare anaemias (RAs) in Europe. Here, we describe the achievements of the second phase of this project, *ENERCA2*, ran from September 2005 to August 2008. *Methods*. The *ENERCA2* Expert Group includes 12 members from several European countries and is organized into 9 working packages (WPs) with specific goals. Each *ENERCA2* WP has been working on a specific field dealing with rare anaemias. The main objectives of the current project are: 1) The provision of a fully revamped website, *ENERCA* website (*www.enerca.org*), launched in July 2006, providing two key information sources: a free-access section for patients, their families and the general public and an extranet section with restricted access for the professional community (WP1); 2) The intensive collection of epidemiological data concerning, i) neonatal screening on hemoglobinopathies in Europe (WP3), ii) membrane disorders, especially dealing with CDA (WP7) and on the indications of splenectomy for patients with hereditary spherocytosis (WP5), iii) red cell enzymopathies and their clinical and therapeutic aspects (WP7); 3) The construction of European databases on CDA (WP7) and thalassemia (WP9); 4) The organisation and the provision of a specialised European EQA dedicated to red cells diagnosis (HbA2 determination, specialized red cells cytology) (WP6); 5) The collection of data on experts European centres specialized in these disorders and the construction of lists of definitions of most of the uncommon anaemias, of support organizations listed by countries (WP1, WP4 and all the WPs). *Results*. The obtained information is available to the public in the free section of the website and to registered professionals, who have access to eight major ENERCA sections: 1) Expert Centres by countries, 2) Diagnosis flowcharts, which allow entering the patient's data and obtaining a basic orientation on the type of anaemia, 3) Professional on-line forum aimed at sharing data on difficult patient's records, 4) Database and registries section, which will allow to identify existing registries on RAs and to promote the development of new ones, 5) Guidelines and recommendations, prepared by several ENERCA WPs, 6) Medical Alert Card (MAC) that can be downloaded from the website, 7) Quality Assurance Programmes for laboratory diagnosis of RAs, 8) Newsletter (ENERCA News), published 3-4 times a year, which provides updated information on rare anaemias and on the ENERCA Expert Group activities. *Conclusions*. ENERCA is intended to promote cross-border cooperation among experts in Europe with the aim of implementing the creation and availability of easy-to-understand information for patients and updated knowledge on RAs in all its scientific, diagnostic, epidemiological and educational aspects for health professionals and services.

0061

SEVERE IRON OVERLOAD IN TRANSFUSION DEPENDENT DIAMOND BLACKFAN ANEMIA PATIENTS

P. Quarello,[1] S. Roggero,[2] T. Vinciguerra,[3] F. Longo,[2] M. Borri,[2] A. Piga,[2] U. Ramenghi[4]

[1]University of Turin, TURIN; [2]San Luigi Gonzaga Hospital, TURIN; [3]Pediatric Department, TURIN; [4]Dept.of Pediatric Hematology, TURIN, Italy

Background. The mainstay of Diamond-Blackfan anemia (DBA) treatment is corticosteroids: approximately 80% of the patients (pts) initially respond. The remaining 20% who are unresponsive to corticosteroids, and others, who may become refractory despite an initial response or encounter unacceptable toxic side-effects, are placed on a chronic transfusion therapy. Iron overload represents a serious complication of potentially lifesaving blood transfusion. *Methods*. We report a retrospective analysis of iron overload and its related complications in a cohort of 29 transfusion dependent Italian DBA pts (16 females and 13 males). These pts performed at least an evaluation of liver iron concentration (LIC) measured by the non invasive technique of magnetic liver susceptometry using a Superconductive QUantum Interference Device (SQUID) between June 2000 and June 2007. All pts were treated with chelation therapy. This population was compared to a group of transfusion dependent pts affected by β-thalassemia major matched for sex and years of transfusion dependence in order to identify potential differences in the amount of iron overload, type and age at onset of iron related complications. *Results*. The median age at the first SQUID evaluation was 11 years (y), the median transfusion therapy duration was 8 y and the median chelation therapy (Deferoxamine s.c.) duration was 6 y. Deferoxamine was well tolerated in all pts. At first SQUID evaluation median ferritin level was 1700±1147 ng/mL, median LIC (Liver Iron Concentration) was 2045±1209 ±g/Fe/g liver w.w.. A severe iron overload (LIC> 2000) was observed in 15 pts (52%), 10 pts (34%) showed a moderate (LIC 1000-2000) and 3 (10%) a mild (LIC 400-1000) iron overload. Iron overload was absent in 1 pt only. We found that DBA pts, compared with the group of β-thalassemic pts, showed a statistically higher LIC (Welch Two Sample t-test; $p<0,01$). At the first SQUID evaluation 9 pts showed at least one complication related to iron overload. The total number of complications was 14: 4 pts had hypothyroidism, 4 hypogonadism, 3 osteoporosis, 2 diabetes mellitus, one strumental cardiopathy and one hypoparathyroidism . The median time of first complication was 14 years from diagnosis, 12,8 years from the beginning of regular transfusions therapy. *Conclusions*. Secondary hemochromatosis is a major medical problem in transfusion-dependent pts. We found a very severe iron overload in transfusion dependent DBA pts, significantly higher if compared with β-thalassemic pts. This difference might be explained by a non optimal chelation therapy (late onset, type, dose, prescription and compliance) in addition to an unknown biological mechanism that lead to an early severe iron overload. Therefore, we suggest that every patient receiving transfusions must have an accurate record of iron intake and a regular monitoring of iron overload and to test the efficacy/compliance of chelation treatment. Physicians taking care of transfusion dependent DBA pts must be concerned about the frequent and early complications such as cardiac toxicity, hypothyroidism and osteoporosis.

0062

PULSED DEXAMETHASONE FOR THE TREATMENT OF PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANEMIA

F.R. Mauro, I. Del Giudice, S. Coluzzi, D. Armiento, M.S. De Propris, M.C. De Nicolò, M.C. Arista, L. Quattrocchi, M.G. Mazzucconi, G. Girelli, R. Foà

University La Sapienza, ROMA, Italy

Background. Continuous steroid administration, as recommended by Damashek *et al*. more than 50 years ago, is still the gold-standard first-line therapy for autoimmune hemolytic anemia (AHA). While the activity of high dose dexamethasone has been largely investigated in immune thrombocytopenic purpura, little is known in AHA. *Aims*. The aim of this study was to evaluate the therapeutic activity and toxicity of dexamethasone pulse therapy in AHA patients. *Methods*. Twenty-five patients with a newly diagnosed AHA (17 cases) or a first relapse of AHA (8 cases) were treated. The median age of patients was 64 years (range: 20-76), 15 patients were females and 10 males. The immunohematologic study revealed an anti-erythrocyte antibody (AeAb) in all cases (IgG: 16; IgM: 4; IgG + IgM: 2; IgA: 1;IgG +IgA: 2). An idiopathic AHA was diagnosed in 17 cases (68%), while in 8 (32%) a concurrent disease was identified. (tumor: 2; autoimmune disease: 5, lymphoproliferative disease: 1

case).Treatment consisted of at least 6 courses of dexamethasone 20 mg, total dose, administered iv or im for 5 consecutive days, every 3 weeks. Between each dexamethasone course, patients received prednisone orally at the initial dose of 0.5 mg/kg/day. During the subsequent courses, prednisone was slowly tapered by 5 mg every 1- 2 weeks to a minimum dose of 10 mg 3 times a week in patients who reached a Hb value ≥12g/dL. After 6 courses of dexamethasone, response was assessed according to the Hb value combined with the evaluation of the direct antiglobulin test (DAT). Patients with no detectable AeAb and persistent Hb values ≥12 g/dL were considered as complete responders (CR) and steroid treatment withdrawn. Patients with persistent AeAb, but with a Hb increase of at least 3 g/dL, were considered as partial responders (PR). Three further dexamethasone courses were given to PR patients with Hb values <12 g/dL, while patients with a persistent Hb value ≥12 g/dL underwent a maintenance dose of 10 mg three times a week. All patients received folic acid supplementation, trimethoprim-cotrimoxazole as Pneumocystis carinii prophylaxis and proton pump inhibitors as prophylaxis of related gastritis. Bisphosphonates were given as prophylaxis of osteoporosis. Red cells were infused in the presence of severe and symptomatic anemia. *Results*. Response was assessed in 20 cases. The OR was 85% (CR: 10%; PR: 75%) and was similar in previously treated and untreated patients, while it was higher, though not significantly, in IgG cases compared to IgM±IgG cases (87% *vs* 75%; p=0.04). To date, an hemolytic relapse was observed in 1 case. The median duration of response of patients with PR and maintained with low doses of prednisone was 25 months (range: 3-64), and 9 and 41 months, respectively, in the 2 patients with idiopathic AHA who achieved a CR. No significant side effects were recorded. *Conclusions*. Pulsed dexamethasone therapy is effective and well tolerated in AHA patients. However, CR are rare. Additional studies need to be designed to explore new dexamethasone schedules and the association with other treatment approaches such as rituximab.

0063

IMPROVEMENT OF HEMOGLOBIN LEVEL AND REDUCTION OF TRANSFUSION REQUIREMENT IN FOUR PATIENTS AFFECTED BY MYELODYSPLASTIC SYNDROMES AND PRIMARY MYELOFIBROSIS RECEIVING DEFERASIROX TREATMENT

E. Messa,[1] R. Catalano,[1] F. Messa,[1] F. Arruga,[1] I. Defilippi,[1] S. Carturan,[1] A. Rotolo,[1] D. Gioia,[2] A. Roetto,[1] E. Bracco,[1] A. Levis,[2] C. Camaschella,[3] D. Cilloni,[1] G. Saglio[1]

[1]University of Turin, TURIN; [2]Hematology Institute, ALESSANDRIA; [3]Vita Salute S.Raffaele, UNIVERSITY AND IRCC OF MILAN, Italy

Background. Transfusional iron overload is a frequent problem that clinicians have to face during treatment of patients affected by both myelodysplastic syndromes (MDS) and primary myelofibrosis (PMF). As a matter of fact, patients affected by low risk MDS or PMF have a relatively long term survival and develop, during the progression of disease, a transfusion requirement . That is why an iron overload and secondary hemosiderosis leading to a multi-organ failure frequently occurs. In order to avoid this complication, patients are now quite routinary treated with iron chelation (ICT), mainly due to the availabilty of oral chelators like Deferasirox. It has been recently reported that chelation therapy can reduced transfusion requirement and improve haemoglobin (Hb) levels in patients affected by MDS and PMF. *Aims.* Aims of our study were to evaluate the effects of ICT with the oral iron chelator Deferasirox on Hb level improvement and transfusion reduction in a population of MDS and PMF patients with transfusional iron overload. *Methods*. We retrospectively analized a population of 10 patients affected (7 MDS and 3 PMF) presenting with transfusional iron overload. Iron parameters and hemocromocytometric values were evaluated before chelation therapy and after one, three, six, and when available nine and twelve months of ICT. Statistical analysis was performed using the Mann-Whitney U test to compare continuous variables. A p<0,05 was considered significant. *Results*. Three MDS and one PMF patients out of 10 receiving ICT with Deferasirox showed an increase in haemoglobin level after few months of treatment. The median haemoglobin improvement calculated at the time of the best response was 2 g/dL (±0,5 g/dL). Two MDS patients reached within three and nine months a complete transfusional independence. The mean ferritin level was 4754 mg/L ±1633 (median 4786) before starting chelation therapy. The response was not correlated with ferritin reduction as in only one case serum ferritin was significantly reduced after three months. In the other cases, stable levels were maintained by low dosage of the drug .There were no statistically significant differences between the responders and non responders groups in terms of age, mean ferritin and Hb level before starting ICT, ferritin reduction during treatment and deferasirox daily dosage. None of the patients receiving chelation therapy for transfusional iron overload affected by thalassemia major or intermedia shows a comparable response in terms of hemoglobin level improvement. *Summary and Conclusions.* Here we describe four cases (three patients affected by MDS and one by PMF) in which iron chelation therapy with Deferasirox results into a significant improvement of the Hb levels and, in two cases, also into a complete transfusion independence. This almost unexpected effect of iron chelation treatment can be observed within few months and even with a very low drug dosage. Our observations confirm what already described sporadically and requires further investigations in order to better understand this peculiar Deferasirox's activity not observed during treatment with other iron chelators.

Chronic lymphocytic leukemia - Biology and Clinical prognosis

0064

P53 MUTATIONS IN A LARGE COHORT OF CLL PATIENTS WITH 17P DELETION: DETAILED ANALYSIS OF MUTATION PROFILE, ALTERNATIVE MECHANISMS OF INACTIVATION, CLONE SIZE AND CLONAL EVOLUTION

T. Zenz,[1] T. Denzel,[1] S. Häbe,[1] J. Mohr,[1] D. Winkler,[1] A. Bühler,[1] N. Patten,[2] S. Truong,[2] H. Döhner,[1] S. Stilgenbauer[1]

[1]University of Ulm, ULM, Germany; [2]Roche Molecular Systems, PLEASANTON, USA

Background. The prognosis of CLL with 17p deletion is very poor. While it is generally accepted that inactivation of p53 (by mutation) underlies refractoriness of CLL with 17p deletion, no study has analysed a large cohort of CLL patients with 17p deletion with respect to TP53 mutations and investigated other mechanisms of p53 inactivation. *Methods.* In order to assess the incidence of TP53 mutations in CLL with 17p deletion we studied TP53 mutations in a large cohort (n=94 patients) of these patients. We used DHPLC to screen for TP53 mutations (Exons 2-11). Aberrant DHPLC profiles lead to sequencing of the respective exons. A sub-group of cases were also studied with an array based p53 mutation platform to confirm the absence of mutations. In addition, detailed genetic studies (VH-mutation status, ZAP70, FISH) were available for the patients. *Results*. We found mutations in the protein coding region of p53 in 71 of 94 CLL patients (76%) with 17p deletion. In the vast majority of the cases the clone size (mutated) correlated very closely with the FISH results. Only few cases had more than one mutation (2/73). The majority of mutations were located in the DNA binding domain of p53. While we found no mutations in exons 2, 3, 10 and 11, 88% of the mutations were located in exon 5 to 8. As further evidence that mutations may also be observed in cases with 17p deletion in a sub-clone, we also observed mutations in cases with less than 20% of cells carrying the 17p deletion. The analysis of follow-up samples in a number of these cases with low grade 17p-deletion showed definite evidence for the selection of the p53 deficient clone (mutation and deletion). Importantly, the mutation occurred prior to exposure to chemotherapy. In spite of this clear evidence for a classical tumor suppressor mechanism underlying the resistance to chemotherapy in cases with 17p deletion, there remain cases where no mutation in the exons of TP53 can be detected by DHPLC, direct sequencing and an array based p53 mutation analysis, suggesting that in these cases alternative mechanisms lead to inactivation of p53. These mechanisms (e.g. alternative splicing, downregulation, functional defect) are currently under investigation (p21/p53 FACS, mRNA analysis). *Conclusions*. The current study supports the role of p53 inactivation (by mutation) underlying the chemo-resistance of CLL with 17p deletion. The extend of mutations of the remaining allele and the demonstration of coexisting mutations even in cases with deletions in only the minority of cells suggests that p53 is the main biological target of 17p deletion and its clinical consequence.

0065

GENOME-WIDE DNA ANALYSIS IDENTIFIES RECURRENT IMBALANCES PREDICTING OUTCOME IN CHRONIC LYMPHOCYTIC LEUKEMIA WITH 17P DELETION

F. Forconi,[1] A. Rinaldi,[2] I. Kwee,[2] E. Sozzi,[1] D. Raspadori,[1] P. Rancoita,[2] D. Rossi,[3] C. Deambrogi,[3] D. Capello,[3] E. Zucca,[2] D. Marconi,[4] R. Bomben,[4] V. Gattei,[4] F. Lauria,[1] G. Gaidano,[3] F. Bertoni[2]

[1]University of Siena, SIENA, Italy; [2]IOSI, BELLINZONA, Switzerland; [3]Ematologia, Università del Piemonte Orientale, NOVARA, Italy; [4]CRO, IRCCS, AVIANO, Italy

Background. Deletion of 17p(p53) identifies a rare subset of chronic lymphocytic leukemia (17p- CLL) with aggressive behavior. However, not all 17p- CLL behave poorly, and intrinsic mechanisms of dismal prognosis cannot simply be recapitulated by the genes deleted at the 17p locus. At present, chromosomal imbalances within the most aggressive 17p- CLL subset have not yet been defined. Genome-wide DNA analysis of copy number (CN) changes and loss of heterozygosity (LOH) is a new tool that can profile common recurrent chromosomal changes of pathogenetic significance in cancer. *Aims*. We aimed at identifying the recurrent genotype regulating tumor behavior of 17p- CLL by using very high-density SNP arrays. *Methods* Genome-wide DNA-profiling was performed in 18 patients with 17p- CLL (17p13.1 deletion in more than 60% nuclei) by means of the Human Mapping 250K-NspI arrays (Affymetrix, Santa Clara, CA, USA). Diagnosis and patients' management was performed according to 1996-NCI guidelines. Samples were collected at diagnosis and investigated for CD38 and ZAP-70 expression, mutational status of IGHV- and TP53-genes, and 13q-, 12+, 11q- and 17p- aberrations. Time from diagnosis to first treatment (TTT) and overall survival (OS) were chosen as indicators of clinical behavior. *Results.* All cases had multiple DNA imbalances with a median of 7 gains (range 2-11) and 10 losses (range 2-21) larger than 1Mb. Imbalances appeared more frequent in 17p- CLL than in unselected CLL. Concordance rate between FISH and SNP-arrays was >98%. Deletions of 13q14.3 locus occurred in 9/18 (50%) cases. The remaining most common deletions affected several loci on 8p (8p12, 8p21.1-p23.1, 8p23.1-p23.3), and the 9q21.33-q22.2 region. The most frequent gains targeted chromosome arms 2p (2p14-p16.1, 2p22.3-pter), 3q (3q24-q29), 8q (8q23.3-qter, 8q24.13-q24.1, 8q24.3), and 17q (17q21.2-q21.32, 17q21.32-q22). Losses at the 8p or 17p13 loci were associated with concomitant gains of 8q or 17q arms in 4/5 8p- or 4/18 17p- CLL, respectively, suggesting the presence of i(8q) or i(17q) isochromosomes. Among all alterations identified in 17p- CLL, 8p-loss (containing TRAIL-R1/2 genes) and 2p16.1-p14 gain (containing REL and BCL11a genes) appeared most interesting. In fact, 8p loss and 2p16.1-p14 gain also predicted significant shorter time from diagnosis to treatment (8p-loss) and overall survival (8p loss and 2p16.1-p14 gain, $p<0.05$). SNP analysis also detected a high frequency of LOH. Six patients displayed one or more copy-neutral LOH regions longer than 5 Mb, suggesting uniparental disomy. Three of these regions (3p12.2-p12.1, 4q25-q8.1, 6q23.3-q25.1) had been previously reported in CLL. *Summary and Conclusions.* The frequency of CN changes points to a highly unstable genome in 17p- CLL. The remarkable significant association of 8p loss and 2p14 gain with prognosis even in this small cohort claims for the role of specific genes other than TP53 in controlling tumor behavior and activity within the 17p- CLL category.

0066

HIGH-RESOLUTION SNP-ARRAY PROFILING OF CHRONIC LYMPHOCYTIC LEUKEMIA

J. Edelmann,[1] C. Schwänen,[1] K. Holzmann,[2] S. Stilgenbauer,[1] H. Döhner[1]

[1]Universitätsklinikum Ulm, ULM; [2]Microarray Core Facility, Universität Ulm, ULM, Germany

Background. Genomic aberrations are important prognostic factors in chronic lymphocytic leukemia (CLL) (*Döhner et al., NEJM, Dec. 2000*). With the development of SNP arrays a new screening method for genomic aberrations is available. Compared to matrix-comparative-genomic-hybridization (matrix-CGH) and chromosome banding, not only a higher resolution but also a detection of uniparental disomies (UPD) as copy number neutral losses of heterozygocity can be achieved. *Material and Methods*. The Affymetrix® 500k SNP array-set was used on samples of 30 CLL patients. Mononuclear cells were separated for CD19 by immunomagnetic beads. The CD19 negative fraction was used as intraindividual reference. DNA was hybridized to the Affymetrix® 500k Array Set and data analysed by the Copy Number Analyser for Gene Chip® (CNAG). FISH analysis (11q, 12, 13q, 14q,17p) was performed on all patients. Matrix - CGH using a 2.8k array was performed on 28 patients. *Results*. SNP array analysis showed genomic aberrations in 80% of all patients. Patients belonging to a good prognostic group defined by normal karyotype or del(13q) single had additional aberrations only at a rare frequency (4 in 18 patients). However patients with a del(17p) showed large genomic complexity with 14 additional aberrations in 4 patients. With respect to common recurrent aberrations, del(13q) was seen in 56.7%, biallelic deletion of the critical region 13q14.2-q14.3 in 13.3%, del(11q) in 16.7%, trisomy 12 as well as del(17p) in 13.3% and del(6q) was seen in 3.3% of all patients enrolled. All results from FISH analysis could be confirmed in SNP arrays. This also applied to the detection of small subclones down to a clone size of 20% aberrant cells. As compared to FISH, 19 additional copy number polymorphisms were detected in 10 patients. In comparison to matrix-CGH, SNP arrays revealed 5 additional copy number changes in 5 out of 28 patients. Altogether 14 UPDs could be detected in 6 patients but failed to be tumor specific except of one copy number neutral loss of heterozygocity (LOH) on chromosome 12. *Discussion*. SNP array analysis is a reliable diagnostic tool to detect genomic aberration in CLL since all aberrations displayed in FISH and matrix-CGH were also detectable by SNP arrays. With respect to FISH analysis, additional aberrations were detected in

33.3% and with respect to matrix-CGH in 17.9% of all patients. The high resolution of SNP arrays not only allows to better define breakpoints but also to detect smaller affected regions. New analysis softwares, validation experiments and larger numbers of patients are necessary to search for new recurrent aberrant regions. Intraindividual reference samples are necessary to rule out germline copy number polymorphisms and germline UPDs.

0067

COMMON HERPESVIRUSES AND CHRONIC LYMPHOCYTIC LEUKEMIA: MOLECULAR EVIDENCE FOR A POTENTIAL LINK WITH A SUBSET OF PATIENTS EXPRESSING STEREOTYPED IGHV4-34 B CELL RECEPTORS

E. Kostareli,[1] A. Hadzidimitriou,[1] N. Stavroyianni,[1] N. Darzentas,[2] A. Athanasiadou,[1] M. Gounari,[1] V. Bikos,[1] A. Agathaggelidis,[1] T. Touloumenidou,[1] I. Zorbas,[1] A. Kouvatsi,[3] N. Laoutaris,[4] A. Fassas,[1] A. Anagnostopoulos,[1] C. Belessi,[4] K. Stamatopoulos[1]

[1]G. Papanicolaou Hospital, THESSALONIKI; [2]INA, CERTH, THESSALONIKI; [3]School of Biology, Aristotle University of Thessaloniki, THESSALONIKI; [4]Nikea General Hospital, PIRAEUS, Greece

The CLL immunoglobulin repertoire is biased and uniquely characterized by the existence of subsets of cases expressing stereotyped B cell receptors (BCRs), suggesting recognition of a putative common - though still unknown- antigen. A major BCR stereotype in CLL is shared by IgG-switched cases utilizing the IGHV4-34 gene. The IGHV4-34 gene is inherently autoreactive and yet very frequent in normal B cells; however, IGHV4-34 antibody secretion is minimal in healthy individuals, suggesting an anergic status of IGHV4-34 cells. All syndromes with increased IGHV4-34 Abs appear to be associated with B cell proliferation and B cell lymphotropic viruses, particularly EBV and CMV. Along these lines, in the present study we explored possible links between persistent activation by EBV and CMV and development of CLL cases expressing the *IGHV4-34* gene. The study group included 80 CLL cases which utilized 24 different IGHV genes, with an intentional bias for the IGHV4-34 gene (23/80 cases). Following the 98% identity cut-off value, 61/80 cases (76.25%) were defined as *mutated*, whereas the remainder (19/80 sequences, 23.75%) had *unmutated* IGHV genes. A median of 3 (2-9) peripheral blood samples per case (overall 280 samples) were tested overtime by Real-Time PCR for the presence of CMV/EBV DNA. The threshold value was based on estimations for viral load corresponding to active (as opposed to latent) infection. Cases were considered as CMV/EBV positive only if two or more samples from different timepoints exceeded the real-time PCR threshold. Based on Real-Time PCR results, CLL cases were assigned to three distinct subgroups: i) group A: 50/80 double negative cases; ii) group B: 21/80 single positive cases (EBV-positive or CMV-positive); iii) group C: 9/80 double positive cases (EBV/CMV-positive). Striking differences with regard to IGHV repertoire were observed between the three subgroups. In particular, the double negative group was characterized by diverse IGHV gene usage (21 different IGHV genes) and a low frequency of the IGHV4-34 gene (5/50 cases; 10%). In contrast, the single positive group utilized 12 different IGHV genes, with a bias for IGHV4-34 (9/21 cases; 42.8%). Finally, all nine double positive cases utilized the IGHV4-34 gene. Remarkably, 7/9 double positive IGHV4-34 cases expressed the major BCR stereotype as described above. Double (EBV/CMV) positive cases with stereotyped IGHV4-34 BCRs also shared unique molecular and clinical features in that they were uniformly negative for CD38 and ZAP-70, had a young age at diagnosis and followed a strikingly indolent disease, indicating diminished responsiveness towards a selecting antigenic element. Based on these findings, we hypothesize that persistence of common herpesviruses could activate CLL progenitors expressing distinctive IGHV4-34 BCRs, promoting survival, expansion and, eventually, malignant transformation.

0068

THE RECEPTOR FOR HYALURONIC ACID MEDIATED MOTILITY (RHAMM): CHARACTERIZATION OF AN IMMUNOTHERAPEUTICAL TARGET WHICH IS ASSOCIATED WITH PROLIFERATION AND SHOWS PROGNOSTIC VALUE IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) PATIENTS

K. Giannopoulos,[1] K. Giannopoulos,[1] A. Buehler,[2] D. Mertens,[2] T. Barth,[2] I. Idler,[2] A. Kroeber,[2] J. Greiner,[2] A. Dmoszynska,[1] J. Rolinski,[1] H. Doehner,[2] S. Stilgenbauer,[2] M. Schmitt[2]

[1]Medical University of Lublin, LUBLIN, Poland; [2]University of Ulm, ULM, Germany

Background. Differential expression of molecules in chronic lymphocytic leukemia (CLL) patients might define prognostic markers and suitable targets for immunotherapy. Elevated levels of CD44, which is another hyaluronic acid receptor, was characterized in B-CLL as negative prognostic marker and correlated with the proliferative potential of CLL. *Aims.* We characterized RHAMM expression in CLL patients. Since the expression of immunogenic TAA might influence the clinical outcome by eliciting an immune response, we further characterized the expression of RHAMM-specific T cells in B-CLL patients. *Patients and methods*. Expression of the tumor-associated antigen (TAA) RHAMM as well as RHAMM-exon4 splice variant was assessed in series of 64 CLL patients. Immunohistochemisty-staining was performed for RHAMM, Ki-67 and CD40L in lymph nodes of CLL patients. Functional studies included CD40L stimulation, mixed lymphocyte cell culture with peptide, Cr release and ELISPOT assays. T regulatory cells were identified as $CD4^+CD25^{hi}FOXP3^+$ (Treg) by flow cytometry. *Results*. Increased RHAMM/TBP ratio was noted in advanced stages of the disease (0.006 in stage A, 0.03 in B and 0.05 in stage C according to Binet classification). qRT-PCR revealed higher RHAMM/TBP ratio in patients with unmutated IgVH status compared with mutated CLL cases (0.03 *vs* 0.005, p=0.003). High expression of RHAMM was observed in a patient with del(17p) (RHAMM/TBP ratio=0.077) when compared to a median RHAMM/TBP ratio of 0.0037 in patients with a normal karyotype. CLL cases with a higher RHAMM expression showed a significantly shorter median treatment-free survival (TFS) (14 *vs* 48.6 months, p=0.0028). In a bivariate analysis, patients with mutated IgV(H) and low RHAMM expression showed a longer TFS compared to IgV(H) unmutated cases with a high RHAMM expression (57.3 *vs* 8.9 months, p<0.0001). Immunohistochemisty-staining revealed that regions with a high RHAMM expression also stain positive for Ki-67 and CD40L. Correspondingly, in a functional in-vitro assay, enhanced expression of RHAMM was observed in CLL cells after stimulation with CD40L. These results suggest that RHAMM might be and indicator of proliferation capacity of CLL cells, which are induced to proliferate by CD40L in lymph nodes. The cytotoxic potential of RHAMM-specific T cells was shown to lyse both cells loaded with a RHAMM-derived epitope and CLL cells expressing RHAMM. We further speculated whether the RHAMM expression is associated with naturally occurring RHAMM-reactive T cells. Unexpectedly, the frequency of these T cells did correlate neither with the RHAMM expression nor with the mutational status nor with the stage of disease. However, patients with higher numbers of RHAMM-specific T cells tended to a have longer TFS when compared to those with limited numbers of RHAMM-specific T cells (24 *vs* 8 months, p=0.3). Therefore, it is possible that T cells specific for the CLL-specific TAA RHAMM have a protective effect. The protective immune effect of RHAMM-specific T cells might be hindered by an excess of Treg in patients with B-CLL when compared to healthy volunteers (HV) (n=18 *vs* n=10). Median Treg % among $CD4^+$ T cells was 11.17% in CLL *vs* 1.87% in HV (p<0.0001). The inverse correlation of R3 specific CD8+ T cells and Treg was noted (r2=-0.59, p=0.3). *Conclusions*. RHAMM expression appears to be of prognostic value and might reflect the proliferate capacity of CLL cells. Our results suggest that RHAMM represents an interesting target for immunotherapy since RHAMM specific T-cell responses have a protective effect resulting in longer TFS and RHAMM-specific T cells might directly lyse CLL cells. In CLL, RHAMM-specific T-cell response might be abrogated by an excess of Tregs.

0069

CCL3 CCL4, THE MAJOR CHEMOKINES PRODUCED BY CD38+ CLL CELLS, FACILITATE MICROENVIRONMENTAL INTERACTIONS OF NEOPLASTIC CELLS VIA THE CD49D/VCAM PAIR

A. Zucchetto,[1] D. Benedetti,[1] R. Bomben,[1] C. Tripodo,[2] F. Bossi,[3] M. Dal Bo,[1] D. Marconi,[4] M. Degan,[1] G. Del Poeta,[5] S. Deaglio,[6] G. Gaidano,[7] F. Tedesco,[3] F. Malavasi,[6] V. Gattei[1]

[1]Centro di Riferimento Oncologico, IRCCS, AVIANO; [2]Università degli Studi di Palermo, PALERMO; [3]Università degli Studi di Trieste, TRIESTE; [4]Università degli Studi di Bologna, BOLOGNA; [5]Ospedale S. Eugenio, Università di Tor Vergata, ROMA; [6]Università degli Studi di Torino, TORINO; [7]Università degli Studi del Piemonte Orientale Amedeo Avogadro, NOVARA, Italy

Background. CD38 is a powerful negative prognostic marker for patients with chronic lymphocytic leukemia (CLL), and has been reported to mediate interactions between CLL cells and the microenvironment, providing proliferation and survival signals for the neoplastic component. *Aims*. Moving from gene expression profiling studies (GEP) comparing CLL cases with different CD38 expression levels, we focused on genes allegedly involved in mechanisms regulating interactions between CLL cells and tumor microenvironment. *Methods*. For GEP studies, we selected purified cells from 12 CD38pos (CD38>30%) and 32 CD38neg (CD38<10%) CLLs. Functional and validation experiments were performed by utilizing flow cytometry, real-time quantitative PCR (RTQ-PCR), ELISA and immunohistochemistry (IHC). *Results*. (i) GEP allowed the identification of 132 differentially expressed genes (44 down-regulated and 88 up-regulated in CD38pos cases). Genes up-regulated in CD38pos cases included the C-C chemokines CCL3 (median-log difference, MLD=1.46) and CCL4 (MLD=1.36), whose over-expression was confirmed by RTQ-PCR in selected CLL (12 cases), and the integrin CD49d (MLD=1.14), that is known to correlate with CD38 protein expression in CLL (*Zucchetto et al. Leukemia, 2006*). ii) In order to evaluate whether CD38 triggering affects the production of CCL3 and CCL4 by CLL, leukemic cells from selected cases of CD38pos CLL (10 cases) were cultured for 14 and 24 hours (t14 and t24) in the presence of either the agonist anti-CD38 monoclonal antibody (mAb) IB4 or the non-agonistic anti-CD38 mAb IB6 as control. RTQ-PCR experiments clearly demonstrated higher levels for both CCL3 and CCL4 transcripts after 14 hours of CD38 engagement as compared to the control condition (t14 mean CCL3 fold increase=18, p=0.041; t14 mean CCL4 fold increase=13.8, p=0.005). Similarly, ELISA experiments performed on supernatants, showed a significant CCL3 and CCL4 enrichment after 24 hours of CD38 engagement (t24 mean CCL3 protein levels=0.9 ng/mL, mean fold increase=14, p=0.003; t24 mean CCL4 protein levels=1.7 ng/mL, mean fold increase=49, p=0.01). Consistently, CCL3 was detected by IHC in neoplastic cells from bone marrow biopsies (BMBs) of CD38pos but not CD38neg CLL. iii) immunophenotypic analysis of peripheral blood samples from CLL cases showed detectable levels of CCL3 and CCL4 receptors (CCR1 and CCR5) in monocytes as well as in cultured macrophages, which may therefore act as target cells for CCL3 and CCL4 released by CD38pos CLL cells. Consistently, high number of infiltrating CD68pos macrophages were found in BMBs of CD38pos but not of CD38neg CLLs. In parallel experiments, conditioned media (CM) from CCL3-stimulated macrophage cultures were collected; these CM were able to induce expression of the CD49d-ligand VCAM in human umbilical vein endothelial cells (HUVEC). According to preliminary ELISA experiments, TNFalpha was shown to be among the cytokines involved in VCAM up-regulation in HUVEC. Again, IHC analysis of CLL BMBs showed a meshwork of VCAM-1-positive cells in the context of lymphoid infiltrates of CD38pos but not of CD38neg cases. *Conclusion*. Altogether, these results identify molecules involved in a functional cross-talk between CD38/CD49d-expressing CLL and cells of the tumor microenvironment. This interplay may eventually affect survival and recirculation of tumor cells via the CD49d/VCAM pair.

0070

NOVEL MOLECULAR AND CLINICAL FEATURES OF CLL EXPRESSING OR NOT EXPRESSING STEREOTYPED B CELL RECEPTORS: RESULTS OF AN ITALIAN MULTICENTRIC STUDY

R. Bomben,[1] M. Dal-Bo,[1] D. Capello,[2] F. Forconi,[3] A. Zucchetto,[1] R. Maffei,[4] L. Laurenti,[5] F. Bertoni,[6] P. Bulian,[1] D. Rossi,[2] M.I. Del-Principe,[7] F. Ilariucci,[8] E. Sozzi,[3] E. Zucca,[6] D. Degan,[1] F. Lauria,[3] G. Del-Poeta,[7] D.G. Efremov,[9] R. Marasca,[4] G. Gaidano,[2] V. Gattei[1]

[1]Centro di Riferimento Oncologico IRCCS, AVIANO, Italy; [2]Amedeo Avogadro University of Eastern Piedmont, NOVARA, Italy; [3]University of Siena, SIENA, Italy; [4]University of Modena and Reggio Emilia, MODENA, Italy; [5]Hematology Institute, Catholic University Sacro Cuore, ROMA, Italy; [6]Oncology Institute of Southern Switzerland, BELLINZONA, Switzerland; [7]S.Eugenio Hospital and University of Tor Vergata, ROMA, Italy; [8]Reggio Emilia Hospital, REGGIO-EMILIA, Italy; [9]ICGEB Outstation, Monterotondo, CNR Campus A. Buzzati-Traverso, ROMA, Italy

Background. Subsets of B-cell chronic lymphocytic leukemia (CLL) carrying a stereotyped B-cell receptor (BCR) have been reported among cases expressing mutated (M) and unmutated (UM) IGHV genes. *Aims.* To correlate the presence/absence of stereotyped BCR, or the expression of particular IGH genes with disease progression or the presence of known prognosticators. *Methods*. A HCDR3-driven clustering was performed by sequence alignment using the ClustalX(1.83) program. HCDR3 amino acid sequences (n=1426; Kabat criteria) were obtained from 1398 unselected CLL patients. Clusters with homologous HCDR3 were selected for having a mean alignment score of at least 60/100. Time to treatment intervals (TTT) were available for 653 cases. Other availlable clinical parameters were Rai staging (n=654), CD38 (n=661), ZAP-70 (n=518), CD49d (n=501), and karyotype abnormalities evaluated by FISH (n=428). *Results*. We identified 71 clusters with homologous HCDR3, utilizing either identical or different IGHV genes. The 32/71 clusters with at least 3 cases/cluster (*confirmed*clusters) comprised 247 CLL (17.3%, UM/M=166/81). Seventeen/32 clusters were *UM clusters* (UM/M=153/3), 14/32 clusters were *M clusters*(UM/M=3/56) and a single cluster (expressing IGHV3-21 gene) was considered a mixed one (UM/M=10/22). Sixteen/32 clusters were common to other CLL datasets whereas 16 were novel clusters. *UM clusters* were significantly more represented in common (UM/M clusters=16/3) than in novel (UM/M clusters=5/11) clusters (p=0.018). By comparing molecular features with clinical parameters, the following findings emerged: i) Patients belonging to the IGHV3-21 cluster, expressing homologous HCDR3 always associated with IGLV3-21 gene, had TTT similar to UM cases and shorter than M CLL (p<0.001). Conversely, *IGHV3-21* CLL with heterologous HCDR3, rarely expressing *IGLV3-21* gene, had TTT closely dependent on their UM/M IGHV mutational status, as in the whole CLL series (p=0.036). Consistently, in M cases belonging to the IGHV3-21 cluster, TTT intervals were shorter than in IGHV3-21 M cases expressing heterologous HCDR3 (p=0.048). In agreement with these observations, IGHV3-21 CLL with homologous HCDR3 more frequently expressed unfavourable prognosticators than IGHV3-21 CLL with heterologous HCDR3 (p=0.002). ii) An UM cluster expressing genes from the IGHV1 family other than IGHV1-69 comprised 30 cases (UM/M=29/1), all but one expressing the IGKV1-39 gene. The prognosis of these patients was poor either if compared to all UM/M remaining cases (p<0.001), or to all the UM/M cases expressing the same IGHV genes but not included in clusters (p=0.009). The presence of unfavourable prognosticators was significantly higher in CLL from this cluster than in their heterologous counterparts (p=0.009). (iii) CLL expressing the IGHV3-23 gene (UM/M=25/109) virtually never belonged to clusters (4/134 cases in clusters); TTT in IGHV3-23 M CLL was significantly shorter than in all remaining M CLL (p=0.019), or in the M CLL subset expressing other IGHV3 family genes (p=0.021). Multivariate Cox proportional hazard analyses on 174 cases (109 cases if circumscribed to the IGHV3 family), selected Rai staging (p=0.0025), FISH (p=0.0025) and IGHV3-23 (p=0.013) as independent biologic prognosticators for M CLL. Conclusion: Novel molecular and clinical features were provided for CLL subsets expressing or not stereotyped BCR.

0071

HOW DOES MBL RELATE TO CLL PATHOGENESIS? A PERSPECTIVE FROM THE IMMUNOGLOBULIN GENE REPERTOIRE ANALYSIS

P.G. Ghia,[1] A. Dagklis,[1] C. Fazi,[1] V. Cantarelli,[1] D. Toniolo,[2] R. Massacane,[3] K. Stamatopoulos,[4] F. Caligaris-Cappio[1]

[1]Università Vita-Salute San Raffaele, MILANO, Italy; [2]Istituto Scientifico San Raffaele, MILANO, Italy; [3]ASL 22, P.O. Novi Ligure, NOVI LIGURE (AL), Italy; [4]G. Papanicolaou Hospital, THESSALONIKI, Greece

Background. The presence of Monoclonal B Lymphocytes circulating in the blood of otherwise healthy individuals is named Monoclonal B Lymphocytosis (MBL). In the majority of cases, MBL cells show a phenotype ($CD5^+$, $CD20^{low}$, IgM^{low}) that is virtually identical to that of chronic lymphocytic leukemia (CLL) cells. This resemblance suggests the idea of MBL as a precursor state of CLL, resembling the relationship between MGUS and Multiple Myeloma. On the one hand, this hypothesis appears to be well grounded as MBL is constantly monoclonal, is more frequent among relatives of CLL patients and affects mainly the elderly male individuals, as it occurs in CLL. On the other hand, this speculation seems rather simplistic since MBL is at least 100 times more frequent than CLL and the progressive restriction of the immune repertoire, including the appearance of monoclonality, is known to be part of the physiological senescence process of the normal immune system. *Aims*. We aimed at defining at molecular level whether an association between CLL and MBL existed, that might support the idea of MBL as precursor of CLL. *Methods*. The blood of 1725 healthy individuals >18 years of age was studied, after informed consent, by flow-cytometric analysis for the presence of MBL. In the positive cases, we amplified by PCR and direct sequenced the Immunoglobulin (IG)HV-D-J rearrangements expressed by 47 MBL cases which were then analyzed on the IMGT database and aligned to a comprehensive panel of IG sequences from CLL cases. *Results*. Eighty-nine MBL cases (5.2% of the total population) showed a CLL-like phenotype and were subjected to IG gene rearrangement analysis. Sequence analysis of the rearrangements showed a predominance of IGHV3 genes usage, followed by IGHV4 subgroup genes, resembling the normal repertoire. Around 80% of MBL cases expressed mutated IGHV genes and frequently used the IGHV4-59/61 gene which is rare in CLL, while the most common CLL-related IGHV genes (IGHV1-69 and IGHV4-34) were absent in MBL repertoire. Less than 7% MBL expressed HCDR3 sequences similar to those identified in CLL (*stereotyped receptors*), that are present in >25% of the cases. *Conclusions*. Our results show that the Immunoglobulin repertoire in MBL is overall different, in terms of IGHV gene usage and gene family preference, from that of CLL. Some MBL cases may express CLL-related *stereotyped* HCDR3 sequences, though at a frequency definitely lower than the one expected in CLL (>25%). Taken together, our results strongly suggest that MBL cells do not represent a pre-leukemic condition in all cases. That notwithstanding, the presence of 2 of these CLL-biased receptors does suggest that the potential to become a CLL is present within MBL cells, though at a low frequency, and this is not determined by the simple fact of showing a typical phenotype or being monoclonal, but is rather linked to the specific BCR expressed by each MBL case, which likely provides a different capacity to respond to distinct (antigenic) signals. A detailed molecular analysis of individual MBL may help to identify those few cases that necessitate a continuous clinical monitoring to anticipate disease progression.

0072

HS1 PROTEIN REVEALS ITS ROLE IN NORMAL AND LEUKEMIC B CELLS MIGRATION

C. Scielzo,[1] M.T.S. Bertilaccio,[2] M. Muzio,[2] M. Frenquelli,[2] B. Apollonio,[2] A. Dagklis,[2] G. Simonetti,[1] C. Fazi,[2] P. Ghia,[1] F. Caligaris-Cappio[1]

[1]Università Vita-Salute, MILANO, Italy; [2]San Raffaele Scientific Institute, MILAN, Italy

Background. In Chronic Lymphocytic Leukemia (CLL) the relationships between cell proliferation and accumulation within lymphoid organs are unclear, as are the rules that control CLL cell migration and re-circulation between peripheral blood and the lymphoid tissues. Given the role played by the cytoskeleton in controlling cell shape and motility, it becomes central to investigate the cytoskeleton organization of CLL cells. The organization of actin-containing microfilaments and vimentin-containing intermediate filaments in CLL reveals that adhesion structures are present and that the *in vitro* adhesion capacity is marked. Previous studies from our group demonstrated that Hematopoietic cell specific Lyn substrate 1 (HS1), a molecule pivotal in the signal transduction triggered by the stimulation through antigen receptor, is a putative prognostic marker in CLL. HS1 function in normal and leukemic B cells was unknown though , based on the sequence of the molecule, an actin-binding activity could be hypothesized, as well as a potential role in cytoskeleton organization. *Aims*. Based on these evidences, we aimed at determining the currently unknown specific role of HS1 in normal and leukemic B cells focusing on a potential role in controlling and regulating cytoskeleton structure, cell shape and movements. *Methods*. By immunoprecipitation experiments, confocal microscopy and mass spectrometry analysis of the co-immunoprecipitated bands, we have investigated HS1 interactions in CLL and normal B cells. To study its function, we silenced HS1 expression in a CLL cell line (MEC1) using an RNA Interference approach, and we utilized B lymphocytes from HS1 Knock-Out mice. Both cell types were then studied for their migration capacity in *in vitro* and *in vivo* assays. *Results*. We demonstrate that HS1 interacts with distinct cytoskeleton adapters, namely HIP-55 and, unexpectedly, Cortactin, which was not known to be present in lymphoid cells, being considered an epithelial-specific molecule. Further we show that HIP-55 interacts also with ZAP70, a protein kinase with a prognostic significance in CLL. Finally we provide evidence that both leukemic and normal B lymphocytes, when lacking HS1 expression, are severely impaired in their spontaneous migration capacity. *Conclusions*. HS1 interacts with several cytoskeleton adapters and components, likely participating in the regulation of cytoskeleton in B-lymphocytes. Specifically, the fact that HS1 complexes with HIP-55, Cortactin and ZAP-70 suggests that it may be involved in coupling antigen receptor induced signaling with cytoskeleton regulation in both normal and leukemic B cells. This might indicate a role in controlling shape and movement in B lymphocytes following activation. The impairment in B lymphocyte spontaneous migration in the absence of HS1, suggests that this molecule might be relevant in the regulation of CLL cells migration and homing into the lymphoid tissues and may represent a novel target for pharmacological intervention in leukemia.

0073

CLL TRANSFORMING TO RICHTER'S SYNDROME CARRY STEROTYPED HCDR3S AT VERY HIGH FREQUENCY (>50%) AND DISPLAY BIASED USAGE OF HOMOLOGOUS IGHV4-39 GENES

S. Valeria,[1] D. Capello,[2] R. Bomben,[3] F. Forconi,[4] L. Laurenti,[5] G. Del Poeta,[6] R. Marasca,[7] S. Pileri,[8] A. Carbone,[9] M. Paulli,[10] F. Bertoni,[11] G. Gaidano,[2] V.S. Gattei,[3] D. Rossi[2]

[1]Amedeo Avogadro University, NOVARA, Italy; [2]Division of Hematology, Amedeo Avogadro University, NOVARA, Italy; [3]Oncohematology Unit, CRO, AVIANO, Italy; [4]Division of Hematology, University of Siena, SIENA, Italy; [5]Division of Hematology, Catholic University of the Sacred Heart, ROME, Italy; [6]Division of Hematology, University of Tor Vergata, ROME, Italy; [7]Division of Hematology, University of Modena and Reggio Emilia, MODENA, Italy; [8]Istituto Seràgnoli, University of Bologna, BOLOGNA, Italy; [9]Division of Pathology, INT, MILAN, Italy; [10]Institute of Pathology, University of Pavia, PAVIA, Italy; [11]IOSI, BELLINZONA, Switzerland

Background. The prognostic value of specific IGHV gene usage is a well recognized feature of CLL. Usage of IGHV4-39 at CLL diagnosis appears to be an independent risk factor of transformation to Richter Syndrome (RS) in multivariate analysis (*Rossi, Br J Haematol 2008, in press*). The potential relevance of specific IGHV gene usage in RS transformation may be further supported by the identification of stereotyped B-cell receptors in RS. *Aims.* To investigate HCDR3 clustering in CLL transformed to RS. *Methods*. The study was based on 57 CLL transformed to RS. A consecutive series of 223 CLL was utilized as internal control group. Analysis of IGHVDJ sequences was based on the International ImMunoGeneTics resource (IMGT®, *http:\\imgt.cines.fr*). Cluster analysis was performed with a blind approach using the multiple sequence alignment ClustalX (1.83) software. HCDR3s of CLL transformed to RS and HCDR3s of CLL belonging to the internal control group were aligned to HCDR3s of 2984 CLL from our collaborative multicentric database (*Bomben, Blood 2007;110:909a*) and from a previously reported database (*Murray, Blood 2008;111:2083-90*). Cluster nomenclature was according to Stamatopoulos (*Blood 2007;109:259-70*) and Murray (*Blood 2008;111:2083-90*). Results. Cluster analysis revealed that 32/57 (56.1%) CLL transformed to RS carried stereotyped HCDR3s. Prevalence of stereotyped HCDR3s was significantly higher in CLL transformed to RS than in CLL of the internal control group considering: i) all cases (RS: 32/57, 56.1% *vs* CLL: 52/223, 22.3%; $p<0.001$); ii)

IGHV unmutated cases (RS: 24/39, 61.5% *vs* CLL: 30/78, 38.4%; p=.018); and iii) IGHV mutated cases (RS: 8/18, 44.4% *vs* CLL: 22/145, 15.1%; p=.006). In addition, the prevalence of stereotyped HCDR3s in CLL transformed to RS was significantly higher than in CLL from the two databases investigated (*Murray, Blood 2008;111:2083-90; Bomben, Blood 2007;110:909a*) when the analysis was conducted: i) for all cases (p=1.2x10^{-16} and p=7.7x^{9}, respectively); ii) for IGHV unmutated cases (p=0.008 and p=8.2x10^{-4}, respectively); and iii) for IGHV mutated cases (p=6.3x10^{-4} and 4.5x10^{-4}, respectively). Analysis of IGHVDJ revealed that CLL transformed to RS displayed a biased usage of IGHV4-39 (8/57; 14.0%) when compared to the consecutive CLL series used as internal control group (8/223, 3.6%) (p=0.002). The biased usage of IGHV4-39 in CLL transformed to RS was also confirmed when CLL from the two databases were used for comparison (Murray database: IGHV4-39 96/1967, 4.8%, p=0.002; Bomben database: IGHV4-39: 55/1426, 3.8%, p=1.6x10^{-4}). IGHV4-39 CLL transformed to RS entered a cluster in 7/8 cases (87.5%). According to the nomenclature by Stamatopoulos and Murray, five cases belonged to subset 8, and 1 case to subsets 10 and 33 each. IGHV4-39 CLL transformed to RS entered a cluster more frequently than CLL from the databases (Murray database: 31/96, 32.2%, p=.003; Bomben database: IGHV4-39: 19/55, 34.5%, p=0.007). *Conclusions*. The implications of these data are twofold. First, occurrence of stereotyped HCDR3s is a frequent feature of CLL that transform to RS, potentially suggesting an important contribution of antigen stimulation in driving CLL transformation to aggressive lymphoma. Second, these data expand the notion that IGHV4-39 preferentially associates with RS. The pathogenetic relevance of IGHV4-39 in RS transformation is further supported by evidence of stereotyped B-cell receptors in the overwhelming majority of RS cases utilizing IGHV4-39.

0074

THE METABOLISM OF MEVALONATE AS A SIGNALING PATHWAY INFLUENCING TUMOR-HOST INTERACTIONS AND DISEASE PROGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

M. Coscia,[1] S. Peola,[1] G. Matta,[1] F. Pantaleoni,[1] M. Foglietta,[1] C. Vitale,[1] D. Angelini,[2] S. Chiaretti,[3] C. Riganti,[4] A. Guarini,[3] D. Drandi,[1] M. Ladetto,[1] A. Bosia,[4] R. Foà,[3] L. Battistini,[2] M. Boccadoro,[1] J.J. Fournie,[5] M. Massaia[1]

[1]Department of Medicine and Experimental Oncology, Section of Hematology, TORINO, Italy; [2]Santa Lucia Foundation, Neuroimmunology Unit, ROMA, Italy; [3]Department of Cellular Biotechnologies and Hematology, Division of Hematology, ROMA, Italy; [4]Department of Genetics, Biology and Biochemistry, TORINO, Italy; [5]Departement Oncogénèse and Signalisation dans les Cellules Hématopoiétiques INSERM, TOULOUSE, France

Background. The mevalonate (Mev) pathway is essential for the post-translational modification of proteins involved in cell growth and differentiation. It also generates intermediate metabolites that are specifically recognized by the Vδ2 γδ T-cell subset. Vδ2 T cells play an important role in immune responses against tumor cells. They represent less than 5% of peripheral blood lymphocytes but can be efficiently expanded *in vitro* upon exposure to Zoledronic acid (Zol). *Aims*. The aim of this work was to evaluate whether the Mev pathway might be regarded as a signaling pathway influencing tumor-host interactions in CLL. *Methods.* Sixtyseven previously untreated CLL patients were evaluated for *in vitro* Vδ2 T cells expansion upon stimulation with Zol + interleukin-2 (IL-2). The IgVH mutational status was analyzed by cDNA PCR amplification and sequencing. The level of activity of the Mev pathway was determined by 1) the analysis of gene expression profile (GEP) data 2) the biochemical quantification of the intermediate metabolite Isopentenyl pirophosphate (IPP). γδ T cells subset distribution and natural killer receptors (NKRs) profile were evaluated by multicolor flowcytometry. *Results*. Based on the *in vitro* Vδ2 proliferative response to Zol+IL2, 60% of CLL patients were defined as responders (R) and 30% as non-responsders (NR). Interestingly, our data have shown a strict and significant correlation among the Vdelta2 proliferative response to Zol + IL-2 and the IgVH mutational status of the leukemic cell. Indeed, 100% of R patients were mutated (M), whereas 70% of NR patients were unmutated (UM). To gain further insight into this association, we evaluated the activity of the Mev pathway in tumor cells of M and UM patients. Both the GEP data and the biochemical analysis have shown a significantly higher activity of the Mev pathway in UM CLL cells. The higher amount of IPP produced by UM CLL cells most likely determines a chronic stimulation of Vδ2 T cells *in vivo*. Indeed, a predominance of effector memory and terminally differentiated effector memory (TEMRA) Vδ2 T cells expressing the inhibitory NKR ILT2 was observed in NR patients. The expression of ILT2 on Vδ2 T cells was paralleled by high level of expression of the corresponding ligand HLA-G on CLL cells. HLA-G expression is associated with an unfavorable outcome in CLL. The association of R/NR status with biological markers of disease aggressiveness (i.e., IgVH mutational status and HLA-G expression) prompted us to determine whether R/NR status might associate to other features of disease aggressiveness and to predict tumor progression in CLL patients. Results from this analysis showed a significantly higher frequency of poor-risk cytogenetic abnormalities and a significantly shorter time to first treatment in NR as compared to R patients. *Conclusions*. Based on these data, we propose that the high rate of activity of the Mev pathway in UM CLL cells might determine a chronic stimulation of Vδ2 T cells thus leading to their *in vivo* differentiation into functionally impaired TEMRA. Therefore, we have identified a novel mechanism of immune escape which is at least partially contributing to disease progression in UM CLL.

0075

THE PROLIFERATIVE RESPONSE TO CPG-ODN STIMULATION PREDICTS DISEASE PROGRESSION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

L. Laurenti,[1] M. Tarnani,[2] I. Innocenti,[2] P.G. Longo,[3] S. De Matteis,[2] P. Chiusolo,[2] A. Mannocci,[2] S. Sica,[1] G. Leone,[2] D.G. Efremov[3]

[1]Policlinico A. Gemelli, ROMA; [2]Policlinico A Gemelli, ROMA, Italy; [3]ICGEB Monterotondo-Outstation, ROMA, Italy

Background. Chronic lymphocytic leukemia (CLL) B-cells from certain patients exhibit marked proliferation following stimulation with unmethylated CpG-oligodeoxynucleotides (CpG-ODN). We observed that proliferation induced by CpG-ODN occurs primarily in leukemic B-cells from patients with progressive disease and unmutated immunoglobulin VH genes. *Aims*. We correlated this proliferative response, IgVH, CD38, ZAP-70 and cytogenetic abnormalities to progression-free survival (PFS) to detect the prognostic value of a new biological marker. *Methods*. The proliferative response to CpG-ODN stimulation was investigated in 65 CLL patients by analysis of [^{3}H] thymidine incorporation. We evaluated the proliferative response based on the stimulation index (SI) (cpmCpG / cpmMedium). A value of SI greater than 3 was considered indicative of a proliferative response. We also studied IgVH mutational status, CD38, ZAP-70 and high risk and low risk chromosome alterations detected by FISH analysis. We performed K test to evaluate the grade of accordance among CpG-ODN and other biological parameters. *Results*. Thirty-nine patients were men and 26 were women. Median age was 65 years. At presentation 49 were Binet stage A, 12 stage B and 4 stage C. Proliferative response to CpG-ODN stimulation showed 31 proliferating cases and 34 non-proliferating. About IgVH 33 cases were mutated and 29 unmutated. CD38 was tested in 63 cases; 14 resulted positive. ZAP-70 was studied in 63 patients; 26 were positive. FISH analysis was performed in 59 patients, 49 patients were low-risk and 10 high risk. The biological data are shown in Table 1.

Table 1.

Biological parameters (cut-off)		*CpG-ODN proliferating*	*CpG-ODN non-proliferating*
IgVH (98%)	Unmutated	22 (71%)	7 (22%)
	Mutated	9 (29%)	24 (78%)
CD38 (30%)	Positive	11 (35%)	3 (9%)
	Negative	20 (65%)	21 (91%)
Zap 70 (20%)	Positive	17 (55%)	9 (29%)
	Negative	14 (45%)	23 (71%)
FISH (HR: 11q-; 17p-) (LR: +12; normal; 13q)	High risk	8 (29%)	2 (7%)
	Low risk	20 (71%)	29 (93)

Patients from the proliferating subset had a significantly shorter median PFS (44 months *vs* 87 months, p=0.002). By univariate analysis, the variables significantly associated with longer PFS were: mutated Ig VH genes (p=0.001), ZAP-70 expression (p=0.009) and proliferative response to CpG ODN stimulation (p=0.002). By multivariate analysis the only independent variable associated to longer PFS was mutated Ig VH genes status (HR = 3.553; 95% CI: 1.628-7.751). Kappa test showed the strongest grade of agreement between proliferative response to CpG ODN stimulation and IgVH genes mutational status (κ=0.47, p<0.0001).

We also evaluated PFS according to proliferative response and VH

gene mutation status. The combination of non-proliferating cases with mutated VH genes (38.7%) had a significantly longer median PFS (not reached at 199 months); non-proliferating cases with unmutated VH genes (11.4%) (60 months); proliferating cases with mutated VH genes (14.5%) (60 months); proliferating cases with unmutated VH genes (35.4%) (30 months), (Logrank=15.249; p=0.002; Breslow=14.978; p=0.002). *Summary and Conclusions*. Cases with a positive proliferative response have significantly shorter PFS, indicating that the proliferative capacity of the leukemic cells is an important determinant of the clinical course in CLL. These data confirm that the proliferative response to CpG-ODN stimulation is strongly correlated with adverse biological prognostic features, in particular with unmutates IgVH genes. A new biological information emerged by combination of IgVH and CpG. About 35% of patients with CpG non proliferating and IgVH mutated genes can be considered as *super stable patients*.

0076

UDP-GLUCURONOSYLTRANSFERASE 2B17 GENE EXPRESSION IS A NOVEL MARKER FOR PROGNOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

M.G. Gruber,[1] M. Bilban,[1] D. Heintel,[1] H. Esterbauer,[1] C. Fonatsch,[1] C. Mannhalter,[1] K. Eigenberger,[1] A. Gaiger,[1] C. Skrabs,[1] E. Porpaczy,[1] T.L. Le,[1] A. Hauswirth,[1] S. Stilgenbauer,[2] U. Kroemer,[2] K. Vanura,[1] U. Jaeger[1]

[1]*Medical University of Vienna, VIENNA, Austria;* [2]*University of Ulm, ULM, Germany*

Background. UDP-glucuronosyltransferase (UGT) 2B17 glucuronidates androgens and their metabolites. UGT2B17 shows by far the most dramatic difference in gene expression between the Asian and Caucasian normal populations and is associated with an increased prostate cancer risk in Caucasians. Interestingly, Asians have a strikingly lower incidence of chronic lymphocytic leukemia (CLL). In previous microarray-analysis we found that UGT2B17 RNA is differentially expressed between high and low risk CLL. *Aims*. i) To evaluate UGT2B17 gene-expression as prognostic marker for CLL; ii) to determine the genetic background (UGT2B17 polymorphism) of Caucasian CLL patients. *Methods*. i) Real-time PCR results of 136 PBMC-samples of Austrian B-CLL patients were compared with clinical data and CLL risk factors. In addition, we analyzed CD19-sorted cells from selected CLL patients and normal controls. ii) Conventional hot start PCR with 214 DNA samples of Austrian CLL patients and 449 samples of Austrian age-matched healthy donors (307 men, 142 women) were compared regarding the distribution of UGT2B17 gene polymorphism. This polymorphism consists of deletions encompassing the entire *UGT2B17* coding region. (genotype: ins/ins, ins/del, or del/del). *Results*. *UGT2B17* gene-expression ranged from 0 - 264,12 (median: 2,026) compared to normal PBMC (set as 1). Expression correlated with LPL-expression (p<0,0002), mutational status (p=0,001), CD38-expression (p=0,007), LDH-elevation (p=0,009), Binet-stages B and C (p=0,013), and 14q aberrations (p=0,044), and showed inverse correlation with 13q-deletion (p=0,013). Time to first treatment was significantly shorter in patients with high UGT2B17-expression (>2,026; p=0,00003) (Figure 1). UGT2B17 levels were up to 3 logs higher in CD19+ selected CLL cells compared to CD19 negative cells. RNA expression was higher in ins/ins compared to ins/del patients, but was absent in del/del patients.

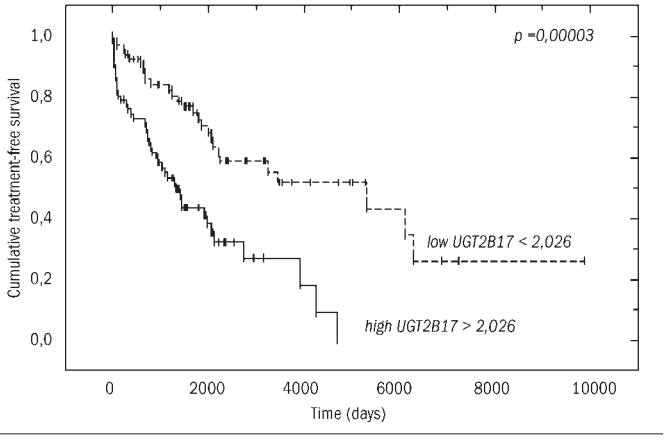


Figure 1.

Genotype frequencies in 214 CLL-patients were 38,97% ins/ins (♂ 35,04%, ♀ 44,87%), 49,74% ins/del (♂ 51,28%, ♀ 47,44%) and 11,28% del/del (♂ 13,68%, ♀ 7,69%), respectively. Within healthy donors we found 42,98% ins/ins (♂ 43,32%, ♀ 42,25%), 46,33% ins/del (♂ 45,60%, ♀ 47,89%) and 10,69% del/del (♂ 11,07%, ♀ 9,86%). There was no significant difference in polymorphism-frequency between CLL-patients and healthy subjects or gender. Double deleted patients showed a tendency for longer time to first treatment compared to ins/ins and ins/del genotypes albeit not statistically significant (p=0,051). *Conclusions*. UGT2B17 gene-expression is a prognostic marker for CLL patients and correlates with genotype. No difference in genotype distribution between CLL patients and controls was found in a Central European population. Impact of UGT2B17-expression on response to treatment is currently being investigated.

0077

ALTERNATIVELY SPLICED TRANSCRIPTS OF ACTIVATION-INDUCED CYTIDINE DEAMINASE (AID) IN CHRONIC LYMPHOCYTIC LEUKEMIA. CORRELATION WITH IGHV GENE MUTATIONAL STATUS AND SURFACE IMMUNOGLOBULIN ISOTYPE EXPRESSION

F. Marantidou,[1] M. Chatzouli,[1] I. Athanasiadou,[2] V. Taxynopoulou,[2] A. Agathagelidis,[2] K. Valianatou,[1] E. Kostarelli,[2] A. Saetta,[3] N. Stavroyianni,[2] E. Manioudaki,[1] P. Korkolopoulou,[3] N. Laoutaris,[1] A. Anagnostopoulos,[2] K. Stamatopoulos,[2] C. Belessi,[1] E. Patsouris[3]

[1]*General Hospital of Nikea, PIRAEUS;* [2]*G. Papanicolaou Hospital, THESSALONIKI;* [3]*Dept. of Pathology, Athens University School of Medicine, ATHENS, Greece*

AID plays a key role in class switch recombination (CSR) and somatic hypermutation (SHM). Normal B-cells carry three different AID isoforms by alternative splicing of AID mRNA. AID1 mRNA encodes for the full-length protein and seems responsible for the enzymatic action; the AID3 isoform lacks amino acid residues critical for AID function. Dimerization of AID1 and AID3 may play a role in controlling AID activity. In the present study, we analyzed the patterns of AID splice variant mRNA expression in CLL and explored possible associations with IGHV gene mutational status and surface immunoglobulin (sIg) isotype expression. The study group included 187 patients with CLL, of which 119 (63.6%)%) carried IGHV genes with <98% homology to the closest germline gene (mutated), whereas the remainder (68/187 cases, 36.4%) carried unmutated IGHV genes. Twenty-two of 130 cases (16.9%) with available data expressed surface IgG; the remainder (108/130 cases; 83.1%) expressed IgM/IgD. Full-length AID transcripts (AID1) and 2 splice variants (AID2/AID3) were amplified by Real-time PCR in three different reactions with primers/probes specific for each isoform. ABL was used as a housekeeping gene and the results were expressed as AID copies /10.000 copies ABL. A cut-off value of 10 AID copies per 10,000 ABL copies was used for AID positivity. AID1/2/3 transcripts were detected in 114/94/99 patients, respectively. Seventy-six of 187 patients (40.5%) carried all three alternatively spliced AID transcripts; 57/187 patients (30.5%) carried one or two splice variants; finally, 54/187 patients (29%) were negative for any AID transcript. Co-expression of all three alternative splice variants was significantly more frequent among cases with unmutated IGHV genes (41/68 cases *vs* 35/119 cases with mutated IGHV genes; p<0.01). On the other hand, complete absence of AID splice variants was significantly more frequent among cases with mutated IGHV genes (42/119 cases *vs* 12/68 cases with unmutated IGHV genes; p=0.01). Expression of at least one AID isoform was significantly more prevalent among IgG *vs* IgM/D cases (20/22 *vs* 59/108 cases, respectively; p<0.01); 19/20 AID positive, IgG-switched cases, carried mutated IGHV genes. Of note, in IgG-switched cases, the AID3 transcript was always co-expressed with the AID1 and/or AID2 transcript. At the cohort level, the median values of AID1/2/3 transcripts were 51.5/48.3/50.3 copies per 10,000 copies ABL, respectively. *AID1/2/3* transcript levels varied between different cases up to 2-log. Significantly higher AID1 transcript levels were observed in IgG-switched cases (median, 138 copies per 10,000 copies ABL). In conclusion, co-expression of all three alternative AID splice variants in CLL patients with unmutated IGHV genes is indicative of functional inactivation of AID, possibly via formation of *AID1/AID3* or *AID2/AID3* dimers. Increased levels of full-length AID1 transcripts in IgG-switched/IGHV-mutated CLL cases alludes to the potential role of the AID1 transcript in both SHM and CSR, in keeping with normal B cells.

0078

REGULATION OF CXCR4 EXPRESSION BY VEGF AND SEMA3A IN B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA

B.P. Power,[1] P.M. Murphy,[2] J.H. Harmey,[1] P.T. Thornton[3]

[1]Royal College of Surgeons Ireland, DUBLIN; [2]Department of Haematology, Beaumont Hospital, DUBLIN; [3]Department of Haematology, Connolly Hospital, DUBLIN, Ireland

B-cell Chronic Lymphocytic Leukemia (B-CLL) is characterized by the accumulation of B-CLL lymphocytes in the blood, marrow and secondary lymphoid tissues. B-CLL cells have a long survival owing to alterations in the normal pathways of apoptosis. In the marrow and lymphoid tissues CLL cells are in close contact with stromal cells that constitute distinct microenvironments. The secretion of the *CXCR4* ligand *CXCL12* by stromal cells attracts B-CLL cells and provides protection from spontaneous or induced apoptosis. Studies in other cell types have shown VEGF signalling to be involved in regulating *CXCR4* expression levels. Our aim in this study was to examine the role of VEGF signalling in regulating CXCR4 levels in CLL cells. Expression levels of VEGF receptors (VEGFRs)-1 and 2 in CLL samples were determined by flow cytometry. Expression of the VEGFR co-receptor Neuropilin-1 (*NRP1*) in CLL samples was examined by Western blot. Informed consent was received from all patients. Treatment of CLL cells from patients with the NRP1 ligand SEMA3A which is a competitive inhibitor of VEGF binding to NRP1 resulted in decreased *CXCR4* expression levels as determined by flow cytometry (n=8, $p<0.05$). Culture of CLL cells with a VEGF blocking antibody resulted in a variable change in *CXCR4* levels which appears to correlate with VEGFR1 expression levels. Treatment of CLL cells with the VEGFR signalling inhibitor SU5416 caused a decrease in cell survival in a number of patient samples which also appears to correlate with VEGFR1 expression levels. These results show that signalling through VEGF receptors and the VEGF co-receptor *NRP1* plays a role in regulating CXCR4 levels in CLL cells. This pathway may therefore represent a target for future treatment in CLL.

0079

ABERRANT GENE EXPRESSION PATTERN OF ANGIOGENESIS-RELATED FACTORS CHARACTERIZES B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

R. Maffei, S. Martinelli, I. Castelli, R. Santachiara, P. Zucchini, M. Fontana, S. Fiorcari, G. Bonacorsi, R. Marasca, G. Torelli

University of Modena and Reggio Emilia, MODENA, Italy

Background. Chronic lymphocytic leukemia (CLL) B cells display defective apoptosis and prolonged survival that are ascribed not only to intrinsic defects but also to aberrant microenvironmental signals. Emerging evidence suggests that angiogenic signalling pathways play important role in CLL. *Aims.* The aim of the study was to define the pattern of angiogenic factors expressed by circulating CLL B cells and then to investigate the ability of CLL to enhance the basal angiogenic potential when exposed to hypoxia condition. *Methods*. The spontaneous gene expression levels of several angiogenesis-related factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiopoietin-1 (Ang1) and -2 (Ang2) and thrombospondin-1 (TSP1) were measured by Real-time PCR in a cohort of 120 CLL patients and 5 normal controls. Ang2 plasma concentrations were detected using ELISA assay. The degree of bone marrow (BM) angiogenesis was quantified in 26 CLL patients by immunohistochemical staining using antibody to CD34 and by measuring the microvessel number. For hypoxia treatment, $CD19^+$ cells from 6 CLL patients were cultured in an air-tight chamber infused with a pre-analyzed air mixture containing 1% O2/5% CO2/94% N2. After 24h culture under either normoxic or hypoxia conditions, the expression levels of angiogenesis-related factors were evaluated by Real-time PCR. *Results.* Circulating CLL B lymphocytes spontaneously expressed increased levels of Ang2 and bFGF, similar level of VEGF and diminished levels of both TSP1 and Ang1, involved in angiogenesis inhibition, compared to normal B cells. Higher expression of Ang2 was detected in high-risk CLL patients characterized by Ig-unmutated genes ($p<0.0001$), CD38/ZAP-70 positivity ($p=0.019$), advanced clinical stage ($p=0.018$) and unfavourable cytogenetics ($p=0.031$). Likewise, 2-fold increase of Ang2 plasma concentration was observed in Ig-unmutated compared to Ig-mutated CLL subsets ($p=0.035$). Of interest, positive correlation was found between Ang2 expression and the degree of vascularization in BM compartment ($p=0.044$). Furthermore, CLL B cells were able to modulate their angiogenic potential in hypoxic conditions. Hypoxic treatment induced brisk up-regulation of VEGF expression, slight increase of Ang2 and down-regulation of bFGF in CLL B cells. Enhancement of VEGF in hypoxia was variable from patient to patient with stronger up-regulation in Ig-unmutated CLL (20-fold to several hundred-fold induction) than in Ig-mutated ones (14-, 3-, and 0-fold induction). Conclusion: We suggest the involvement of Ang2 and VEGF dysregulation in precocious switch to a vascular phase and in CLL disease progression.

0080

A PROTEOMIC APPROACH TO INVESTIGATE TARGETS ASSOCIATED WITH B-CELL RECEPTOR SIGNALING IN CHRONIC LYMPHOCYTIC LEUKAEMIA

L. Eagle,[1] L. Cawkwell,[2] D. Allsup[3]

[1]University of Hull, HULL; [2]Post-Graduate Medical Institute, the University of Hull, HULL; [3]Department of Haematology, Hull Royal Infirmary, HULL, UK

Chronic Lymphocytic Leukaemia (CLL) results from the progressive accumulation of tumour cells which do not proliferate rapidly but fail to undergo death. Stimulation of the B-cell receptor (BCR) by antigen may prevent apoptosis of the malignant cells and has a relevant role in the disease with a poor prognosis. A good prognosis is associated with a characteristic BCR unresponsiveness and a bad prognosis is associated with a BCR that maintains the full capacity to respond to a triggering antigen, which the cells may be chronically exposed to. We have utilised proteomics as a way of investigating the BCR signaling pathways by identifying proteins which are differentially expressed in cells which have undergone sustained BCR stimulation. Protein was extracted from stimulated and unstimulated cells from clinical CLL samples. The extracts were separated using conventional two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). The gels were stained with a visible total protein stain and analysed with statistical software. Proteins with a two-fold change in expression between stimulated and control samples were excised from the gels and analysed by matrix assisted laser desorption/ionisation with time of flight mass spectrometry (MALDI-TOF-MS). Changes in protein expression were detected in response to prolonged BCR stimulation. Targets found include ones which are associated with the activation of anaplastic lymphoma kinase (ALK), the plasma kallikrein-kinin system (KKS), the AKT-1 pathway, the MAPK pathways, the adenylate kinase system and involvement in the CD40-dependant activation of B-CLL cells. One of the protein targets found was increased by over two-fold in two independent clinical samples after sustained BCR stimulation. Confirmation work on this novel protein is presently being undertaken in a number of clinical samples. These biomarkers may be of interest for future therapeutic intervention or could be utilised as prognostic markers in a novel routine test.

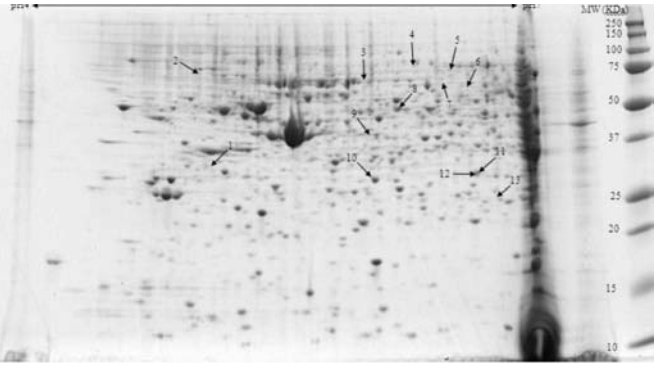


Figure 1. 2D gel showing differentially expressed proteins.

0081

CLL WITH T(14;18) : A DISTINCT DISEASE?

N.M.J. Put,[1] P. Meeus,[2] B. Chatelain,[3] M. Stul,[1] N. Boeckx,[4] C. Graux,[3] G. Bries,[5] C. Doyen,[3] C. Dubois,[6] S. Marichal,[7] P. Pierre,[8] A. Van Hoof,[9] J. Verschuere,[10] L. Harlet,[11] I. Wlodarska,[1] P. Vandenberghe,[1] L. Michaux[12]

[1]UZ Leuven / KULeuven, LEUVEN; [2]OLV Hospital/Centreof Human Genetics, AALST / LEUVEN; [3]University Hospital of Mont-Godinne, YVOIR; [4]UZ Leuven, LEUVEN; [5]Virga Jesse Hospital, HASSELT; [6]Europe Hospitals, BRUSSELS; [7]St. Jean Hospital, BRUSSELS; [8]St. Joseph Hospital, ARLON; [9]AZ Sint-Jan AV, BRUGGE; [10]AZ Zusters van Barmhartigheid, RONSE; [11]H. Hart Hospital, ROESELARE - MENEN; [12]UZ Leuven/UCL St. Luc, LEUVEN / BRUSSELS, Belgium

Background. Balanced translocations are uncommon in chronic lymphocytic leukemia (CLL), and their significance remains poorly understood. *Aims.* The aim of the present study was to identify the characteristics of a population of patients with B-CLL and a t(14;18) or variant. Methods. Clinical and cytogenetic files from patients referred between 1990 and 2007 for cytogenetic characterization of CLL, and displaying a t(14;18)(q32;q21) or its molecular equivalent were reviewed. Morphology was centrally reviewed. FISH was performed to confirm involvement of BCL2. IgVH mutational status, VDJ family usage, and BCL2 breakpoint were assessed. *Results*. Thirty patients displayed a t(14;18) or variant in the karyotype and 3 in molecular analysis (representing < 2% of CLLs referred during the period of study). These were mainly males (ratio M/F: 23/10) ranging in age from 34 to 88 years (median 67). Staging data were available in 24 patients: 19 presented with Binet stage A, 2 with stage B and 3 with stage C. Follow-up data were available in 29 patients over a period ranging from 1-161 months (median 43). Sixteen/28 required therapy; two were refractory to therapy. Five died due to disease evolution, and 3 due to another disease, whereas 17 are still alive and 4 are lost to follow-up. Immunophenotype was compatible with CLL (Catov*s*ky score ≥3). Morphological review confirmed the diagnosis of classical CLL in all cases. The t(14;18) was the sole aberration in 10 cases, and was associated with other changes in 23 cases, i.e. +12 in 13 patients and del(13q) in 5 patients. Other abnormalities included +X, -X and del(6q) in one case each. Complexity (≥3 changes) was rare (n=4). The translocation involved most frequently the IgH locus, but variant cases involving the Ig κ or λ locus were observed in 3 and 5 cases, respectively. The *BCL2* breakpoint was in the major breakpoint region in 14 cases and in the minor cluster region in 1 case. In 10 cases, the breakpoint was located elsewhere. VH was mutated in 17 and unmutated in 3 cases. In 20 patients, the 4 following Ig VH families were used : VH3 in 14, VH5 in 4 (all 5.51), VH4 and VH1 each in one case. J and D usage was known for 18 patients. J4 was used in 11, J6 in 3, J5 in 2 cases and J1 and J3 each in one case. D usage was variable. In three patients D2-8 was seen, D3-10 in 3 patients, D3-16 and D1-26 each in 2 patients. The 8 remaining patients all displayed a different D usage. There were no recurrent VDJ combinations. *Conclusions*. CLL with t(14;18) represents a rare entity. Population characteristics are comparable to those of CLL patients in general (middle-aged patients, male predominance, typical immunophenotype and classical morphology). The t(14;18) was neither associated with aggressive disease nor with complex cytogenetic changes. In addition, the majority of the patients displayed mutated IgVH status, and responded to therapy.

0082

ANALYSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA CASES WITH STEREOTYPIC IMMUNOGLOBULIN'S RECEPTORS IN UKRAINIAN COHORT

N. Bilous, I. Abramenko, A. Chumak

Research Centre for Radiation Medicine, KIEV, Ukraine

Background. The biased repertoire of immunoglobulin heavy-chain variable (IgHV) gene found in CLL and additional identification of CLL subsets with stereotyped heavy chain complementarity determining region 3 (*HCDR3*) suggest about possible role of antigen(s) in the disease pathogenesis. The nature of antigens remains to be identified, however some data suggest that it might be auto-antigens, and microbial or viral antigens. *Aims*. Our aim was to analyze of CLL cases in Ukrainian cohort for distinguishing cases with stereotyped HCDR3s and gain inside the nature of possible antigens involved. *Methods*. Reverse transcription, polymerase chain reaction, and direct sequence.

Table 1.

Patient no	GenBank Access no.	IgHV gene	HCDR3 aa sequence	Antigen specificity
E16	EF583678	IgHV4-30-4	AR YGPSS YYDFWSGY P YYFDY	
	DQ535056	IgHV3-33	-- A- -D ------- - -----	anti-PPS Ig
	EU099124	IgHV3-49	T- S---D --------Y G G----	CLL case
E53	EF091926	IgHV4-61	AR HRGD YDFWSGYY P YYWFDP	
	DQ322846	IgHV3-11	-- DTRP -------- - -----	anti-PPS Ig
	EU099131	IgHV4-31	-- YPPPF Y-------- - -----	CLL case
F47	EF407656	IgHV3-15	TT LT YYDFWSGY WSQ YYYYYYMDV	
	AY909677	IgHV3-48	AR Q --------Y TGH --------	anti-PPS14 IgG
	EU099159	IgHV3-30	AS AYP- -------- - Y-----G---	CLL case
F85	EU433870	IgHV3-11	AR DT LYFGEF HA YYFDY	
	DQ322780	IgHV3-33	-R PQ ----- LN -----	anti-PPS4 Ig
C36	EF583667	IgHV1-69	AR RQL LWGGD FDY	
	AF115114	IgHV1-69	-- GA --F-- F ---	antibodies that recognize the capsular polysaccharide of Neisseria meningitidis
F35	EF175440	IgHV4-59	AR AR YYYDSSGYY FDY	
	AB021532	IgHV3-30-3	-- --H -------S ---	anti- staphylococcal protein A Ig
F74	EU433868	IgHV1-02	AK PSF YCTHGVCY TD YYYGMDV	
	EF177969	IgHV3-30-3	-- DHR ---G---Y DG Y-------	rotavirus specific Ig
	DQ100687	IgHV4-39	-R GCR ------- V Y-------	CLL case
E70	EF091912	IgHV1-02	AR ? YSSSY PW YYYGMDV	
	AY944713	IgHV1-02	-- Y ----- - Y-------	rotavirus specific Ig
E73	EF175415	IgHV3-30-3	AR DTT YYVDSSGYYYYYYGMDV	
	AY941901	IgHV3-30	-- -PE ---E----P--------	anti-rabies virus Ig
	46379	IgVH3-33	-- -N ----------	natural human anti-Sm antibody VH chain
	EU099240	IgHV3-30-3	-- -E- ----------	CLL case

Results. Single productive IgHV gene rearrangements from 218 CLL patients were analyzed. Unmutated IgHV sequences predominated in the group - 155 (71.1%) cases *vs* 63 (28.9%) mutated. Ten IgHV genes accounted for more than 60% of all cases, the most frequent genes were IgHV1-69 (20.6%), IgHV4-34 (7.3%), and IgHV1-02 (6%). Alignment analyses of the HCDR3 sequences revealed that sixty seven (30.7%) had HCDR3 homology (>60%) with sequences inside CLL cohort or with CLL sequences available from GenBank database. At that, 54 of them were referred to 23 of 48 previously described stereotyped CLL subsets. Another 13 cases along with homologous CLL cases available from database were referred to 8 subsets, which to our knowledge, had not been reported earlier. As each of that groups consisted of at least 3 cases, they might be considered as true CLL subsets. We found also, that several CLL cases from the cohort showed HCDR3 homology with anti-bacterial or anti-viral Igs (Table 1). Specifically, 4 cases (IgHV4-30-4, IgHV4-61, IgHV3-15, and IgHV3-11) shared similar HCDR3 with Igs specific to Streptococcus pneumoniae polysaccharides (PPS), at that, for 3 first cases we found homological CLL case from GenBank database. One IgHV-69 case showed *HCDR3* homology with Ig that recognizes capsular polysaccharide of Neisseria meningitides, and one IgHV4-59 case - with anti - staphylococcal protein A Ig. Three cases showed homology with anti-viral Igs: two IgHV1-02 sequences - with rotavirus-specific Igs, and one IgHV3-30-3 - with anti-rabies virus Ig. *Summary*. The restricted IgHV repertoire and high quantity of CLL cases with stereotyped immunoglobulin receptors strongly suggests a selection by restricted set of antigens. It is possible that bacterial and viral antigens might play a role in CLL pathogenesis.

0083

CD200 AS A TOOL FOR DIFFERENTIAL DIAGNOSIS BETWEEN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AND MANTLE CELL LYMPHOMA

G.A. Palumbo,[1] G. Fargione,[1] N. Parrinello,[1] K. Cardillo,[2] A. Chiarenza,[1] S. Berretta,[1] C. Conticello,[3] L. Villari,[2] F. Di Raimondo[1]

[1]University of Catania, CATANIA; [2]Pathology Unit - Ospedale V. Emanuele, CATANIA; [3]Istituto Oncologico del Mediterraneo, VIAGRANDE (CT), Italy

A wide spectrum of B-cell lymphoproliferative disorders may become evident in leukemic phase and be misdiagnosed as Chronic Lymphocytic Leukemia (B-CLL), because of overlapping disease features. In particular, in the presence of a CD5 positive B-cell lymphocytosis, it is important to differentiate B-CLL from mantle cell lymphoma (MCL) as the latter is a more aggressive disease with poor response to conventional chemotherapy. Differential diagnosis between B-CLL and MCL is assessed by immunophenotypic analysis on freshly isolated cells and in this respect CD23 is considered a reliable marker because of its positivity in CLL and negativity in MCL. However, some cases where CD23 is not discriminant have been reported. In any case the diagnosis of MCL has to be confirmed by demonstration of cyclin D1 positivity or by the presence of the t(11;14)(q13;q32) chromosomal translocation detected by cytogenetics, fluorescence *in situ* hybridization (FISH), Western blot or Polymerase Chain Reaction (PCR) analysis. Even though these represent reliable methods, they are not widely available and time-consuming. Cyclin D1 detection can be accomplished on tissue biopsy only and not in cell suspension by flow cytometry; moreover, performing analysis on fixed tissues, CD5 and cyclin D1 may show equivocal or even negative results. In addition, the presence of the chromosomal translocation t(11;14) is not patognomonic for MCL. In fact, MCL patients without it are reported and the same translocation can be rarely found in B-CLL. Therefore, there is a need for finding new markers that allow an easier differential diagnosis between the two diseases. In this study we attempt to define the role of CD200 as a diagnostic tool. This antigen (previously referred to as OX2) is a membrane glycoprotein, belonging to the immunoglobulin superfamily, that seems to play an immunosuppressive role and regulates myeloid cell activity in a variety of tissues. It is expressed on a subset of T and all $CD19^+$ B lymphocytes (but not on NK cells), and highly on central and peripheral nerve tissue. Its expression has also been reported on human myeloma plasmacells and B-CLL cells. We evaluated CD200 in 91 patients with a CD5+ lymphoproliferative disease (79 B-CLL and 12 MCL in leukemic phase) by flow cytometry on fresh neoplastic cells. CD200 was positive in all B-CLL patients, while it was totally absent in 9 MCL patients and positive in 4, 7 and 16% of $CD5^+$ cells in the remaining 3. To confirm these results, we examined CD200 by immunohistochemistry on paraffin-embedded lympoid tissues and bone marrow (BM) trephine biopsies from 23 B-CLL and 44 MCL patients. Also with this technique all B-CLL neoplastic cells were positive for CD200 both in lymphnodes and in BM while all MCL cells were negative. Notably, in all MCL CD200-negative lympoid tissue biopsies, it was possible to observe CD200+ residual dendritic cells (useful as immunohistochemistry reaction positive control), as already known in tonsil and splenic follicular dendritic cells. Thus, in our series, there is a clearcut between MCL cells, in which CD200 is not expressed *vs* B-CLL, with high expression as reported by other authors. Therefore, adding CD200 in flow cytometry and immunohistochemistry routine panels could be of great help in distinguishing between these two entities, in particular in patients with a prevalent leukemic expression.

Chronic lymphocytic leukemia - Clinical

0084

COMBINED THALIDOMIDE AND FLUDARABINE THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA RESULTS IN DISTINCT MOLECULAR AND ANTILEUKEMIC EFFECTS

K. Giannopoulos,[1] K. Giannopoulos,[1] A. Dmoszynska,[1] M. Kowal,[1] E.W.K. Wasik-Szczepanek,[1] P. Wlasiuk,[1] A. Bojarska-Junak,[1] J. Rolinski,[1] S. Stilgenbauer,[2] L. Bullinger[2]

[1]Medical University of Lublin, LUBLIN, Poland; [2]University of Ulm, ULM, Germany

Background. Thalidomide (THAL) is a promising immunomodulatory drug that might not only target leukemia cells but also the tumor microenvironment. Moreover since angiogenesis plays important role in chronic lymphocytic leukemia (CLL) THAL based regiments might effectively treat disease. *Aims*. We aimed to treat CLL patients (pts) with THAL and fludarabine (FLU) and monitored effects of THAL treatment *in vivo* in sequential blood samples. *Patients and methods*. Forty patients were included in this study (median age 67, range 43-75 years). In total, 20 patients received THAL+FLU as first line (arm A) and 20 as second or third line therapy (arm B). Pts received THAL (100 mg po/day) starting at day 0 (d0). FLU was added for 5 consecutive days every 28 days (25 mg/m^2 iv/day) starting at day 7 (d7) for up to 6 cycles. TNF expression levels were assessed using a high sensitivity ELISA test, and T-cell subpopulations were analyzed by FACS analysis on d0, d7, and d12. To evaluate the influence of THAL on gene expression (GEP), 20 (10 from arm A and 10 from arm B) paired d0 and d7 samples were analyzed using U133plus2.0 microarrays. *Results*. The overall response rate (ORR) was 52,5%, 80% in arm A and 25% in arm B. 25% in arm A achieved CR but no CR in arm B was observed. THAL therapy was effective in decreasing the number of CLL cells ($CD5^+CD19^+$) as assessed by FACS from 51.75 G/L to 31.7 G/L after treatment on day 7. Subsequent 5 days of therapy with FLU reduced significantly the number of CLL cells to 16.8 G/L. The number of CD3 lymphocytes showed no significant change during THAL therapy (2599 cells/mL on day 0 and 2166 cells/mL on day 7). Addition of FLU decreased $CD3^+$ cells to 853 cells/mL on day 12 (p=0.01). Both $CD4^+$ and $CD8^+$ T cell subsets were significantly reduced after FLU therapy (p=0.008 and p=0.009, respectively). THAL induced no effect on $CD4^+$ as well as CD8+ T cell populations (p=0.44 and p=0.29, respectively). Interestingly, analyzing in detail CD4 T cells we found significant decrease in the number of CD4+CD25hiFOXP3+ T regulatory cells (Tregs) after THAL from 182 cells/mL to 114 cells/mL (p=0.05). Tregs were further reduced by FLU to 43.9 after FLU on day 12 (p=0.001). THAL therapy did not reduce the number of other T-cell subpopulations reported to possess regulatory properties such as $CD8^+CD28^-$, $CD4+GITR^+$, $CD4^+CD62L^+$ and $CD3^+TCR\gamma\delta^+$. In contrast addition of FLU to therapy decreased the number of $CD8^+CD28^-$ (p=0.012), $CD3^+TCR\gamma\delta^+$ (p=0.005) as well as $CD4^+GITR^+$ (p=0.058) and $CD4^+CD62L^+$ (p=0.27). The changes in Tregs during THAL therapy differed in responder *vs* nonresponder groups of CLL patients showing greater fold of Tregs reduction in patients who responded to therapy. However, we observed no significant changes in the plasma levels of TNF neither after THAL alone nor after FLU. No correlation was observed between serum TNF level and expression of TNF receptor 1 (*TNF R1*) nor TNF R2. Interestingly, the expression of TNF R1 was significantly higher in patients who did not responded to THAL as determined by WBC changes after 7 days of THAL therapy (19.98 *vs* 9.7, p=0.03).The nonresponder group tended to present with higher levels of TNF R2 when compared to patients who responded (51.6 *vs* 33.1, p=0.17). Paired supervised analysis of GEP data based on sample comparison before and during THAL treatment revealed a THAL induced signature comprising 123 differentially expressed genes (p<0.001). Comparison of GEP changes observed *in vivo* with the data contained in Connectivity Map, a public repository of GEP changes induced by *in vitro* treatment of cells with a wealth of different drugs, revealed a significant correlation of our signature with thalidomide induced changes in breast cancer cells (MCF7 cells). Interestingly, there was also a significant correlation with signatures induced by imatinib, valproic acid and prednisolone in MCF7 cells. Gene set enrichment analyses showed 22 deregulated pathways, including the apoptosis and FAS signaling pathway which involved expression changes of FAS, FADD, caspase 8 and caspase 3 (p<0.005). Furthermore, we observed a significant association with CD40L signaling which has been previously reported to play a role in immunomodulatory drug action. *Conclusions*. Combined THAL+FLU

therapy demonstrated significant efficacy previously untreated B-CLL and limited efficacy as salvage therapy with acceptable safety profile. Furthermore, our study provides novel biological insights into the molecular effects of THAL, which might act (i) by enhancing apoptosis of CLL cells and (ii) by reducing Tregs, thereby enabling T-cell dependent tumor rejection. THAL therapy revealed a superior safety profile in interaction with T cells when compared to FLU. Therefore THAL might represent an interesting therapeutic option for CLL patients.

0085

RITUXIMAB CONSOLIDATION AND MAINTENANCE THERAPY PROLONG RESPONSE DURATION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

G. Del Poeta,[1] M.I. Del Principe,[1] A. Siniscalchi,[1] L. Maurillo,[1] F. Buccisano,[1] A. Venditti,[1] F. Luciano,[1] P. Niscola,[1] A. Zucchetto,[2] V. Gattei,[2] A.P. Perrotti,[1] P. De Fabritiis,[1] S. Amadori[1]

[1]Department of Hematology, University Tor Vergata, ROMA; [2]Clinical and Experimental Hematology Unit, IRCCS, AVIANO (PN), Italy

Monoclonal antibodies in combination with chemotherapy resulted in more complete responses and longer response duration in B-cell chronic lymphocytic leukemia (B-CLL), reducing disease burden to levels detectable only by flow cytometry. Moreover, low-dose rituximab decreases CD20 antigen loss and promotes enhanced targeting in CLL (*Williams, 2006*). We performed a phase II study adding rituximab to fludarabine (Flu) as therapy for symptomatic, untreated CLL. Remission status was assessed also by a multiparametric flow cytometric approach based on the detection of $CD19^+CD5^+CD79b$- B-CLL lymphocytes. IgVH mutational status, CD38, ZAP-70 and cytogenetics were obtained in all patients (pts) before treatment. We defined as high risk pts having at least two of these markers: unmutated IgVH, CD38>30%, ZAP-70>20%, intermediate/unfavorable cytogenetics (trisomy 12 or del11q or del17p). Ninety-five CLL pts, median age 61 years (range 37-79) received six monthly courses of Flu (25 mg/sm^2 for 5 days) and four weekly doses of rituximab (375 mg/m^2) starting after completion of Flu therapy.

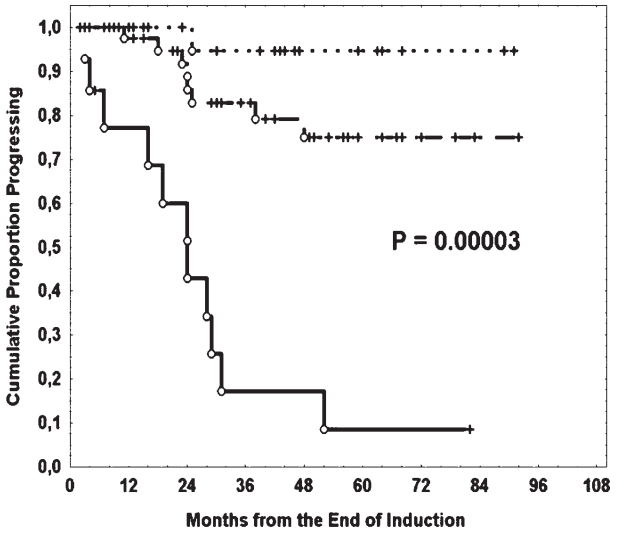


Figure 1. Response duration by Rituximab consolidation/maintenance.

Informed consent was obtained from all pts. According to modified Rai stages, 11 pts had a low stage, 81 an intermediate stage and 3 a high stage. Based on NCI criteria, 75/95 (79%) pts achieved a complete remission (CR), 16/95 (17%) a partial remission (PR) and 4/95 (4%) no response or progression. Four pts underwent grade 3 (WHO) infective lung toxicity, 1 patient acute fatal B hepatitis and 1 patient progressed towards Richter's syndrome. Hematologic toxicity included mainly neutropenia (grade 3 and/or 4 in 47 pts) and thrombocytopenia (grade 3 and/or 4 in 5 pts). Forty-one pts either in CR with $CD5^+CD19^+CD79b$- bone marrow (BM) cells >1% (MRD^+, n=15 pts) or in CR MRD negative with $CD5^+CD19^+$ peripheral blood lymphocytes (PBL) >1000/μL (n=14 pts) within 1 year after completion of the induction treatment or in PR (n=12 pts), underwent consolidation and maintenance therapy with four monthly cycles of rituximab at 375 mg/m^2 followed by twelve monthly doses of rituximab at 150 mg/m^2. The median follow-up duration was 48 months. All pts experienced a long progression-free survival (PFS) from the end of induction treatment (67% at 7 years). Nevertheless, CLL pts that underwent consolidation and maintenance therapy (n=41) showed a significantly longer response duration compared to MRD^+ not consolidated pts (n=14; 75% *vs* 9% at 6 years; p=0.00003, Figure 1). Noteworthy, BM and PBL persistently MRD negative (>1 year) pts (n=36) showed a very long response duration (95% at 6 years, Figure 1). A significant shorter PFS was observed within $CD38^+$ pts (29% *vs* 81% at 6 years, p=0.0002), unmutated pts (58% *vs* 89% at 4 years, p=0.003) and ZAP-70^+ pts (44% *vs* 85% at 6 years; p=0.00002). Notably, within the high risk subset (n=34), considering only MRD^+ pts in CR or PR (n=18), MRD^+ consolidated pts (n=11) showed a significantly longer response duration (58% *vs* 18% at 2 years, p=0.01) in comparison with MRD^+ not consolidated pts (n=7). Therefore, consolidation and maintenance therapy with rituximab prolong significantly the response duration in B-CLL, also within the high risk subset, thus potentially increasing overall survival.

0086

FLUDARABINE PLUS THALIDOMIDE AS FRONTLINE THERAPY FOR NEWLY DIAGNOSED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL).

S. Ailawadhi, K.C. Miller, D. Depaolo, A. Whitworth, W. Brady, M. Czuczman, Z.P. Bernstein, F. Hernandez-Ilizaliturri, S. Padmanabhan, A. Chanan-Khan

Roswell Park Cancer Institute, BUFFALO, USA

Introduction. Chronic lymphocytic leukemia (CLL) is a malignant hematologic disorder characterized by proliferation of morphologically mature appearing lymphocytes and a dysregulated tumor microenvironment that supports tumor cell growth. Fludarabine (F) has demonstrated its clinical efficacy in CLL and is often used as the first therapeutic option in these patients. To further improve the clinical responses to F we combined it with thalidomide (T). We hypothesized that T will target the tumor microenvironment resulting in increased sensitivity of the tumor cell to F. Thus the FT regimen will concurrently target the tumor cell as well as its microenvironment. Here we report the efficacy and toxicity profile of the FT regimen in treatment naive CLL patients enrolled on a phase I/II clinical study at our center. *Methods*. All previously untreated CLL patients requiring treatment as per the NCI-WG (1996) were eligible for this study. F was given at a dose of 25 mg/m^2 IV for 5 consecutive days starting day 8 of each 28-day cycle for a maximum of 6 cycles. T was initiated on day 1 and was given every day continuously throughout the treatment (6 cycles). The phase I part of the study was concluded and did not demonstrate any dose limiting toxicity of this combination (*Chanan-Khan et al Blood 2006*). The phase II investigated T at 200 mg/day in combination with F. Response assessment was done as per the Revised NCI-sponsored Working Group Guidelines. Acyclovir 400 mg PO BID, Bactrim DS PO 3 times/week and weight-adjusted low-dose warfarin were given for prophylaxis of herpes zoster, PCP and venous thromboembolism, respectively. *Results*. A total of 35 patients (11 females, 24 males) with a median age of 63 years (range 35-78) have been enrolled. Rai stage distribution at initiation of treatment was stage I 31% (n=11) stage II 20% (n=7), stage III 29% (n=10) and stage IV 20% (n=7), respectively. The phase I part of accrual included 13 patients while 22 patients were accrued on the phase II of the protocol. Most common ≥ grade 3 hematologic toxicities included neutropenia (20%) and thrombocytopenia (11%). There were no ≥ grade 3 non-hematological toxicities with an incidence > 10%. Most common non-hematological toxicities were grade 1 or 2 nausea (23%) and constipation (20%). Tumor flare was noted in 14% patients (n=5). Response analysis showed that 29 patients were evaluable. Of these, 15 (52%) achieved a CR and 13 (45%) achieved a PR. Thus the ORR was 97%. *Conclusions*. FT is a novel and efficacious regimen for treatment naive CLL. This regimen demonstrated high CR rate compared to historical control (fludarabine alone).Toxicity was manageable and acceptable. Detailed and updated results of this study will be presented at the meeting.

0087

LUMILIXIMAB IN COMBINATION WITH FCR FOR THE TREATMENT OF RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): RESULTS FROM A PHASE I/II MULTICENTER STUDY

J. Byrd,[1] J. Castro,[2] I. Flinn,[3] A. Forero-Torres,[4] T. Kipps,[2] N. Heerema,[1] Y. Kasamon,[5] W. Wierda,[6] T. Lin,[1] H. Mu,[7] S. Tangri,[7] S. O'Brien[6]

[1]The Ohio State University Comprehensive Cancer Center, COLUMBUS; [2]Moores Cancer Center University of California San Diego, SAN DIEGO; [3]Sarah Cannon Research Institute, NASHVILLE [4]University of Alabama Birmingham Comprehensive Cancer Center, BIRMINGHAM; [5]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, BALTIMORE;[6]The University of Texas/MD Anderson Cancer Center, HOUSTON; [7]Biogen Idec, SAN DIEGO, USA

Background. Lumiliximab is an anti-CD23 monoclonal antibody that is being investigated for relapsed CLL. In a previous study, lumiliximab monotherapy given weekly was well tolerated, achieved sustainable CD23 receptor occupancy and showed clinical activity. *Aims.* A phase I/II, multicenter study was conducted to evaluate the safety and efficacy of lumiliximab in combination with fludarabine, cyclophosphamide, and rituximab (L+FCR) for patients (pts) with relapsed $CD23^+$ CLL. In addition, CD23 receptor occupancy on CLL cells and possible effects of elevated serum CD23 were evaluated. *Methods*. 31 pts received either 375 mg/m^2 (n=3) or 500 mg/m^2 (n=28) of L+FCR for up to six 28-day cycles. All pts completed treatment and follow-up is ongoing. A semi-quantitative flow cytometry method was used to measure CD23 receptor occupancy and serum CD23 was analyzed using an enzyme-linked immunosorbent assay. *Results*. Median age at study entry: 58 yrs, Rai Stage I/II: 71%, median # of prior regimens: 2 (1-10). Overall response rate was 65%: complete response (CR) 52% and partial response13%. 5 of the 8 pts with del (11q22.3) achieved CR. Based on median follow-up of 16.8 mos (1.5-37.6), KM estimated median progression-free survival (PFS) for all pts was 19.3 mos. Median PFS for all responders and CR pts were 23.4 mos and 30.4 mos, respectively. 23 pts (74%) reported a Grade 3 or 4 event. Compared with published FCR data, L+FCR has a similar safety profile with no additional toxicities. L+FCR sustained CD23 receptor occupancy which was not affected by elevated levels of serum CD23. *Conclusions*. L+FCR is an effective regimen for pts with relapsed CLL. L+FCR produced an impressive CR rate, an encouraging PFS and a similar safety profile to that of FCR. A large, randomized, global study of L+FCR *vs* FCR (LUCID) is ongoing to further evaluate the safety and efficacy of this regimen.

0088

IDENTIFICATION OF AN IN VIVO MOLECULAR SIGNATURE OF SENSITIVITY VS RESISTANCE TO FLUDARABINE IN B CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

E. Moussay,[1] V. El Khoury,[1] N. Aouali,[1] K. Van Moer,[1] B. Leners,[1] F. Bernardin,[1] A. Muller,[1] P. Nazarov,[1] M. Yatskou,[2] L. Vallar,[1] V. Palissot,[1] G. Berchem[3]

[1]CRP-Santé, LUXEMBOURG; [2]Université du Luxembourg, LUXEMBOURG; [3]Centre Hospitalier de Luxembourg, LUXEMBOURG, Luxembourg

Background. The B chronic lymphocytic leukemia (B-CLL), the most common hematological malignancy in Western countries, is characterized by the accumulation of $CD5^+$ $CD19^+$ $CD23^+$ B lymphocytes arrested in G0/G1 phase of the cell cycle. Leukemic B-cells do not fulfill their immunological role and do not undergo apoptosis anymore due to up-regulation of anti-apoptotic factors, thus resulting in progressive obstruction of organs, gradually leading to death of the patients. Due to their potential to kill non-dividing cells, purine nucleoside analogs, such as fludarabine, are often used in first line therapy to treat hematological malignancies. Although the effect of fludarabine against CLL cells is widely accepted, its mode of action on the molecular level *in vivo* is poorly understood. A short list of genes regulated by fludarabine *in vivo* was previously reported; all of them induced in B cells by p53 after 5 days of treatment. *Aims*. In this study, we compared the response, at the transcriptomic level, of B cells from CLL patients sensitive or resistant to fludarabine in the clinic. We investigated their response to fludarabine up to 9 days after the beginning of the treatment. *Design and Methods*. For this purpose, we purified B cells from blood puncture before fludarabine administration (day 0) and after 1, 2, and 9 days of treatment. All patients had signed a written informed consent. We then performed a whole-genome analysis with cDNA microarrays (loop design). Selected genes were submitted to real-time PCR confirmation. Furthermore, an array-based Comparative Genomic Hybridization (CGH) analysis was used to determine the genetic aberrations of the B cells from these CLL patients. Additionally, we compared the genomic and transcriptomic data obtained by high-throughput methods with the clinical data of the patients. Gene Ontology softwares were used to functionally classify these genes. *Results*. Statistical analysis and clustering led us to identify for each time-point tested a gene expression signature of *in vivo* sensitivity and/or resistance to fludarabine of B-CLL patients. Most of *in vivo*-regulated genes identified could be classified with gene ontology softwares as part of the cell death mechanisms, cell cycle regulation, DNA damage and repair, kinase activity, enzymatic activity, translation and the RNA and nucleotide metabolism. In addition to common losses (del13q14; del17p13), cytogenetic analysis revealed the presence of isochromosomes together with other abnormalities in fludarabine-resistant patients. *Conclusions*. Taken together, our data show significant distinct profiles between sensitive and resistant patients in response to fludarabine treatment and strongly argue in favor of the establishment of targeted PCR after 1-2 days of treatment as a reliable tool to early determine the outcome of the therapy of B-CLL patients by fludarabine.

0089

ABNORMAL SERUM FREE LIGHT CHAIN RATIOS IN CHRONIC LYMPHOCYTIC LEUKAEMIA ARE SIGNIFICANTLY ASSOCIATED WITH EARLIER DISEASE-SPECIFIC DEATH

J. Harding,[1] G. Pratt,[2] R. Holder,[2] C. Fegan,[3] C. Pepper,[3] D. Oscier,[4] G. Mead[2]

[1]The Binding Site, BIRMINGHAM, UK; [2]University of Birmingham, BIRMINGHAM; [3]Cardiff University, CARDIFF; [4]Royal Bournemouth Hospital, BOURNEMOUTH, UK

Background. Serum free light chains (FLC) have prognostic significance in monoclonal gammopathy of undetermined significance, solitary plasmacytoma of bone, smouldering myeloma, multiple myeloma, Waldenstroms macroglobulinaemia and AL amyloidosis. The incidence of abnormal FLC in other lymphoid malignancies including chronic lymphocytic leukaemia (CLL) is unclear with only one published report which found 8/18 CLL patients with an abnormal FLC. There have been no studies correlating FLC with other biological variables and clinical outcomes in CLL or lymphoma. *Methods*. This is a retrospective study that analysed 259 (Stage: A, 209; B, 23; C, 21; Male:Female ratio 1.6:1, mean age 75: range 29-98) archived patient sera. Sera had been collected before (n=181) or during (n=79) treatment. The levels of FLC and B2M were assessed using nephelometric immunoassays (The Binding Site) on the Dade-Behring BNTMII analyser. Previously recorded measurements for biological and clinical markers (age, sex, CD38, ZAP70, and VH mutational status) were used in Kaplan Meier Survival Hazards and Cox Regression analysis, outcome was described as alive, cause of death (COD) related to disease or COD unknown/unrelated.

Table 1. Serum free light chain and B2M in CLL.

	Patient Status			
	All	Alive	Disease specific COD	Unrelated COD
Kappa κ (mg/L)	28.0 (0.32-382)	22.5 (0.45-309)	56 (0.32-382)	30 (2.14-177)
Lambda λ (mg/L)	21 (0.82-216)	19.4 (1.63-216)	28 (6.82-179)	24 (2.83-113)
κ / λ ratio	3.5 (0.2-156.1)	2.4 (0.02-54)	11.2 (0.02-156)	1.9 (0.09-10)
B2M (mg/L)	4.5 (0.13-74.2)	3.6 (0.13-21.9)	9.3 (1.6-72.4)	4.9 (1.56-15.8)

Results. The population of CLL patients were analysed (Table 1). Using Kaplan Meier Survival Hazards, abnormal FLC ratio was a significant indicator of poorer survival (n=255, Log Rank Mantle-Cox p=0.006). Using Kaplan Meier Survival Hazards, abnormal FLC ratio was a significant indicator of poorer survival (n=255, Log Rank Mantle-Cox p=0.006). Using Cox Regression analysis (n=179, missing =63, with 17

cases excluded before the first event) we analysed age, stage, sex, CD38, ZAP70, mutational status and B2M and found 4 significant, independent, prognostic factors ZAP70 (p<0.001), mutational status (p<0.002), B2M (p<0.003) and abnormal ratio (p<0.006). Furthermore, analysis of patients with an abnormal λ ratio showed significant correlation (Spearmans ρ 0.396 p<0.01) with VH3-21, 48 and 53 usage. *Conclusions*. Elevated levels of κ and λ FLC are evident in CLL patients. An abnormal FLC ratio associated significantly to worse outcome and is independent of the established markers ZAP70, CD38 and mutational status. VH3-21, 48 and 53 are known to be indicators worse outcome in mutated patients, here we provide the first indication that an abnormal λ FLC ratio may be indicative of the VH usage. The importance of abnormal FLC ratios in CLL requires further studies as does the biological rationale for their occurrence.

0090

RAI AND BINET CLASSIFICATIONS FOR CHRONIC LYMPHOCYTIC LEUKEMIA SHOULD RECOGNIZE A ROLE FOR IMMUNE THROMBOCYTOPENIA, BUT NOT FOR AUTOIMMUNE HAEMOLYTIC ANEMIA

C. Visco, I. Giaretta, M. Ruggeri, E. Novella, E. Albiero, E. Pacquola, C. Borghero, F. Rodeghiero

Ospedale S Bortolo, VICENZA, Italy

Background. In the era of biologic markers, and of immuno-chemotherapy, Rai and Binet clinical staging systems provide reliable classification of patients with chronic lymphocytic leukaemia (CLL) into well-defined risk-groups. Patients presenting with a haemoglobin level inferior to 10 (Binet C) or 11 g/dL (Rai 3), and/or a platelet count less than 100x10^9/L (Rai 4, Binet C) are still classified as high risk. However, both Rai and Binet classifications do not distinguish between autoimmune haemolytic anemia (AHA)/immune thrombocytopenia (IT), and marrow infiltration as the cause for anemia or thrombocytopenia. *Aims*. To verify the prevalence and prognostic significance of IT and AHA in patients with CLL at presentation. *Methods*. Four-hundred twenty-five patients with CLL consecutively diagnosed and followed at our Institution in a 9-year period were analyzed for the occurrence of AHA and IT at presentation, or during the first year following diagnosis in those not requiring treatment. Overall survival (OS) of patients developing AHA or IT within the first year from CLL diagnosis was compared with remaining patients using Kaplan-Meyer method, starting from the time of CLL diagnosis. Criteria for diagnosis of IT and AHA were as reported in our previously published series (*Blood 2008;111:1110-6*). Rai and Binet stage was 3/4 or C in 15% and 14% of patients, respectively. Median follow-up was 62 months, with 60% of patients requiring treatment for CLL. *Results*. Considering the whole follow-up period, 23 patients were diagnosed with AHA (5,4%), and 25 with IT (5,8%). Median time to AHA was 44 months (range 0-94), while median time to IT was significantly inferior (14 months, range 0-82, p=0.01, by Log-Rank test). Thus, of the 23 patients with AHA, three developed this complication at the time of CLL presentation, and one in the first year after diagnosis (4/23, 17%). Conversely, IT occurred at CLL diagnosis in six patients, and in the first year after diagnosis in five (11/25, 44%), significantly more frequently than AHA (p=0.04). Median OS of the four patients developing AHA within the first year from CLL diagnosis was not different from other patients with CLL (90 *vs* 112 months, p=0.25), whereas patients with IT within the first year from CLL diagnosis shared a remarkably shorter OS (50 *vs* 112 months, p=0.0001), which was similar to patients with Rai 4 or Binet C (44 and 48 months, p=0.23). An un-mutated immunoglobulin heavy-chain variable-region was frequently observed both among CLL patients with IT (80%), and AHA (55%). *Conclusions*. Differently from AHA, rarely occurring at CLL presentation, and whose prevalence increased when advanced stages were more frequently observed, IT was not related to disease activity. According to our findings, IT should be recognized as a possible cause of thrombocytopenia at CLL presentation, and patients classified as high risk in Rai and Binet classifications, while AHA doesn't seem to retain a prognostic role in this setting.

0091

COMPARISON OF CLADRIBINE PLUS CYCLOPHSOPHAMIDE OR FLUDARABINE PLUS CYCLOPHOSPHAMIDE IN DIFFERENT AGE GROUPS IN PREVIOUSLY UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (PALG-CLL3 STUDY)

T. Robak,[1] J.Z. Blonski,[1] J. Gora-Tybor,[1] K. Jamroziak,[1] L. Konopka,[2] B. Ceglarek,[2] M. Calbecka,[2] A. Kostyra,[2] B. Stella-Holowiecka,[2] J. Kloczko,[2] K. Warzocha,[2] I. Seferynska,[2] A. Dmoszynska,[2] M. Kowal,[2] K. Lewandowski,[2] J. Dwilewicz-Trojaczek,[2] E. Wiater,[2] K. Kuliczkowski,[2] S. Potoczek,[2] A. Hellmann,[2] A. Mital,[2] A. Skotnicki,[2] W. Nowak,[2] K. Sulek,[2] A. Zdunczyk,[2] J. Dybowicz,[2] K. Zawilska[2]

[1]*Med Univ of Lodz, LODZ, Poland;* [2]*PALG-CLL3, LODZ, Poland*

Background. Purine analogues, Cladribine (2-CdA) and Fludarabine (FA), are highly effective in treatment of chronic lymphocytic leukemia (CLL). This trial was designed to compare the efficacy and toxicity of 2-CdA and cyclophsophamide (CC regimen) with FA and cyclophsophamide (FC regimen) in different age groups of patients with CLL. *Patients and Methods*. The study was started in January 2004 and requirement was ended in May 2005. The efficacy and toxicity of CC *vs* FC were compared in two groups previously untreated patients with progressive or symptomatic CLL: aged less than 65 years (134 pts) and aged 65 years and more (151 pts). The primary endpoints of the analysis were overall response (OR) and complete response (CR). The secondary endpoints were progression free survival (PFS), overall survival (OS) and treatment-related toxicity. The treatment response and side effects were evaluated according to NCI-SWOG guidelines. The details of the treatment schedule were published previously (*Blood, 2006;108(2):473*). Results. The results of study are shown in *Table 1*.

Table 1

Characteristics		Treatment CC	Treatment FC	p value
Pts enrolled		145	168	
Pts evaluated		134	151	
	<65y	91	111	
	≥65y	43	40	
OR	<65y	77 (45%)	95 (55%)	0.50
	≥65y	41 (54%)	35 (46%)	0.19
CR	<65y	34 (39%)	54 (61%)	0.25
	≥65y	25 (54%)	21 (46%)	0.43
PFS (median)	<65y	2.294y	2.688y	0.33
	≥65y	2.676y	2.297y	0.60
Neutropenia	<65y	17 (37%)	29 (63%)	0.15
	≥65y	12 (60%)	8 (40%)	0.22
Thrombocytopenia	<65y	11 (48%)	12 (52%)	0.46
	≥65y	6 (55%)	5 (45%)	0.50
Infections	<65y	22 (42%)	30 (58%)	0.41
	≥65y	19 (56%)	15 (44%)	0.25
Death	<65y	11 (46%)	13 (54%)	0.33
	≥65y	5 (42%)	7 (58%)	0.60

Conclusions. CC and FC programmes used as first line therapy in B-CLL give similar response rates and PFS and OS in younger and older CLL patients. The chemotherapy toxicity is acceptable in both age groups and does not differ significantly between patients treated with FC and CC.

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0092

INCREASED SERUM BAFF (B-CELL ACTIVATING FACTOR OF THE TNF FAMILY) LEVEL IS A PECULIAR FEATURE ASSOCIATED WITH FAMILIAL CHRONIC LYMPHOCYTIC LEUKEMIA

S. Molica,[1] G. Digiesi,[2] F. Mauro,[3] R. Mirabelli,[1] G. Cutrona,[4] G. Vitelli,[2] F. Iuliano,[5] F. Morabito,[6] R. Foà,[3] M. Ferrarini[4]

[1]Azienda ospedaliera Pugliese-Ciaccio, CATANZARO; [2]Clinical Pathology, IRCCS REgina Elena, ROMA; [3]Hematology Institute, University La Sapienza, Rome, ROMA; [4]Medical Oncology, Istituto Nazionale Tumori Genova, GENOVA; [5]Medical Oncology, Ospedale Giannettasio, ROSSANO (CS); [6]Department Hematology, Az. Ospedaliera, COSENZA, Italy

Although serum levels of BAFF (B-cell activating factor of the TNF family) have been evaluated in a number of lymphoproliferative disorders information on B-cell chronic lymphocytic leukemia (CLL) are limited. With this background we sought to establish whether BAFF levels correlated with clinical characteristics of disease in a series of 84 CLL patients. In this patients' cohort we found that BAFF serum levels, as measured at the time of diagnosis using a commercial ELISA assay (R & D Systems, USA), were significantly higher in age- and sex-macthed healthy controls (i.e.,median, 695 ng/mL; range, 389-1040) in comparison to the general population of CLL patients (median, 376; range, 93-8914; $p<0.0001$). Moreover, we observed significantly lower levels of BAFF among patients with unmutated IgVH (295.5 ng/mL; range, 138-1823) in comparison with patients with mutated IgVH (415 ng/mL; range 93-8914: $p=0.0001$). These differences translated into a shorter time to first treatment observed in Binet stage A patients with lower soluble BAFF levels in comparison to patients with higher concentration ($p<0.0001$). When we examined the clinical characteristics of CLL patients with elevated BAFF levels (after setting as a cut-off the median value observed in healthy controls; i.e., 695 ng/mL) we found that 6 out of 15 (40%) of individuals with increased levels had a first-degree relative diagnosed with CLL. In contrast, only 5 out of 64 (7.2%; $p=0.0007$) patients with sporadic CLL displayed BAFF concentrations higher than median value found in controls. This difference translates into a six-fold hingher frequency of elevated BAFF levels in the familial CLL in comparison to sporadic CLL. To determine whether elevated BAFF levels correlated with features other than familial incidence, we compared clinico- biological caracteristics between patients with familial and sproradic CLL, respectively. The two groups were alike with respect to age ($p=0.82$), sex distribution ($p=0.97$), mutational status of IgVH ($p=0.682$), CD38-expression ($p=0.529$) and ZAP-70-expression ($p=0.85$). Finally, the only feature differentiating sporadic from familial CLL was serum BAFF level which was significantly higher in the latter (Sporadic CLL, 336 ng/mL; range 93-925; Familial CLL, 601 ng/mL; range, 138-8914; $p=0.002$). In conclusion our results indicate that in early B-cell CLL clinico-biological profile including among other parameters BAFF may provide a useful insight into the complex interrelationship of prognostic variables. As demonstrated by others in patients with familial lymphoproliferative disorders (J. Clin Oncol 2006, 24 : 983-987) we provide clear evidence that serum BAFF levels represent a peculiar biological feature of familial CLL.

0093

LOW DOSE ORAL FLUDARABINE PLUS CYCLOPHOSPHAMIDE IN ELDERLY PATIENTS WITH UNTREATED AND REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

F. Forconi, A. Fabbri, M. Lenoci, E. Sozzi, M. Bocchia, A. Gozzetti, M. Tassi, D. Raspadori, F. Lauria

University of Siena, SIENA, Italy

Background. Fludarabine plus cyclophosphamide (FC) at conventional doses is highly effective in Chronic lymphocytic leukemia (CLL) and is currently indicated in first line therapy. Oral FC at reduced doses remains highly effective in elderly patients with low-grade non Hodgkin lymphomas other than CLL. *Aims.* to test tolerability and efficacy of oral FC at reduced doses in patients unfit for standard regimens. *Methods.* Twenty-five elderly patients with untreated (UT-CLL, n=13) or refractory CLL (R-CLL, n=12), requiring treatment according to 1996-NCI criteria, were given oral F (25 mg/m^2/day) and C (120 mg/m^2/day) in an outpatient regimen for 4 consecutive days every 4 weeks for a maximum of 4 cycles. Median age of the whole population was 70 years (range 61-80). Performance status was ≥1 in 80% patients. Tumor IGHV gene was unmutated in 90% cases. CD38 was positive in 68% and ZAP-70 in 68%. Chromosomal 11q or 17p deletions were documented in 25.5% patients. Median time from diagnosis or from prior treatment to FC was 24 months (range 1-77). Overall, biological risk and clinical characteristics were indicative of an aggressive behavior of the investigated cohort. Patients were evaluated after every cycle for toxicity and hematological response. Toxicity and responses were defined according to NCI criteria, with the exception of not repeating bone marrow aspirate after treatment termination. Progression, new treatment or death was defined as "event". *Results.* Patients received median 3 cycles (4 in UT-CLL, 3 in R-CLL). Nine-of-25 (36%) reduced the number of cycles because of fatigue (1 patient), heart failure (1), idiopathic thrombocytopenic purpura (1) in the UT-CLL (3/13) or infection (2), femur fracture (1), prolonged isolated thrombocytopenia or pancitopenia (2) and idiopathic thrombocytopenic purpura (1) in the R-CLL (6/12). Overall, 23/25 patients (92%) obtained a response (11/25 CR, 44%; 12/25 PR, 48%). Among UT-CLL, all responded to treatment (8/13 CR, 61.5%; 5/13 PR, 38.5%). Among R-CLL, 10/12 (83.5%) responded (3/12 CR, 25%; 7/12 PR, 58.5%; 2/12 NR, 16.5%). With a median follow-up of 23 months, 14/25 patients (56%) were event-free and 23/25 (92%) were alive. Among UT-CLL (median follow-up 12 months), 9/13 (69%) were event-free and all were alive. Among R-CLL (median follow-up 25 months), 5/12 (42%) were event-free and 10/12 (83%) were alive. Deaths occurred in the 2 R-CLL that had not responded to treatment, after 2 and 4 years, respectively. *Summary and Conclusions.* Despite the poor risk of the investigated population, this low-dose oral FC treatment showed good efficacy both in untreated and refractory/relapsed CLL. The treatment may be useful in elderly patients who cannot benefit of more aggressive or eradicating strategies and is easy to administer on an outpatient basis.

0094

ORAL FLUDARABINE PLUS RITUXIMAB RESULT IN HIGH COMPLETE REMISSION RATE IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

G. Del Poeta,[1] M.I. Del Principe,[1] L. Maurillo,[1] A. Siniscalchi,[1] F. Buccisano,[1] A. Coletta,[1] P. Bulian,[2] A. Venditti,[1] P. Niscola,[1] V. Gattei,[2] C. Simotti,[1] A.P. Perrotti,[1] P. De Fabritiis,[1] S. Amadori[1]

[1]Department of Hematology, University Tor Vergata, ROMA; [2]Clinical and Experimental Hematology Unit, IRCCS, AVIANO (PN), Italy

In the past two decades the treatment goal of chronic lymphocytic leukemia (CLL) shifted from symptom palliation to the attainment of maximal disease control, using the chemoimmunotherapy combinations incorporating purine analogs and monoclonal antibodies. Although intravenous (IV) fludarabine is effective against CLL, IV administration is inconvenient in an outpatient setting. The aim of this phase II study was to evaluate the efficacy and the toxicity of a regimen combining sequentially oral fludarabine phosphate (Flu) and rituximab in symptomatic, untreated CLL patients (pts). The primary end point was the overall response rate. Minimal residual disease (MRD) was assessed by a multiparametric flow cytometric method based on the detection of CD19$^+$CD5$^+$CD79b- residual B-CLL lymphocytes in bone marrow (BM). VH mutational status, CD38, ZAP-70 and cytogenetics by fluorescence *in situ* hybridization were obtained in all pts before treatment. Forty-six CLL pts, median age 65 years (range 50-79) received six monthly courses of Flu (35 mg/m^2 for 5 days) and four weekly doses of rituximab (375 mg/m^2) starting soon after completion of Flu therapy (median time 40 days). Informed consent was obtained from all pts. According to modified Rai stages, 9 pts had a low stage, 35 an intermediate stage and 2 a high stage. Eighteen of 46 (39%) pts were ZAP-70 positive, 13/46 (28%) were CD38 positive and 16/45 (36%) were IgVH unmutated. Eighteen of 46 pts (39%) showed a normal karyotype, 12/46 (26%) presented del13q and 16/46 (35%) an intermediate/high risk cytogenetic pattern (6 pts: del17p; 5 pts: del11q; 5 pts: trisomy 12). Based on NCI criteria, 37/46 (80%) patients achieved a complete remission (CR) and 9/46 (20%) a partial remission (PR). There were no differences between VH mutated and unmutated, or between ZAP-70$^+$ and ZAP-70 negative, or between CD38+ and CD38- subsets with regard to CR rate. Twenty-nine of 36 pts (80%) in clinical CR presented CD5$^+$CD19$^+$CD79b- BM cells <1% (MRD-). The main side effects were myelotoxicity and infections. Hematologic toxicity included mainly neutropenia (grade 3 and/or 4 in 16 pts), thrombocytopenia (grade 4 in 2 pts) and anemia (grade 4 in 1 patient). Five pts presented grade 3/4 (WHO) infective lung toxicity and 5 patients herpes zoster virus infection. Three patients received only oral fludarabine because grade 4 neutropenia and serious lung infections. Gastrointestinal toxicity was generally mild to moderate and did not require treatment. Although the median follow-up duration was still short (19 months), all B-CLL pts experienced a long progression-free survival from the end of induction treatment (90% at 2 years). In conclusion, the combination of oral fludarabine/rituximab provides a highly effective and well tolerable first-line option allowing us to achieve MRD negativity with a potential increase in survival time.

0095

RETROSPECTIVE COMPARISION OF EFFICASY AND TOXICITY OF FC AND FCR REGIMENS IN UNTREATED PATIENTS WITH B -CELL CHRONIC LYMPHOCYTIC LEUKEMIA

A. Nikitin,[1] A. Stadnik,[2] N. Salogub,[3] Yu. Lorie,[1] N. Tsyba,[1] A. Alexeeva,[4] N. Mashuk,[1] V. Biderman,[1] A. Zaritsky[2]

[1]Hematology Research Center of Russia, MOSCOW; [2]Center for Heart, Blood and Endocrihology, ST.PETERSBURG; [3]Pavlov Medical University, ST.PETERSBURG; [4]City Hospital 31, ST.PETERSBURG, Russian Federation

Background. Although no randomised trials have been published so far, phase II studies and retrospective comparisions have demonstrated that addition of rituximab to fludarabine containing regimens provides a significant advantage over FC or fludarabine alone in terms of response, PFS and even OS. In this retrospective study we have compared treatment outcomes in patients who received FC or FCR as initial therapy. *Aims*. The main tasks were assesement of toxicity, PFS and OS in patients treated with FC or FCR in real clinical practice. *Methods*. The study included 102 B-CLL patients who started treatment before the 1st of October, 2006 in four Russian centers. There were 69 males and 33 females. The median age was 56,5 yrs (range 40-78 yrs). Sixty two patients received FC and 40 FCR. The decision between FC and FCR was based predominantly on the availability of rituximab. FC was given in a standard 3-day schedule either intravenously (F 25 mg/m^2/d and C 250 mg/m^2/d) or per os (F 40 mg/m^2/d, C 250 mg/m^2/d). All patients in FCR group received 375 mg/m^2 of rituximab on day 1 of each cycle. *Results*. Overall and complete response rates were 87% and 39% in the FC group *vs* 95% and 60% in the FCR goup, respectively (p=0.03). The median progression free survival was 24 months in FC group and has not been reached in FCR group and the survival probability at 2 years was 75% (p=0,009). There was a clear trend in overal survival in favour of FCR, although differences were not significant (p=0,11). Pretreatment characteristics of two groups, such as stages, blood count, lymphocyte doubling time, LDH level, % of patients with bulk lymph nodes and mutational status were well matched, although there were two exceptions. First, the median time to treatment in FCR group was significantly shorter (14 months *vs* 28,5 months). Second, 35% (n=14) of patients in FCR group were younger than 50 years, while in FC group only 17% (n=11) were younger than 50 years. Multivariate Cox regression analysis using baseline characteristics showed that the addition of rituximab to FC reduced the risk of disease progression across all patient subgroups (age, time to treatment, stages) compared with FC. With adjustment on age and time to treatment, stage C and treatment without rituximab were independently associated with disease progression with hazard ratios of respectively 2.65 (CI-95% 1,7-4,0; p=0.02) and 2.3 (CI-95% 1,5-3,6; p=0.05). More patients in the FCR group experienced neutropenia grade III - IV (39% *vs* 29%). The rate of anemia and thrombocytopenia were similar in both treatment groups. Two patients in FC group developed autoimmine hemolytic anemia (AIHA). There were no cases of AIHA in patients receiving FCR. Infectious toxicity was similar, with febrile neutropenia being the most frequent event (12% of cycles in FC group and 9,5% of cycles in FCR group). *Conclusion*. The addition of rituximab to the FC regimen improves the clinical outcome in previously untreated patients with B-CLL without increased toxicity.

0096

CHEMOIMMUNOTHERAPY REGIMEN OF FLUDARABINE, CYCLOPHOSPHAMIDE, AND RITUXIMAB FOLLOWED BY ALEMTUZUMAB AS INITIAL THERAPY FOR CHRONIC LYMPHOCYTIC LEUKAEMIA: A VALID APPROACH FOR ERADICATION OF MINIMAL RESIDUAL DISEASE

S. Galimberti, N. Cecconi, G. Cervetti, G. Buda, E. Orciuolo, F. Papineschi, F. Caracciolo, E. Benedetti, M. Petrini

Hematology, PISA, Italy

Background. Association of cyclophosphamide, fludarabine and rituximab has been reported as a very effective treatment for patients affected by chronic lymphocytic leukaemia (CLL), with 95% of overall responses (ORR) and 70% of complete remissions (CR) (*Keating MJ, JCO 2005*). Our Group previously reported that 62% of cases, persistently IgH-positive after fludarabine, achieved molecular remission after alemtuzumab administered subcutaneously, 10 mg, three times a week, for 12 weeks (*Galimberti S, J Immunotherapy 2004*). Moreover, Moreton and co-workers reported that alemtuzumab offered 20% of minimal residual disease (MRD)-negative responses, with median survivals significantly longer in MRD-negative patients compared with those achieving an MRD-positive complete remission, partial remission (PR), or not responsive (NR) (*Moreton P, JCO 2005*). *Patients*. On these bases, in the 2004 a new protocol for treatment of patients affected by advanced CLL, aged ≤65 years, started at Pisa Haematology. This protocol scheduled 4 cycles of fludarabine-cyclophosphamide-rituximab; cases in PR received further 2 cycles. Cases in CR MRD-negative underwent leukapheresis after Aracytin plus G-CSF, whereas patients in CR but still MRD-positive or in PR received alemtuzumab at the schedule above reported. After 60 days, patients were re-evaluated by molecular assays also; cases still MDR-positive received Rituximab again as *in vivo* purging before stem cell mobilization procedure. Patients MRD-negative after alemtuzumab directly underwent the stem cell mobilization. Cases achieving an MRD-negative CR stopped treatment and underwent clinical and molecular follow-up every three months for the first 3 years. Patients in CR but still positive or that became IgH-positive again after a PCR-negative phase or who harvested contaminated CD34$^+$ cells had to be considered for autologous transplantation. *Results*. Until December 2007, 16 patients have been treated according to this protocol; 11 were male, and the median age was 50 years. The median lymphocytic count was 13.1x10^6/L, median bone marrow infiltration was 43%; 25% of cases presented high β2-microglobulin levels or splenomegaly >5cm or were scored at high risk for CD38 high levels. No deletions of chromosome 17 were documented after FISH analysis. After 4/6 R-FC cycles, the ORR was 87%, with 74% of CRs. All patients completed treatment: toxicities included in half of patients WHO grade 3-4 neutropenia, 12% infections, and 10% of CMV reactivations (without disease). Ten out of the 16 patients resulted IgH-negative by fluorescent PCR assays and then underwent stem cell harvest. Seven of them mobilized >3×10^6 CD34$^+$ stem cells and 85% of harvests were uncontaminated. One patient in CR but MRD-positive, 3 patients in PR and one resistant received alemtuzumab. The patient in CR achieved the MRD-negativity, and the resistant patient achieved a good PR. Three cases received Rituximab before stem cells harvest, but they collected contaminated aphereses. With a median follow-up of 20 months, 11 patients are still in CR, 9 of them (82%) also PCR-negative. In univariate analysis, quality of response (CR *vs* PR/NR), splenomegaly and MRD-negativity after R-FC treatment were the most significant parameters conditioning PFS. In multivariate analysis, the molecular status and splenomegaly retained this significance. *Conclusions*. In conclusion, the R-FC regimen, followed by administration of alemtuzumab, allowed to achieve a very high rate of haematological complete responses and MRD eradication. Because even in the *alemtuzumab era* the eradication of minimal residual disease is associated with better overall and treatment-free survivals, the above reported combination could represent a valid approach for patients affected by advanced CLL.

0097

DEMOGRAPHICS AND SURVIVAL OF PATIENTS WITH STAGE 0 CHRONIC LYMPHOCYTIC LEUKEMIA (CLL-0) AND MONOCLONAL B CELL LYMPHOCYTOSIS WITH CLL-PHENOTYPE (MBL) IN A SINGLE CENTRE 1992-2007: EFFECT OF PROPOSED NEW DIAGNOSTIC CRITERIA

A. Yong,[1] G. White,[2] J. Stanley,[3] D. Buhrkuhl,[2] J. Phillips[2]

[1]Otago University, WELLINGTON; [2]Haematology, Wellington Hospital, WELLINGTON; [3]Public Health, University of Otago, WELLINGTON, New Zealand

Background. Little is known about the nature and prognosis of MBL or how this condition differs from early stage CLL. New diagnostic criteria to distinguish between CLL-0 and MBL have been proposed and are currently controversial.[1] *Aims*. To compare the demographics and survival of patients with CLL-0 and MBL diagnosed over 15 years in a single centre using both current and proposed new diagnostic criteria. Methods. We established a retrospective database from diagnostic immunophenotyping records and hospital clinical records for all patients with CLL or MBL (IWLLG criteria) diagnosed at Wellington Hospital 1992-2007. Dates of death were obtained from New Zealand Health Information Service. Results. 388 CLL and 44 MBL patients were identified. 203 CLL patients were Rai stage 0 at diagnosis. 154 were of unknown stage. Peripheral clone size at diagnosis was reported for 207/247 patients with CLL-0 or MBL. No significant difference was seen in demographics, CD38 status or overall survival between CLL-0 and MBL patients using current diagnostic criteria in which a lymphocyte count of 5x10^9/L is used to distinguish CLL-0 and MBL or proposed new criteria in which peripheral blood B cell clone size of 5×10^9/L is used (*Table 1*). For the 247 patients with either CLL-0 or MBL, projected medi-

an survival (Kaplan-Meier) was 8.2 yrs for patients with CD38 negative disease and 4.1 yrs for patients with CD38 positive disease (p=0.065, log-rank test). Conclusion. No significant difference in age distribution, sex ratio or proportion with CD38 positive disease was found between patients with CLL-0 and those with MBL using either current or proposed new diagnostic criteria. No significant difference in survival was found between CLL-0 and MBL patients regardless of whether peripheral blood clone size of 5×10^9/l or peripheral blood lymphocyte count of 5×10^9/l was used as a diagnostic criterion. In patients with early lymphoproliferative disease expressing a CLL immunophenotype (either CLL-0 or MBL), there was a non-significant trend to greater survival in patients with CD38 negative disease.

Reference

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0098

HCNT1 EXPRESSION AND PURINE ANALOGUE ACTIVITY: PHARMACOGENOMIC AND CLINICAL RESULTS IN CLL/SLL PATIENTS TREATED WITH 2-CDA AND RITUXIMAB

P. Bertazzoni, C. Rabascio, D. Laszlo, L. Calabrese, D. Radice, L. Orlando, L. Preda, P. Rafaniello, F. Gigli, S. Bassi, L. Nassi, G. Martinelli

European Institute of Oncology, MILAN, Italy

Background. The combination of purine analogue and rituximab represents an efficacy treatment for CLL/SLL pts. According to NCI-WG updating guidelines, a treatment failure or disease progression within 6 months to a purine analogue based regimen is defined as refractory disease (*high risk* pts) with a poor prognosis. The mechanisms of resistance are still unclear despite some preliminary suggestions seem to involve several proteins responsible for uptake and transport of nucleoside analogue into the tumour cells. *Aims*. To investigate the activity of 2-CDA and rituximab combination in CLL/SLL pts and to identify, by pharmacogenomic approaches, genetic factors that may predict clinical response for a risk adapted therapy. *Methods*. 35 pts with an active CLL (24 pts) or SLL (11 pts) were treated with a combination therapy with rituximab at a dose of 375 mg/m^2 on day 1 and 2-CdA at a dose of 0.1 mg/kg (subcutaneously) per day for 5 days. The treatment was repeated every 4 weeks for 4 cycles. The median age was 59 years (31-76). 43% of pts were pre-treated. All pts performed a CT scan of the abdomen at baseline and at the and of treatment. Minimal residual disease (MRD) assessment was evaluated by flow-cytometry and PCR methods. Using ABI PRISM 7000 Real Time PCR platform we investigated the hCNT1 expression encoding for concentrative nucleoside transporters protein. Relative quantification was performed using the Delta Ct calculation: the value of gene expression was normalised to the calibrator (healthy tissue cells and healthy donors peripheral blood cells). The pharmacogenomic analysis was done in bone marrow for SLL and in peripheral blood for CLL, in 19 pts (14 CLL and 5 SLL). Results. The overall response rate was 92% (49% CR and 43% PR). SLL pts achieved 100% of ORR. At baseline the abdomen CT scan was abnormal in 33 pts and it remains abormal at revaluation in 16 pts. 8 pts achieved a negative MRD by flow-cytometry. With a median follow-up of 16 months (1,5- 44) 13 pts progressed with a median TTP of 9,8 months (2-40). 50% of pts with abnormal CT scan experienced a PD with a median TTP of 3 months while 29% with a normal CT scan at the and of therapy progressed with a median TTP of 13 months. The pharmacogenomic analysis showed a significance difference in terms of hCNT1 expression levels between patients with a refractory and no refractory disease (p=0,0214). There was no statistical difference in terms of hCNT1 expression between CLL and SLL pts. *Conclusions*. The combination of 2-CDA and rituximab is associated with a high response rate. The high rate of relapse observed in pts with abnormal CT scan underlies the relevant role of this radiological examination. The low levels of hCNT1 expression measured in high risk patients need to be confirmed in a larger studies and correlated, if possible, with other negative prognostic factors such as the presence of del (17) (p13.1) to better understand the reduced efficacy of purine analogue in pts with refractory disease.

Chronic myeloid leukemia - Biology

0099

BCR/ABL ONCOGENIC KINASE INHIBITS MISMATCH REPAIR TO PROTECT FROM APOPTOSIS AND INDUCE POINT MUTATIONS

T.S. Stoklosa,[1] T. Poplawski,[2] M. Koptyra,[3] M. Nieborowska-Skorska,[3] G. Basak,[1] A. Slupianek,[3] M. Rayevskaya,[4] I. Seferynska,[5] L. Herrera,[4] J. Blasiak,[2] T. Skorski[3]

[1]The Medical University of Warsaw, WARSAW, Poland; [2]Department of Molecular Genetics, University of Lodz, LODZ, Poland; [3]Department of Microbiology and Immunology, Temple University, PHILADELPHIA, USA; [4]Department of Pediatrics/Hematology, Temple University, PHILADELPHIA, USA; [5]Department of Hematology, Institute of Hematology and Blood Transfusion, WARSAW, Poland

Background. BCR/ABL oncogenic tyrosine kinase transforms hematopoietic stem cells to induce chronic myelogenous leukemia (CML) and acute lymphocytic leukemia (ALL). BCR/ABL kinase can also increase DNA damage and compromise the fidelity of DNA repair causing genomic instability. Accumulation of genetic abnormalities is believed to be responsible for resistance to the small molecule inhibitors such as imatinib, dasatinib and nilotinib, and for transition of a relatively benign chronic phase (CML-CP) to the fatal blast crisis (CML-BC). Mismatch repair (MMR) system maintains genomic stability by removing mismatched bases from DNA and by inducing apoptosis in cells with excessive DNA damage, the role of MMR activity in leukemia cells remains unclear. *Aims*. Examining the role of mismatch repair system in genetic instability of CML cells. *Methods*. 32Dcl3, Baf3 murine IL-3-dependent cell lines and their BCR/ABL-transformed counterparts were used. Primary CML CD34$^+$ patient cells were used after receiving informed consent. For measurement of MMR activity the plasmid substrate was used containing EGFP gene with T:G mismatch-corrupted start codon. The mismatch can be corrected to T:A by MMR, which restores T-A match and EGFP expression as determined by flow cytometry measuring the number of EGFP$^+$ cells. N-methyl-N-nitro-N-nitrosoguanidine (MNNG) was used as a mutagen inducing MMR-sensitive DNA lesions and ouabain-resistance as an indicator of mutagenesis in Na^+/K^+ ATPase to further assess mutation rate and phenotype. Cell viability was assessed by trypan blue exclusion, clonogenic tests of cells surviving MNNG challenge were done in methylcellulose. MMR proteins expression was analyzed by Western blotting, while nuclear localization by immunofluorescence microscopy. *Results*. The efficacy of MMR was reduced 2-fold in BCR/ABL-positive cell lines and CD34$^+$ CML cells in comparison to normal counterparts as measured by MMR activity assay. Impaired MMR activity in leukemia cells was associated with better survival, resistance to apoptosis and accumulation of p53 but not of p73 after treatment with MNNG, which induces DNA lesions that recognized by the MMR pathway. In contrast, parental cells displayed accumulation of p53, p73, and activation of caspase 3 resulting in cell death (Figure 1).

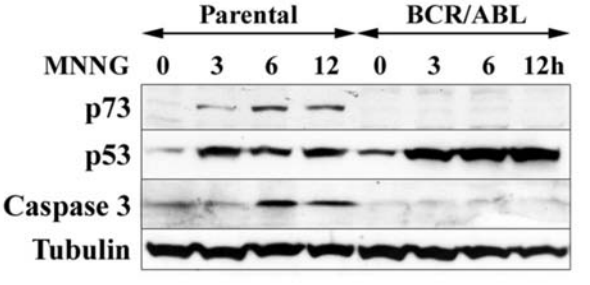

Figure 1.

BCR/ABL-positive cells, surviving the treatment with MNNG, displayed 15-fold higher mutation frequency than parental counterparts as determined by ouabain-resistance test. More than 70% of mutations in BCR/ABL cells were G:C'A:T and A:T'G:C transitions. Western blotting and immunofluorescence studies indicated that total and nuclear expression of MMR proteins (MSH2, MSH6, MLH1, PMS2) did not differ in BCR/ABL-positive and normal cells, but their nuclear co-localization in response to MNNG was severely impaired in the former cells. The

defects in activity of MMR system and interaction of MMR proteins in leukemia cells were reversed by inhibition of BCR/ABL kinase with imatinib. *Summary and Conclusions*. We observed reduced MMR activity in CML cells associated with higher mutation frequency. Deregulated MMR activity in CML cells may be directly responsible for generation of point mutations in bcr/abl kinase sequence (and in other genes) causing resistance to small molecule inhibitors and malignant progression of the disease. The precise effect of BCR/ABL kinase on MMR pathways requires further studies.

0100

HIGH-RESOLUTION MAPPING OF DELETIONS OF CHROMOSOME 9Q+ IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA BY SNP-ARRAYS: WHICH GENES MATTER?

S. Colarossi,[1] A. Gnani,[1] S. Soverini,[1] F. Castagnetti,[1] G. Marzocchi,[1] S. Luatti,[1] A. Astolfi,[2] S. Formica,[2] I. Iacobucci,[1] E. Ottaviani,[1] F. Palandri,[1] A. Poerio,[1] M. Amabile,[1] G. Rosti,[1] A. Pession,[2] N. Testoni,[1] M. Baccarani,[1] G. Martinelli[1]

[1]Institute of Hematology and Medical Oncology Seràgnoli, BOLOGNA; [2]Pediatric Oncology and Hematology L. Seràgnoli, BOLOGNA, Italy

Background. Extensive submicroscopic deletions 5' to ABL and/or 3' to BCR on the derivative chromosome 9 (9q+) may be detected at diagnosis in a proportion of patients (pts) with chronic myeloid leukemia (CML). 9q+ deletions (del9q+) have been associated with adverse outcome in pts treated with chemotherapy of interferon, but whether or not they retain prognostic significance in the imatinib era still remains a matter of controversy. *Aims*. Last year we presented the results of a study conducted on 442 newly diagnosed CML pts enrolled in GIMEMA CML WP studies of imatinib between Jan 2004 and Jan 2006. The rates of complete cytogenetic response and major molecular response were shown not to differ significantly between pts with (n=55) and without (n=387) del9q+. It can be hypothesized that the size of the deleted region may be a confounding variable and that one or more critical genes lost in some cases and retained in others may be responsible for differences in pt response and outcome. In order to address this issue, we planned to use SNP-arrays to perform a high-resolution mapping the deleted region in the 55 pts who were known to carry del9q+ as assessed by FISH analysis. *Methods*. Genomic DNA could be extracted from bone marrow mononuclear cells archived at diagnosis in 42/55 pts. SNP-array-based karyotyping was perfomed using Affymetrix GeneChip Human Mapping 250K Nsp arrays. Copy number analyses were performed using 48 Hapmap normal individuals as reference set and Partek Genomic Suite software. *Results*. Results obtained in the subset of 13 pts analyzed so far show that the extension of genomic loss is variable in size. The deleted region spanned 600 kb to 2.3 Mb 5' to ABL and/or 150 kb to 7.8 Mb 3' to BCR, with overall deletion size ranging from 3 to 8 Mb. Detailed maps of genes involved were generated for each pt. The comparison of these maps highlighted clusters of genes (8 in the region 5' to ABL and 13 in the region 3' to BCR) that were lost in different subgroups of pts. On chromosome 9, they included the PP2A inhibitor SET; PPP2R4, encoding the regulatory subunit of PP2A; the oncogenic transcription factor *PRDM12*; *TOR1A* and *TOR1B*, two ATPases involved protein folding; hsa-mir-199b and hsa-mir-219-2 micro RNAs; on chromosome 22, they included MIF, a lymphokine involved in tumor cell motility; the splicing factors *TFIP11* and SF3A1; the *PIB5PA, PTPNS1L* and DUSP18 phosphatases; the *ADRBK2* receptor kinase; the mitotic spindle assembly checkpoint regulator *MAD1*; the regulator of chromatin *SMARCB1*; the Rgr oncogene, encoding a Ras activator; the *CHEK2* ser/thr kinase; the oncogenic transcriptional co-activator MN1. *Conclusions*. Molecular characterization of del9q+ has so far been performed only by FISH. To the best of our knowledge, this is the first study allowing a high-resolution mapping of sequences involved in del9q+. Analysis of pts is ongoing and complete results, including clinical correlations with Sokal risk and response to imatinib treatment will be presented at the meeting.

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0101

PHILADELPHIA -POSITIVE LEUKAMIA PATIENTS ALREADY HARBOURING ABL KINASE DOMAIN MUTATIONS HAVE A HIGHER LIKELIHOOD OF DEVELOPING FURTHER MUTATIONS UNDER THE SELECTIVE PRESSURE OF NOVEL TYROSINE KINASE INHIBITORS

S. Soverini,[1] A. Gnani,[1] S. Colarossi,[1] F. Castagnetti,[1] F. Palandri,[1] S. Paolini,[1] E. Abruzzese,[2] S. Merante,[3] M. Rondoni,[4] I. Iacobucci,[1] A. Poerio,[1] M. Amabile,[1] C. Papayannidis,[1] P. Giannoulia,[1] G. Rosti,[1] M. Baccarani,[1] G. Martinelli[1]

[1]Institute of Hematology and Medical Oncology L.e A. Seràgnoli, BOLOGNA; [2]Department of Hematology Tor Vergata UNIVERSITY OF ROMA; [3]Division of Hematology, PAVIA; [4]Hematology Unit, RAVENNA, Italy

Background. Resistance to the Bcr-Abl tyrosine kinase inhibitor (TKI) imatinib mesylate (IM) in patients (pts) with chronic myeloid leukemia (CML) and Philadelphia-positive (Ph^+) acute lymphoblastic leukemia (ALL) is often caused by selection of point mutations in the Abl kinase domain (KD) altering residues that are directly or indirectly critical for IM binding. To circumvent this problem, novel TKIs have been rationally developed. Each of them, however, can be expected to retain its own Achilles heels, including novel inhibitor-specific mutations. *Aims*. To assess a) which mutations develop *in vivo* under dasatinib or nilotinib treatment and b) how Abl KD sequences evolve under the selective pressure of sequential therapy with novel TKIs. *Methods*. We have monitored the mutation status of 95 IM-resistant pts before and during treatment with up to two consecutive novel TKIs (dasatinib, nilotinib). Forty-five pts (47%) had CML in chronic phase; 50 pts (53%) had CML in accelerated/blastic phase (AP/BP) or Ph^+ ALL. *Results*. At the time of IM failure, 51/95 (54%) pts had KD mutations. After switching to a 2nd TKI (n=95 pts), 19/51 (38%) pts who had mutations at baseline as against 7/44 (16%; p=.02) pts who did not have mutations at baseline subsequently relapsed with newly acquired mutations. Median time to relapse was 8 months (range, 1-22). Cloning showed that these mutations could either be acquired by the pre-existing mutated subclone or arise in an independent one. In addition, 14/51 mutated pts did not respond to the 2nd TKI because of the mutation they were harbouring at baseline. After switching to a 3rd TKI (n=16 pts), 10/13 mutated pts as against 0/3 non-mutated pts relapsed with newly acquired mutations. Median time to relapse was 3 months (range, 1-5). Switch to a 4th TKI (MK-0457, PHA-739358) has so far been attempted in 3 mutated pts, but observation time is still too short. Newly acquired mutations in pts who failed dasatinib were *T315I, F317L, V299L, T315A, F317I/S/V*. Newly acquired mutations in pts who failed nilotinib were *Y253H, E255V/K, L273M, T315I*. More detailed analyses will be presented. *Conclusions*. (a) In IM-resistant pts treated with 2nd/3rd TKIs, Abl KD mutations are often the mechanism by which the Ph^+ clone tries to escape from inhibition (90% of failures associated with presence/emergence of mutations). However, the spectrum of *critical* mutants is very limited as compared to that of IM. b) Pts already harbouring mutations, especially those with CML in AP/BC or with Ph^+ ALL have a higher likelihood of developing further mutations under the selective pressure of novel TKIs. It can be hypothesized that in these pts a higher genetic instability may foster rapid emergence of multiple mutations over time within the same or different Bcr-Abl-positive subclones, which are selected or de-selected depending on the sensitivity profile of the specific TKI employed. In this clinical setting combination therapies would probably be more effective than single-agent treatment for long-term disease control.

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0102

P210-DERIVED PEPTIDE VACCINATIONS IN CML PATIENTS INDUCE *NOT REGULATORY* $CD4^+$/ $CD25^{High+}$/$FOXP3^+$ AND $CD4^+$/$PERFORIN^+$ PEPTIDE-SPECIFIC T CELLS

M.B. Bocchia,[1] M. Ippoliti,[1] M. Tassi,[1] E. Abruzzese,[2] M. Defina,[1] M.M. Trawinska,[2] A. Gozzetti,[1] D. Raspadori,[1] R. Crupi,[1] F. Lauria[1]

[1]University of Siena, SIENA, Italy; [2]S. Eugenio Hospital, ROMA, Italy

Background. We have previously shown that vaccination of chronic myelogenous leukaemia (CML) patients with CMLVAX100 (a mixture of 5 p210-b3a2 breakpoint derived peptides) plus GM-CSF was able to induce an evident and durable peptide-specific $CD4^+$ T cell response in the majority of patients. Peptide-specific $CD4^+$ T cell proliferation was measured by standard [^{3}H] thymidine incorporation assay and this response was mainly mediated by a 25 mer long breakpoint peptide

(b3a2-25) included in the vaccine. In this pilot clinical trial we showed that about 60% of 32 CML patients vaccinated while on imatinib, showed a reduction of their long lasting molecular residual disease after immunization (first 6 vaccinations) and about 25% of them achieved in addition a complete molecular response. *Aims*. To investigate the contribute of this peptide specific $CD4^+$ T cell response in antitumor activity, we further characterize peptide-induced proliferating $CD4^+$ cells. *Methods*. Briefly, in 10 *CMLVAX100* vaccinated patients in which a strong b3a2-25 specific $CD4^+$ proliferation was previously observed, we freshly isolated peripheral blood $CD4^+$ T cells and we cultured them 4 days only in the presence or not of b3a2-25 vaccine peptide or control PR-25 peptide. Afterwards, each culture condition was analyzed both for the co-expression of CD25, perforin and *FOXP3* molecules and for standard [^{3}H] thymidine incorporation assay. *Results*. As expected all patients showed a strong b3a2-25 specific $CD4^+$ proliferation (mean stimulation index 43 -range 19 to 81-). When flow cytometric analysis was performed, we observed a consistent increase of two main $CD4^+$ T cells subsets only in the b3a2-25 stimulated $CD4^+$ T cells, not in *no peptide* or *control peptide* stimulated $CD4^+$ T cells. 1) a *small-size* $CD4^+$/perforin$^+$ T cell population (raising from a median of 1.% (range 0.21-5.2) to 2.3% (range 0.49-6.2) with potential cytotoxic activity 2) a *large-size* $CD4^+$/$CD25^{+high}$/$FOXP3^+$ T cell population (raising from 0.29 % (range 0.03-0.66) to 5.65% (range 0.71-9.5) with potential regulatory function. Since the large size of the latter cells and their high proliferative activity would argue against a regulatory features and considering that the immunophenotypic profile of Tregs in human is not yet fully defined, in 3 selected vaccinated patients we expanded the $CD4^+$/$CD25^{+high}$/$FOXP3^+$ population with further *in vitro* b3a2-25 peptide stimulations and then we performed a *functional test* to measure its potential regulatory activity. Hence, we evaluated the ability of expanded b3a2-25 peptide specific $CD4^+$/$CD25^{+high}$/$FOXP3^+$ T cells to inhibit the growth of CFSE labelled normal subjects naïve $CD4^+$ T cells stimulated with autologous CD3-depleted APCs and antiCD3 plus antiCD28 MoAbs. In our experimental conditions, naïve CFSE $CD4^+$ T cells equally proliferated in the presence of b3a2-25 specific $CD4^+$/$CD25^{+high}$/$FOXP3^+$ T cells (at various peptide-specific: naïve T cell ratios), *no peptide* $CD4^+$ cells or PR-25 control peptide stimulated $CD4^+$ cells and the rate of proliferation was similar to the one observed in co-culture experiments with allogeneic normal $CD4^+$ cells. *Conclusions*. Our data confirmed that CMLVAX100 vaccinated CML patients mount *in vivo* a strong $CD4^+$ proliferative response specific for the b3a2-25 peptide contained in the vaccine. Some $CD4^+$ cell are perforin+, but the majority are $CD4^+$/$CD25^{+high}$/$FOXP3^+$. The latter display no *regulatory* activity despite their phenotype. Whether and how these peptide-specific $CD4^+$ T cells directly exert an antitumor activity against CML cells is now under evaluation.

0103

PATIENT-SPECIFIC IC50 ASSAYS IN IMATINIB-RESISTANT CML PATIENTS ARE HIGHLY VARIABLE AND MAY BE CLINICALLY RELEVANT

D. White,[1] V. Saunders,[1] S. Quinn,[2] T. Hughes[1]

[1]IMVS and Hanson Institute, ADELAIDE; [2]Novartis Pharmaceuticals, SYDNEY, Australia

Background. We have previously demonstrated that in-vitro measurement of patient specific sensitivity to imatinib induced kinase inhibition (IC50imatinib) provides a key predictor of molecular response in imatinib treated *de novo* CML patients.[1] While overall responses to imatinib in chronic phase CML patients are excellent, some 15-20% of patients fail to respond or develop secondary resistance. The choice of second line therapy has, until recently, been guided largely by drug availability or clinical trial access. *In vitro* modelling of more common kinase domain mutants may provide an indicator for second-line drug choice, but this approach provides little guidance in patients with less common mutations, multiple mutations, in resistant patients without mutations, or in whom mutations are undetectable. *Aims.* To assess the predictive value of the patient specific IC50 assay, for achievement of 1 and 2 log reduction in BCR-ABL respectively, and progression in a cohort of imatinib-resistant patients treated with nilotinib for up to 24 months. *Methods*. Blood was collected from 12 imatinib resistant patients, immediately prior to nilotinib therapy. The IC50nilotinib was determined *in vitro* by assessing the reduction in phosphorylation of the adaptor protein Crkl (p-Crkl), an immediate downstream partner of BCR-ABL1. *Results*. The median IC50nilotinib was 78nM (Range:35-250nM). Seven of 12 (58%) patients achieved a 1 log reduction in BCR-ABL over the treatment course and 5 of the 12 (42%) achieved a 2 log reduction in BCR-ABL. There was a marked difference in the IC50nilotinib between those patients who achieved, and those that failed to achieve these milestones (Table 1). Four of the 12 patients progressed on nilotinib (median time to progression 3 months). There was a significant difference in IC50nilotinib between these patients and those who did not progress (median time on study 21months) $p<0.001$ Table 1. Five patients had baseline mutations. Four of these mutations have been assessed by in-vitro modeling, and all 4 have low IC50 75, 13, 67 and 35 nM. The patient specific IC50nilotinib for this cohort were 66, 180, 210, 250 and 180nM. Interestingly, the IC50 in patients without baseline mutations range from 30 to105nM (Median 60nM), which is not significantly different from that observed in *de novo* CML patients (median 57nM; range 12-85nM n=40). Hence, substantial interpatient variation in IC50, not attributable to assay variability, is observed in patients with mutations, but not significantly in resistant patients without mutations. *Conclusion*. This small dataset suggests that the IC50 assay, developed for *de novo* patients, is also applicable to imatinib-resistant patients. This assay provides a measure of the intrinsic sensitivity to nilotinib induced kinase inhibition. It may provide a more accurate predictor of the subsequent response or risk of progression for individual patients than published IC50 data from modeled constructs, which address only the nilotinib sensitivity of the predominant mutated clone. This assay warrants further assessment regarding its predictive value for second line therapy in imatinib resistant patients.

Table 1. Demonstrating the median IC50nilotinib for patient.

	Median $IC50^{nilotinib}$	
Milestone :-	**Yes**	**No**
1 log reduction in BCR-ABL	66nM (45-78nM)* n=7	180nM (89-220nM)* n=5
2 log reduction in BCR-ABL	66nM (55-76nM)* n=5	110nM (56-203nM)* n=7
Progression	195nM {Range 105-250nM} n=4	63nM {Range 35-120nM} n=8

*25th to 75th percentile

Reference

1. White D, Saunders V, Lyons AB, et al: Blood 2005;106:2520-6.

0104

PHILADELPHIA CHROMOSOME POSITIVE (PH^+) LEUKEMIA CELLS ARE NOT ENRICHED IN THE PRIMITIVE ($CD34^+CD38^-$) STEM CELL FRACTION DURING TYROSINE KINASE INHIBITOR THERAPY

S. Mustjoki,[1] P. Rohon,[1] K. Rapakko,[2] S. Hernesniemi,[3] P. Koskenvesa,[3] T. Lundan,[3] K. Porkka[3]

[1]Hematology Research Unit, HELSINKI; [2]Genetics Laboratory, Department of Laboratory, Oulu University Hospital, OULU; [3]Hematology Research Unit, Department of Medicine, HELSINKI, Finland

Background. Targeted tyrosine kinase inhibitors (TKIs) efficiently induce rapid hematologic and cytogenetic remission in most chronic myeloid leukemia (CML) patients. However, a small population of resistant primitive leukemia stem cells remains even after years on therapy, as rapid leukemia relapse occurs in patients who discontinue TKI therapy. *In vitro* experiments have suggested that the most primitive CML stem cells reside in the $CD34^+CD38^-$ fraction of bone marrow (BM) and are relatively resistant to TKIs, but thus far there are no reports describing the prevalence of these cells in patients under TKI therapy. *Aims.* The aim of this project was to analyze the effect of TKI therapy on Ph^+ leukemia stem cells *in vivo*. *Methods*. Large volume (median 26 mL) of BM aspirate samples from chronic phase CML patients under TKI therapy were collected (n=14) and CD34 positive cells were separated with immunomagnetic columns. CD34 positive cells were further sorted into $CD34^+CD38^+$ and $CD34^+CD38^-$ cell populations with multicolor flow cytometry in order to analyze progenitor cell fractions of different mat-

uration stage. Proportion of Ph+ cells was determined with fluorescence *in situ* hybridization (FISH) by counting 1000 cells in each fraction. *Results*. Of 14 patients with CML 11 were in complete cytogenetic remission (CCyR) when assessed by metaphase FISH of non-fractionated BM cells. Only one patient had Ph^+ cells detectable in the $CD34^+$ fractions. In remainder of patients, all progenitor cell fractions, including the most primitive $CD34^+CD38$- cells, were negative for Ph^+ cells. Of 4 patients not in CCyR and thus with detectable Ph^+ cells in total BM fraction, three had Ph^+ cells in $CD34^+$ progenitor cell fractions. However, the proportion of Ph^+ cells was not increased in the most primitive $CD34^+CD38^-$ cell fraction, as expected, but was at the same (patient 5, Table 1) or lower level (patients 13 and 14, Table 1), as compared to non-fractionated BM. *Conclusions*. Based on our data, in chronic phase CML patients, TKI therapy eradicates most Ph^+ $CD34^+$ progenitor cells. Unexpectedly, leukemic stem cells were not enriched in the most primitive $CD34^+CD38^-$ cell fraction *in vivo*. These results differ from the *in vitro* studies, where $CD34^+CD38^-$ cells have been shown to be resistant to TKIs. This could be due to non-physiological conditions (growth factor sensitivity, other cytokines) in cell culture assays. Our data underline the tremendous proliferative potential of very rare stem cells in CML patients in CCyR, as is evident after discontinuation of TKI therapy. Future studies evaluating the kinetics of disappearance of Ph^+ cells from stem cell fractions during TKI therapy are warranted and may give important information on the depth of the therapy response.

Table 1.

Pt	Treatment	Months on TKI	% BCR-ABL/GUS	BM FISH (% of Ph+ cells)			
				Non-fractionated	CD34+ $CD38^{high}$	CD34+ $CD38^{med}$	CD34+ $CD38^{neg}$
1	Imatinib	21	Neg	0.00	0.00	0.00	0.00
2	IM+IFN	19	Neg	0.00	0.00	0.00	0.00
3	Dasatinib	72	0.003	0.00	NE	0.00	0.00
4	Imatinib	17	0.007	0.00	0.00	0.00	0.00
5	Imatinib	19	0.009	0.38	0.00	0.50	0.19
6	Imatinib	7	0.025	0.00	0.00	0.00	0.00
7	Imatinib	23	0.032	0.59	0.00	0.00	0.00
8	Dasatinib	32	0.090	0.00	NE	0.00	0.00
9	Dasatinib	27	0.131	0.00	0.00	0.00	0.00
10	IM+IFN	6	0.151	0.00	0.63	0.45	0.00
11	Imatinib	9	0.366	0.00	0.00	0.00	0.00
12	Imatinib	17	NE	0.00	NE	NE	0.47
13	Imatinib	21	1.077	20.18	0.10	0.10	0.09
14	IM+IFN	18	1.425	9.26	1.01	2.54	0.00

IM, Imatinib; IFN, interferon-α; NE, not evaluated. CD34+ cells were divided in 3 different fractions based on the CD38 expression: high, medium, negative (<5%).

0105

FOXO TRANSCRIPTION FACTOR IS DELOCALIZED AND INACTIVATED IN CML PATIENTS BY BCR-ABL ONCOGENIC SIGNALLING

F. Messa,[1] C. Panuzzo,[1] F. Arruga,[1] P. Nicoli,[1] E. Messa,[1] A. Morotti,[1] A. Rotolo,[1] S. Carturan,[1] T. Kalebic,[2] I. Iacobucci,[3] G. Martinelli,[3] E. Bracco,[1] G. Saglio,[1] D. Cilloni[1]

[1]University of Turin, TORINO; [2]Novartis Oncology, EAST HANOVER, USA; [3]University of Bologna, BOLOGNA, Italy

Background. The FoxO family of transcription factors is regulated by PI3K/Akt induced phosphorylation resulting in nuclear exclusion and degradation. Nuclear FoxO transcribes proapoptotic molecules and cell cycle inhibitors. In CML cells the TK activity of Bcr-Abl leads to the abnormal activation of downstream effectors including PI3K/Akt. The aim of this study was to investigate the role of FoxO in Bcr-Abl induced apoptotic arrest and cell growth and the consequence of imatinib (IM) treatment on FoxO activity. *Methods*. BM cells were collected from 30 CML patients and 20 healthy donors. The expression levels of FoxO were tested by RQ-PCR, FoxO protein amount and localization by Western blot (WB) and immunofluorescence and DNA binding activity by EMSA. Foxo was analyzed also in CML cells and Ph+ cell lines after incubation with IM and LY294002. Finally, we transfected cells with FoxO constitutively activated and we evaluated cell growth and apoptosis. Finally we used our already set up model of Drosophila melanogaster (Dm) transgenic for human Bcr-Abl to study the pathways leading to FoxO inactivation. *Results.* We found that the amount of FoxO mRNA and protein is similar in CML cells and controls. FoxO protein is equally distributed in the nucleous and cytoplasm in controls but is completely cytoplasmatic in CML cells and it enters the nucleous during IM treatment.Additionally, FoxO DNA binding activity in CML patients is completely absent at diagnosis and reappears after IM or LY294002 treatment. Cells transfected with the mutant form of FoxO showed a decreased rate of apoptosis and increased proliferation. The progeny obtained from the crossbreeding of Bcr-Abl flies and flies transgenic for FoxO showed a rescue of FoxO phenotype demonstrating that FoxO inactivation is Bcr-Abl mediated. In addition, the crossbreeding between Bcr-Abl flies and flies carrying inactivated FoxO mutant results into a progeny showing a mild phenotype suggesting that FoxO mediates Bcr-Abl oncogenic pathway. *Conclusions*. Taken together these observations suggest that FoxO is inactivated in CML cells and its delocalization is mainly dependant from Bcr-Abl activity. This results in increased proliferation and reduced apoptosis and it may be crucial for Bcr-Abl oncogenic pathway. The antiproliferative activity of IM may be mediated by FoxO re-localization.

0106

DOWN-REGULATION OF PROTEIN TYROSINE PHOSPHATASE γ (*PTPRG*) IN CHRONIC MYELOID LEUKEMIA (CML)

M. Della Peruta,[1] M. Martinelli,[2] M. Monne,[3] D. Pintani,[4] T. Grafone,[2] I. Iacobucci,[2] M. Murineddu,[3] F. Vinante,[5] C. Tecchio,[5] M. Vezzalini,[4] G. Piras,[3] A. Gabbas,[3] A. Mafficini,[4] C. Sorio[4]

[1]University of Verona, VERONA; [2]Institute of Hematology and Medical Oncology Lorenzo e Ariosto Seragnoli, BOLOGNA; [3]Centro di Diagnostica Biomolecolare e Citogenetica Emato-Oncologica San France, NUORO; [4]Department of Pathology/General Pathology Section; University of Verona, VERONA; [5]Department of Clinical and Experimental Medicine/Hematology Section; University, VERONA, Italy

Background. Chronic Myelogenous Leukemia (CML) is the most common myeloproliferative disease while Receptor Protein Tyosine Phosphatase Gamma (PTPRG) has been implicated as a candidate tumor suppressor gene being its expression reduced in various neoplasms and somatic mutation detected in colon cancer. *PTPRG* regulates murine hemopoiesis and is expressed in specific hematopoietic lineages including $CD34^+$ cells. *Aims*. Explore the possibility that *PTPRG* could be involved in the pathogenesis of CML. *Methods*. mRNA and protein levels were measured in cell lines, bone marrow and peripheral blood cells by real-time PCR and flow cytometry using a newly developed antibody specific for the extracellular domain of *PTPRG*. Proliferation, clonogenic and xenografting assays were used to study the effect of *PTPRG* cDNA transfection in CML cell lines. *Results*. We found that PTPRG expression was undetectable in two of four CML cell lines analyzed. Its loss correlates with higher clonogenicity and proliferation capabilities, while re-expression inhibits both parameters, reduces tyrosine phosphorylation and induces myeloid differentiation in stably transfected K562 cells. The oncosuppressive and differentiation-inducing effect of PTPRG was confirmed *in vivo* after xenotransplantation in a nude mice model. *PTPRG* is down regulated at mRNA and protein levels in leukocytes of CML (but not of chronic lymphocytic leukemia-CLL) patients in both peripheral blood and bone marrow, including $CD34^+$ cells, and is reexpressed following molecular remission of the disease. *Conclusions*. Loss of *PTPRG* expression is associated to the pathogenesis of CML and designate *PTPRG* as a new pharmacological target. Measurement of *PTPRG* expression levels might find clinical application for confirming diagnosis and for following progression of disease and treatment.

0107

ABL KINASE DOMAIN MUTATIONS ARE INFREQUENT IN EARLY-CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS RESISTANT TO IMATINIB

S. Soverini,[1] A. Gnani,[1] S. Colarossi,[1] F. Castagnetti,[1] F. Palandri,[1] E. Abruzzese,[2] E. Orlandi,[3] M. Breccia,[4] G. Specchia,[5] F. Gherlinzoni,[6] A. Poerio,[1] M. Amabile,[1] I. Iacobucci,[1] G. Rosti,[1] M. Baccarani,[1] G. Martinelli[1]

[1]Institute of Hematology and Medical Oncology L.e A. Seràgnoli, BOLOGNA; [2]Department of Hematology Tor Vergata, UNIVERSITY OF ROME; [3]Division of Hematology, PAVIA; [4]Department of Hematology La Sapienza, ROMA; [5]Department of Hematology, BARI; [6]Hematology Unit, TREVISO, Italy

Background. Point mutations in the kinase domain (KD) of the Bcr-Abl gene are generally regarded as the most frequent mechanism of resistance to the tyrosine kinase inhibitor (TKI) imatinib mesylate (IM) in patients (pts) with chronic myeloid leukemia (CML). Nearly all studies, however, have focused mainly on pts with advanced disease, where resistance is most often observed. Nowadays, the great majority of pts on IM are early chronic phase (ECP) pts receiving IM as front-line treatment. If, on one hand, the IRIS study demonstrated that response rates are high and relapse is infrequent in ECP, on the other hand we still know very little on the contribution of KD mutations to resistance in this subset of pts. *Aims*. To assess the incidence of ABL KD mutations in ECP. *Methods*. Between January 2005 and January 2008 we analyzed for the presence of Abl KD mutations one hundred and six ECP pts on IM who were referred to our laboratory because their response was defined either as failure (n=72 pts) or as suboptimal (n=34 pts) according to recently published recommendations (*Baccarani et al., Blood 2006*). *Results*. Twenty mutations were detected in 18/72 (25%) pts who failed IM. In particular, mutations were observed in 1/2 pts who showed no hematologic response (HR) at 3 months, 1/10 (10%) pts who showed less than partial cytogenetic response (PCgR) at 12 months, 4/25 (16%) pts who showed less than complete cytogenetic response (CCgR) at 18 months, 7/25 (28%) pts who lost CCgR, 5/10 (50%) pts who lost HR. Mutations were M244V (n=2), G250E (n=1), Y253H (n=4), E255K (n=1), T277A (n=1), E279K (n=1), F311I (n=1), T315I (n=1), M351T (n=3), E355D (n=1), F359V (n=1), H396R (n=3). In 7 pts who progressed to accelerated or blastic phase shortly after, four had mutations: Y253H (n=2 pts), E255K (n=1 pt) and T315I (n=1 pt). Four mutations were detected in 4/34 (13%) pts who had a suboptimal response to IM. In particular, a mutation was observed in 1/13 (9%) pts who showed less than PCgR at 6 months and in 3/21 (14%) pts who showed less than CCgR at 12 months. Mutations were E255K, F317L, M351T, F359V. In both groups no correlation was observed between likelihood of mutation selection and Sokal risk score. *Conclusions*. In ECP pts who receive IM as front-line treatment Abl KD mutations are not the major mechanism of drug-resistance, probably because mutations tend to accumulate during the natural course of the disease as a result of a progressively increasing genetic instability and are therefore a feature of CML clinical deterioration rather than a phenomenon observed only against a background of IM exposure. Our data highlight the need to find out which is the actual predominant mechanism(s) of resistance acting in the setting of ECP - which now gathers the overwhelming majority of CML pts on IM therapy - as a mandatory step towards the development of effective second-line treatment strategies.

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0108

THE EFFECT OF IMATINIB MESYLATE ON THE SIGNAL TRANSDUCTION CASCADE REGULATING TELOMERASE ACTIVITY IN K562 CELLS AND IN K562 CELLS RESISTANT TO IMATINIB MESYLATE

R. Mor,[1] O. Uziel,[2] O. Shpilberg,[3] J. Lahav,[4] P. Raanani,[3] E. Rabizadeh,[4] Y. Zimra,[4] M. Lahav,[5] G. Granot[2]

[1]Felsenstein Medical Research Center, PETAH-TIKVA; [2]Felsenstein Medical Research Center, Beilinson Hospital, PETAH-TIKVA; [3]Felsenstein Medical Research Center, Institute of Hematology, Beilinson Hospital, PETAH-TIKVA; [4]Felsenstein Medical Research Center, Hematology Laboratory, Beilinson Hospital, PETAH-TIKVA; [5]Felsenstein Medical Research Center, Medicine A, Beilinson Hospital, PETAH-TIKVA, Israel

Background. Imatinib mesylate (IM) is a tyrosine kinase inhibitor selective for bcr-abl, c-Kit, and PDGFR. IM inhibits kinase activity through competition for ATP binding and is indicated for the treatment of newly diagnosed chronic myeloid leukemia (CML) patients. Recent publications have demonstrated that IM may also target other cellular components. In light of the important role of telomerase in malignant transformation, we were interested in the effect of IM on telomerase activity (TA) and on the signal transduction cascade upstream of it. The signal transduction cascade we focused on was: PI3K activation occurs following growth factor binding to its receptor. Active PI3K phosphorylates PIP2 generating PIP3. The balance of cellular PIP3 is also regulated by PTEN, a phosphatase that reduces PIP3 levels. PIP3 promotes AKT translocation to the plasma membrane. There, altered conformation of the protein allows subsequent phosphorylation by PDK-1. AKT is one of the kinases known to phosphorylate and activate telomerase. Aims To evaluated the effect of IM on the signal transduction cascade leading to modulation of TA in the bcr-abl positive K562 cell line and also in an IM resistant K562 cell line (K562res). The effect of IM on TA in the K562res cells is unknown. Using these cells will enable us to study the connection between the signal transduction cascade activated by bcr-abl and the cascade leading to telomerase activation. *Methods*. TRAP assay was used for detecting TA. RT-PCR and Western blots were used to detect levels of RNA and protein of the different cascade members. FACS analysis was used for cell-cycle studies. *Results*. IM caused an 80% inhibition of TA in both K562 and K562res cell. Inhibition of TA was not caused by changes in the transcription level of hTERT (the catalytic subunit of the enzyme). Telomerase is also regulated at the post-translational level through phosphorylation by p-AKT. A 60-85% reduction in p-AKT level was observed following IM incubation in both cell lines. In addition, the expression of PDK-1, known to phosphorylate AKT, was downregulated, explaining the dephosphorylation of AKT observed in the presence of IM. The expression of PI3K, known to positively regulate AKT and PDK-1, was downregulated in both K562 and K562res cell following exposure to IM. *Conclusions*. Our results demonstrate the ability of IM to inhibit TA in bcr-abl expressing cell lines. TA inhibition was demonstrated in K562 cells as well as in K562 cells resistant to high concentrations of IM. Therefore, the inhibitory effect of IM on TA isn't necessarily mediated through the known tyrosine kinase targets of IM. The signal transduction cascade in both the K562 and the K562res cell lines appears to go through downregulation of PI3K which causes a downregulation of PDK-1 and p-AKT levels leading to inhibition of TA. This study supports previous studies demonstrating telomerase as an additional target of IM. In addition, this study shows that cells, known to be resistant to IM with regards to its effect on bcr-abl, could still be sensitive to IM treatment regarding other cellular components.

0109

DYNAMICS OF MUTANT BCR-ABL POSITIVE CLONES AFTER CESSATION OF IMATINIB TREATMENT

B. Hanfstein, M.C. Mueller, S. Kreil, T. Schenk, C. Lorentz, U. Schwindel, A. Leitner, R.. Hehlmann, A. Hochhaus

Medizinische Fakultät Mannheim der Universität Heidelberg, MANNHEIM, Germany

Background. Mutations of the BCR-ABL tyrosine kinase are considered a major cause of imatinib resistance in chronic myeloid leukemia (CML). More than 50 different mutations have been described, conferring resistance by different molecular mechanisms. The concept of selection of resistant clones in the presence of imatinib is well understood. However, only few data have been presented on the changes of mutant BCR-ABL positive clones after cessation of imatinib treatment. The assumption of reversibility of clonal selection after removing the selective pressure of ABL tyrosine kinase inhibition seems to be plausible, but a general concept of deselection has not yet been established. *Aims*. We sought to determine the decline of resistant clones after cessation of imatinib treatment in CML patients expressing different BCR-ABL mutations. *Methods*. We examined peripheral blood samples of 53 patients (pts; 31 m, 22 f, median age 61 yrs) with imatinib resistant CML bearing BCR-ABL mutations detected by direct sequencing and receiving a subsequent treatment without tyrosine kinase inhibitors after cessation of imatinib (median interval of imatinib treatment 1.6 yrs, median interval to resistance 1.1 yrs). BCR-ABL specific nested RT-PCR of total leukocyte RNA was performed. The amplicon was subjected to mutation specific restriction digests. The proportion of mutant BCR-ABL was defined by a densitometry assay following agarose gel electrophoresis. *Results*. 16 pts (9 m, 7 f, median age 63 yrs; in chronic (n=8), accelerated (n=4) or blastic phase (n=4); mutations Y253F, n=1; Y253H, n=4; E255K, n=5; T315I, n=4; M351T, n=2) had a minimum follow up of 0.3 yrs after cessation of imatinib (subsequent treatment: hydroxyurea, n=10; cytarabine, n=2; mercaptopurine and anagrelide, n=1; thioguanine, n=1; busul-

fan, n=1; interferon alpha, n=1) and could therefore be monitored for deselection of the mutant clone. Median follow up after cessation of imatinib was 1.3 yrs (range 0.3-2.8). Median proportion of mutant BCR-ABL expression in the presence of imatinib was 98% (range 38-100). The difference between the proportions before and after cessation at the lowest point was 61% (median, range -5-100) over a period of 0.4 yrs (median, range 0.2-2.1). We observed a decline of 93% within 2.1 yrs (Y253F), medians of 97%/0.5 yrs (Y253H), 67%/0.4 yrs (E255K), 0%/0.6 yrs (T315I) and 48%/1.1 yrs (M351T). Three pts expressing the T315I mutation revealed the persistence of a 100% mutant BCR-ABL clone during follow up. Two pts (Y253H and E255K) showed a pattern of repeated rapid selection and deselection after resumption and second cessation of imatinib therapy. 6 of 16 pts (CP, n=1; AP, n=3; BC, n=2; Y253H, n=2; E255K, n=1; T315I, n=2; M351T, n=1) died during a median observation period of 3.6 yrs after onset of imatinib treatment, five of them dying in blast crisis. *Conclusions*. Deselection of mutant BCR-ABL clones after cessation of tyrosine kinase inhibition seems to be common and reproducible except in patients harboring T315I.

0110

GENE EXPRESSION DOWNREGULATION BY HAPLOINSUFFICIENCY MECHANISM IN CHRONIC MYELOID LEUKEMIA WITH DELETIONS ON DER(9)

F. Albano,[1] A. Zagaria,[1] L. Anelli,[1] A. Pannunzio,[1] A. Russo Rossi,[1] F. Manodoro,[1] N. Coccaro,[1] V. Liso,[1] M. Rocchi,[2] G. Specchia[1]

[1]Hematology-University of Bari, BARI; [2]DI.GE.MI University of Bari, BARI, Italy

Background. Genomic deletions flanking the breakpoint on der(9)t(9;22) occur in 10%-15% of patients with chronic myeloid leukemia (CML). These microdeletions have a variable extension and involve sequences on chromosomes 9 and/or 22, located proximally to the ABL and distally to the BCR gene. The deletions on der(9) are associated with a poor prognosis on interferon α (IFN-α) therapy whereas controversal data are available about their influence on the response to imatinib. The molecular mechanisms responsible for this unfavourable prognosis are still unclear. We report a gene expression study performed by quantitative real-time polymerase chain reaction (qRT-PCR) on 30 CML patients bearing genomic microdeletions on der(9) chromosome. To date, an expression study of genes mapping within the deleted regions on der(9) has never been performed. *Methods*. 334 CML patients in chronic phase were analyzed by FISH experiments with probes specific for ABL and BCR genes. Sixty (18%) out of 334 cases showed genomic deletions on der(9) chromosome; a detailed characterization of deletions extension was performed by using specific BAC contigs. Because of RNA sample availability, gene expression studies were performed in 30 out of 60 CML patients bearing der(9) deletions and in a pool of 10 CML cases without microdeletions. A total number of 47 genes with known functions were found located inside the 9 and 22 chromosome regions most frequently deleted in CML patients. Among all selected genes, 37 resulted to be homogeneously expressed in normal bone marrow samples and were analyzed by qRT-PCR experiments. A total number of 28 genes was validated by efficient primers pairs; their expression was evaluated in 30 CML cases bearing der(9) deletions and compared to a pool of cDNA samples derived from 10 CML patients without sequence deletions (calibrator). To standardize the quantification of each target gene, β-glucuronidase (β-GUS) gene served as endogenous control. All samples were run in triplets as technical replicates. Results. All the 28 analyzed genes were found downregualted with respect to CML cases without deletions. However, the difference was statistically significant only for six: protein kinase PKN3 (*PKN3*, p=0.003), SH3-domain GRB2-like endophilin B2 (*SH3GLB2*, p=0.0018), protein phosphatase 2A regulatory subunit B' (*PPP2R4*, p=0.007), ankyrin repeat and SOCS box-containing 6 isoform (*ASB6*, p=0.002), ubiquitin specific protease 20 (USP20, p=0.010), and torsin family 1 member B (*TOR1B*, p=0.009). The expression levels of the downregulated genes were 0.096, 0.222, 0.223, 0.198, 0.222, and 0.293 for *PKN3*, *SH3GLB2*, *PPP2R4*, *ASB6*, *USP20* and *TOR1B*, respectively. All 6 downregulated genes are located on chromosome 9 sequences, centromeric to the ABL gene, and are implicated in crucial cellular pathways. *Conclusions*. We have showed for the first time an expression downregulation of genes located on der(9) chromosome in CML patients bearing genomic microdeletions. These findings support the haploinsufficiency hypothesis, suggesting that in these cases one allele is always not sufficient to ensure an adequate gene expression dosage. However, the biological meaning and clinical implications of this gene downregulation remain to be investigated.

0111

EVIDENCE THAT PPAR ACTIVATION MAY CONTROL THE EXPRESSION OF THE IMATINIB TRANSPORTER HOCT1 IN CHRONIC MYELOID LEUKAEMIA

L. Wang, M. Austin, E. Clark

Department of Haematology, LIVERPOOL, UK

The tyrosine kinase inhibitor imatinib (Glivec) produces remarkable responses in chronic myeloid leukaemia (CML). However, many CML patients may not achieve cytogenetic benefit, or lose an initial cytogenetic response. We recently reported that the expression level of the imatinib uptake transporter human organic cation transporter 1 (hOCT1) is a critical determinant of outcome in imatinib treated CML. Patients with high pre-treatment hOCT1 expression had superior complete cytogenetic response (CCR) rates, progression free and overall survival. Upregulation of hOCT1 in hOCT1-low patients could potentially improve the efficacy of imatinib treatment for these patients, but nothing is known about transcriptional regulation of human OCT1 in haematopoietic or CML cells. It has been reported that in liver cells, the hOCT1 gene is transactivated by hepatocyte nuclear factor-4α (HNF-4α), and that peroxisome proliferator-activated receptors (PPAR) α and γ activation increases OCT1 expression in mouse hepatocytes. Here we investigate HNF-4α and PPAR in primary CML cells and cell lines. Hepatocyte nuclear factor-4α (HNF-4α) ---- HNF-4α gene expression was studies by RT-PCR in CML (*K562*, *KCL22* and *LAMA84*) and HepG2 (hepatocyte) cell lines. HepG2 cells which express HNF-4α isoforms 1,2,3,7,8 and 9 were used as a positive control for detecting HNF4α expression. It was found that HNF-4α was not expressed in K562, KCL22, *LAMA84* or 11 CML primary cell samples. Using two HNF-4α antibodies (G162 and E410) which bind to the D domain and C-terminal F domain on Western Blot (and thus cover all possible isoforms), no HNF-4α protein was detected in either CML primary cells or CML cell lines. These results indicate that hOCT1 expression is not regulated by HNF-4α in CML. Peroxisome proliferator-activated receptors (PPAR) ---- RT-PCR studies showed that both PPAR α and γ are expressed in 10 primary CML cell samples and in 4 CML cell lines (*K562, KCL22, LAMA84* and *KYO1*). KCL22 cells (low basal hOCT1 expression) and mononuclear cells from 5 chronic phase CML patients were treated with the PPAR α agonists clofibrate or WY14643 and the PPAR Á agonist ciglitazone. A treatment dose and time was chosen that had no effect on the viability of the cells (assessed by the MTT assay). hOCT1 gene expression levels were measured using real-time quantitative RT-PCR on LightCycler. Both PPAR α and γ agonists increase hOCT1 expression in KCL22 cells to 1.5-3.0 fold of the control level. In all 5 primary CML cell samples, the PPAR γ agonist ciglitazone increased *hOCT1* expression to 1.7 to 4.2 fold of control. In 3 of 5 cases, the PPAR α agonist clofibrate also increased hOCT1 expression. In summary, PPAR α/γ agonists can increase hOCT1 expression in primary CML cells and CML cell lines. PPAR agonists may potentially be of clinical use in CML patients failing imatinib.

0112

EFFECT OF TYROSINE KINASE INHIBITORS ON REGULATORY T-CELLS (TREGS) IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML)

P. Rohon,[1] K. Porkka,[2] S. Mustjoki[3]

[1]Hematology Research Unit, Biomedicum, Helsinki University Central Hospital (HUCH, HELSINKI, Finland; [2]Hematology Research Unit, Dept of Medicine, HUCH, HELSINKI, Finland; [3]Hematology Research Unit, Depts of Medicine and Clinical Chemistry, HUCH, HELSINKI, Finland

Background. Tyrosine kinase inhibitors (TKIs) are currently the standard treatment in patients with chronic myeloid leukemia (CML). In addition to BCR-ABL oncoprotein, they also inhibit other kinases (e.g. C-kit, *TEC, BTK, PDGFR, SRC*), some of which have important physiological functions in immune responses. However, the *in vivo* effects of TKIs on immune effector cells are mostly unknown. T regulatory cells (Tregs) play the key role in immune homeostasis and protection against autoimmunity. Tregs are involved in the immune recognition of tumor-related antigens and may suppress anti-tumor immune responses by inhibiting the function of other effector cells. Increased amounts of Tregs have been observed in patients with solid tumors, but in hematological malignancies only a few studies exist. *Aims*. We aimed at the characterization of the Tregs at diagnosis and during TKI therapy (imatinib, dasatinib) in chronic phase CML patients. *Methods*. A total of 130 peripheral blood (PB) and bone marrow (BM) samples were studied from CML patients at diagnosis (n=13) and during TKI therapy (n=28). Samples

from 12 healthy volunteers served as controls. Tregs were analyzed with multicolor flow cytometry using antibodies for CD4, CD25 and FoxP3. The proportion of Tregs is presented as a percentage of $CD4^+$ T-cells. *Results*. Proportions of Tregs in PB and BM were similar (median percentages 5.2 and 4.3, respectively, p=0.14) and the values correlated significantly (rs=0.46, p<0.001). In BM, the median percentage (range) of Tregs at diagnosis, during imatinib and dasatinib therapy was 2.0 (0.9-5.3), 5.6 (0.2-9.8) and 2.7 (1.6-5.8), respectively (p=0.008). The median percentage of Tregs in PB at diagnosis, during imatinib and dasatinib therapy and in healthy controls was 4.7 (1.3-8.8), 5.7 (0.6-10.3), 3.6 (1.0-7.6) and 5.3 (3.8-6.9), respectively (p=0.42). Proportion of Tregs was lower in females than in males (3.7 and 5.5, respectively, p=0.003). No correlation with age or the Sokal risk score at diagnosis was observed. *Conclusions*. We show that the relative numbers of BM Tregs are decreased at diagnosis in CML patients. Imatinib induces a rapid increase in the proportion of Tregs to levels found in healthy controls. In contrast, dasatinib therapy was associated with similarly low Treg levels as at the time of diagnosis. This dissimilarity may reflect varying effects of imatinib and dasatinib on immune effector cells and on immune responses. In contrast to studies in solid tumors, increased proportions of Tregs were not observed, which may reflect a different function of these cells in hematological malignancies.

0113

OMACETAXINE INDUCES THE RAPID LOSS OF MCL-1 BUT NOT OTHER ANTI-APOPTOTIC BCL-2 FAMILY PROTEINS IN BCR-ABL POSITIVE CELLS

H. Segal,[1] S. Michaels,[2] D. Brown[2]

[1]Deakin University, GEELONG, Australia; [2]ChemGenex Pharmaceuticals, MENLO PARK, CA, USA

Background. The natural product alkaloid omacetaxine mepesuccinate (OMA; formerly homoharringtonine) is currently undergoing evaluation in clinical trials as a therapy for patients with tyrosine kinase inhibitor resistant chronic myeloid leukemia (CML). OMA has been shown to inhibit protein synthesis (putatively via inhibition the elongation step of polypeptide synthesis) and induce apoptosis in leukemic cells. Overnight treatment (>16 hr) of BCR-ABL positive leukemia cells with OMA modulates expression of bcl-2 family proteins and activates apoptosis via proapoptotic caspases; however the key early proapoptotic mechanisms activated by OMA have been incompletely characterized. *Aims*. To further delineate the early (<8hr) cellular events affected by OMA, we have investigated the expression of pro- and anti-apoptotic bcl-2 family proteins following OMA treatment in BCR-ABL positive leukemia cells. For comparison, bcl-2 family protein expression was also assessed in cells treated with imatinib mesylate. *Methods*. Using the K562 leukemia cell line, the expression of bcl-2 family proteins that are anti-apoptosis (Bcl-2, Mcl-1 and Bcl-XL) and pro-apoptosis (Bim and Bax) was examined by Western blot after exposure to 10nM OMA or 10uM imatinib mesylate for 1, 2, 4 and 6 hr. *Results*. Treatment of K562 cells with OMA rapidly induced the loss of Mcl-1 protein expression, which was readily detectable after 2 hr and was more pronounced 4 and 6hr after addition of OMA. In contrast, treatment with imatinib mesylate did not alter Mcl-1 expression in the first 6 hr after treatment. Expression of other anti-apoptotic proteins Bcl-2 and Bcl-XL or pro-apoptotic Bim or Bax proteins were not affected by OMA or imatinib mesylate. The OMA induced loss of Mcl-1 was dependent on proteasome activity as co-treatment of K562 cells with the proteasome inhibitor MG-132 (10 uM) prevented OMA induced Mcl-1 degradation. *Summary/Conclusion*. These results indicate that OMA but not imatinib mesylate induces a rapid loss of the anti-apoptiotic protein Mcl-1 protein in CML cells. Follow up studies are currently underway to further define the molecular pathways affected by OMA treatment of BCR-ABL positive leukemic cells.

0114

ROLE OF IMATINIB MESYLATE IN OSTEOBLASTOGENESIS

D. Tibullo,[1] C. Giallongo,[1] P. La Cava,[1] S. Berretta,[1] F. Stagno,[1] A. Chiarenza,[1] C. Conticello,[2] G. Palumbo,[1] F. Di Raimondo1

[1]Division of Hematology, University of Catania, CATANIA; [2]Department of Experimental Oncology, Mediterranean Institute of Oncology, VIAGRANDE (CATANIA), Italy

Background. Imatinib mesylate (IM) is a tyrosine kinase inhibitor currently used in chronic myeloid leukaemia (CML). It has been reported that IM may affect bone tissue remodeling mainly by an inhibitory activity on osteoclastogenesis. We therefore evaluated possible effects of IM on osteoblatic differentiation of Mesenchymal Stem Cells derived from bone marrow (BM-MSCs). Methods. Osteogenic differentiation of hBM-MSCS was induced in presence of 0.2 mM ascorbic acid, 0.1 µm dexamethasone and 10 mM β-glycerophosphate (osteogenic medium, OM). Expression of osteoblast-associated genes such as osteocalcin (OCN), RUNX2 and Bone morphogenetic protein (*BMP-2*) were evaluated by reverse transcription-polymerase chain reaction (RT-PCR). Culture surnatant and serum levels OPG and RANKL were assayed using specific ELISA tests. *Results*. At 21 days, an extracellular mineralization was present in hBM-MSC cultures with OM or IM alone but it was more evident with the combination. The levels of OCN, Runx2/cfba1 and OPN expression were increased in cells treated with OM, IM and OM + IM in respect to control. In particular, levels of BMP2 were significantly higher in IM treated cultures respect to control (p<0.0007) and they were also higher in OM + IM treated culture respect to OM alone (p<0.002). The same differences applied for OCN and Runx2/Cfba1, although at less significat p values. *OPG/RANKL* ratio in the surnatant of the culture (p<0,005) and OCN levels (p<0,005) were increased in BM-MSCs samples cultured with IM in comparison to control (OM alone). In addition, we examined the *OPG/RANKL* ratio in serum of 41 serum samples from CML patients at diagnosis or treated with IM 400 mg from 3 to 24 months. The *OPG/RANKL* ratio in patients at diagnosis was higher in comparison to healthy voluntaries (p<0.004) and the ratio further increased after 3 and 6 months of IM treatment (p<0.004). However. the *OPG/RANKL* ratio returned at the basal level after 24 months of treatment with IM. *Conclusions*. In summary, our data show that IM increases osteogenic markers mRNA expression in BM-MSCs and increase the *OPG/RANKL* ratio and OCN level in surnatant of BM-MSCs cultured with IM. In addition, we have shown that CML patients treated with conventional doses of IM have a transient elevation of the serum *OPG/RANKL* ratio that return to the basal level after 24 months. Our data show that IM increased the expression of BMP-2 and Runx2/cfba1 mRNA and we hypothesize that the BMP/Runx2 axis can have an important role in the IM-mediated induction of osteoblasts.

0115

NCK β ADAPTER COORDINATES BCR-ABL/SAM68 INTERMOLECULAR INTERACTION

E. Bracco,[1] E. Bracco,[2] E. Deklic,[1] V. Rosso,[2] S. Mussino,[2] F. Arruga,[2] R. Catalano,[2] S. Carturan,[2] F. Messa,[2] I. Defilippi,[3] C. Panuzzo,[2] P. Nicoli,[2] E. Messa,[2] A. Rotolo,[2] R. Pedrola,[2] D. Cilloni,[2] G. Saglio[2]

[1]University, ORBASSANO; [2]University of Turin, ORBASSANO; [3]University of Turin, ORBASSANO, Italy

Background. Ph^+ disorders, such as chronic myelogenous leukemia (CML) are characterized by the presence of abnormal chromosome rising from translocation between chromosome 9 and 22 thus giving birth to a chimeric oncogenic protein named Bcr-Abl. This oncogenic kinase displays constitutive tyrosine kinase activity which leads to tyrosine residues autophosphorylation, in turn recruiting SH2 and/or PTP containing proteins. Bcr-Abl targeted therapy has been successfully used in the last decade and among currently available drugs inhibiting Bcr-Abl activity Imatinb mesylate represents the most efficient. Despite the vast amount of data describing the effects of Imatinib on signal transduction pathways (ERK1/2, CrkL phosphorylation, PI3K) in Ph^+ leukemic cells rather few experimental evidences are available on the effects of Imatinib on adapter molecules. *Aims and Methods*. The significance of interactions occurring between Bcr-Abl and adapter molecules is still debated matter. Most of the interactions so far described (CrkL, Grb2, PI3K p85 regulative subunit etc) appear to play a role in mediating and integrating signals which lead to proliferation, cell survival and/or cytoskeletal organization and more recently few data are presented supporting the hypothesis of adapter molecules as Abl catalytic regulators. By means of an interactomic approach, based on proteomic strategy using GST-Pull Down assay with an array of SH2 containing proteins, we attempted to gain insight into the role played by adapter molecules and Bcr-Abl interactions. *Results and conclusions*. The data herein presented aims to demonstrate the presence of quaternary complex involving the SH2-SH3 containing adapter protein Nck beta, the oncogenic tyrosine kinase Bcr-Abl and the RNA binding protein Sam68. The experimental evidences we have collected support the hypothesis for an Imatinib-dependent interaction between Nck beta and Bcr-Abl. Furthermore, Pull Down experiments indicate intermolecular interaction between Nck-beta and Sam68, supporting the idea of a complex Bcr-Abl/Nck-beta/Sam68. Interestingly, Sam68 may modulate splicing of gene regulating apoptosis event, such as BclX. These data represent the first experimental evidences showing an interaction between Bcr-Abl and Sam68 leading to speculate a putative role played by Bcr-Abl in mRNA splicing sc

0116

UNUSUAL SINGLE BAC COPY NUMBER CHANGES IN CHRONIC MYELOID LEUKEMIA: EXAMPLES FROM CELL LINES AND PATIENTS

A. Chanalaris, A. Chanalaris, D. Brazma, M. Valgañón, C. Grace, E.P. Nacheva

Royal Free & UC Medical School, LONDON, UK

Background. The advent of aCGH has enabled us compare two or more genomes and led to impressive discoveries in the variation of our genetic material. The plethora of cryptic numerical aberrations in diseases are discovered with relative ease, the discovery of copy number variations and the discovery of disease associated genomic loci by single nucleotide polymorphisms are the contributions of this technology. Previous studies have used aCGH in CML to discover common features among CML patients in order to elucidate the relevance of the genetic background in CML disease progression. By employing aCGH in CML patients and CML cell lines we came across consistent numerical changes of large areas adjacent to the BCR/ABL1 fusion and also single BAC numerical changes interspersed throughout the genome that are not compliant with the numerical changes of the neighbouring region. *Aims.* To characterise the unusual copy number changes we observed and to eradicate the possibility of aCGH artefact results. *Methods*. Patient and cell line material was analysed by 1Mb BAC array (SGI). The unusual numerical aberrations were confirmed by FISH and quantitative real time PCR. *Results*. In addition to detection of genome imbalances, affecting regions both large (detectable at chromosome level) and cryptic (as small as 100Kb) array CGH was instrumental in revealing complex structural rearrangements. (i) Amplification of the region downstream of ABL1 gene: A several fold copy number gain of an 800 kb region downstream of the ABL1 breakpoint at 9q34.12 region was identified by high resolution array CGH in the cell lines *MEG-01, MC3, EM2* and CML-T1. FISH mapping revealed that this genomic gain is a result of a structural change, namely a tandem duplication of the same amplicon. The latter shows a consistent proximal breakpoint at the ABL1, while its distal boundaries fall within the *RAPGEF1* gene. This amplicon contains several genes, including *NUP214, LAMC3* and *RAPGEF1*. Similar gains/amplifications were also identified by FISH in a subclone in the cell line KYO-1 and were seen in 5 out of 12 cases with double Ph. (ii) Discordant copy number changes: loss of single BAC locus within a gained region and visa versa. This phenomenon, clearly detectable at the graphic presentation of the array data, has been detected in several patients and cell line genome profiles alike. The most frequently seen are the ones at the regions of 9p21, 6q23-27 and 1q22-qter were further investigated and confirmed by FISH and qPCR. *Conclusions*. We are presenting some cases of unusual copy number aberrations, unusual in that they go against the numerical changes of the surrounding genomic area. These changes are not artefacts and we are currently investigating the possibility of a mechanistic reason behind their appearance.

Chronic myeloid leukemia - Clinical I

0117

NILOTINIB IN CHRONIC MYELOGENOUS LEUKAEMIA IN BLAST CRISIS (CML-BC) PATIENTS WITH IMATINIB-RESISTANCE OR -INTOLERANCE: UPDATED PHASE 2 RESULTS

J. Giles,[1] R.A. Larson,[2] H.M. Kantarjian,[3] P. Le Coutre,[4] F. Palandrini,[5] A. Haque,[6] N. Gallagher,[6] O.G. Ottmann[7]

[1]CTRC at the UT Health Science Center San Antonio, SAN ANTONIO, USA; [2]The University of Chicago Hospitals, CHICAGO, USA; [3]MD Anderson Cancer Center, HOUSTON, USA; [4]Humboldt-Universität, BERLIN, Germany; [5]Department of Hematology-Oncology, University of Bologna, BOLOGNA, Italy; [6]Novartis Pharmaceuticals, EAST HANOVER, USA; [7]Medizinische Klinik III, FRANKFURT, Germany

Background. Patients (pts) with CML-BC, for whom prognosis remains poor, may benefit from treatment with nilotinib, a potent and highly selective BCR-ABL inhibitor. Nilotinib has been approved in several countries including the US and Europe for the treatment of patients (pts) with Philadelphia chromosome-positive chronic myelogenous leukaemia (Ph^+ CML) in chronic phase (CP) and accelerated phase (AP) who are resistant or intolerant to prior therapy including imatinib. *Methods*. This phase 2, open-label study evaluated the efficacy and safety of nilotinib in adult pts with Ph^+ CML-BC resistant or intolerant to imatinib. The primary endpoint was confirmed haematological response (HR). Imatinib resistance was defined as either treatment with imatinib > 600 mg/d with disease progression or no HR in bone marrow after 4 weeks. Imatinib intolerance was defined as no major cytogenetic response (MCyR) and discontinuation due to grade 3/4 adverse events (AEs), or grade 2 AEs persisting for > 1 month while on imatinib. In the absence of safety concerns, the initial dose of nilotinib 400 mg twice daily (BID) on an empty stomach, could be escalated to 600 mg BID for: failure to achieve a return to CP, loss of an achieved haematological or cytogenetic response, or progressive disease. *Results*. Safety and efficacy data are reported for 136 BC pts (myeloid, n=105; lymphoid, n=31), 82% were imatinib-resistant and 18% were imatinib-intolerant. Many had also received other prior therapies including interferon (34%), cytarabine (37%) and BMT (13%). At study entry, 38% of pts had ≥95% Ph^+ metaphases, with additional chromosomal abnormalities noted in 53% of pts. Confirmed HR occurred in 29 (21%) pts, with 15 (11%) achieving complete HR. MCyR was achieved in 55 pts (40%), with 40 (29%) having complete cytogenetic response (CCyR). Overall survival at 12 months was 42%. Patients with myeloid and lymphoid blast crises had similar response rates. Treatment with nilotinib is ongoing in 13 pts (10%); 71 (52%) pts discontinued treatment due to disease progression. The median duration of treatment was 84 days. Median dose intensity was 800 mg/day, the same as the planned dose. Although dose interruptions occurred in 38% of pts, the median total duration of dose interruptions was only 10 days. The most common grade 3/4 laboratory abnormalities were neutropenia (67%), thrombocytopenia (62%), and anaemia (42%). Non-haematological toxicities were mostly mild to moderate and included rash and nausea. Grade 3/4 pleural/pericardial effusions and pulmonary edemas were not observed. <1% experience had grade 3/4 GI bleeding and no CNS bleeding occurred. *Conclusions*. Nilotinib demonstrates clinical activity in pts with imatinib-resistant or -intolerant CML-BC and is generally well tolerated. Nilotinib represents a novel treatment option in a group of CML pts with very advanced disease and limited treatment options.

0118

NILOTINIB IN IMATINIB-RESISTANT OR -INTOLERANT PATIENTS WITH CHRONIC MYELOGENOUS LEUKAEMIA IN ACCELERATED PHASE (CML-AP): UPDATE OF A PHASE 2 STUDY

P. le Coutre,[1] F.J. Giles,[3] A. Hochhaus,[2] J. Apperley,[3] G. Ossenkoppele,[4] A. Haque,[5] N. Gallagher,[5] M. Baccarani,[6] J. Cortes,[7] H.M. Kantarjian[7]

[1]Charité, BERLIN, Germany; [2]Med. Fakultät Mannheim der Universität Heidelberg, Mannheim, Germany, MANNHEIM, Germany; [3]Imperial College School of Medicine, Hammersmith Hospital, LONDON, UK; [4]Free University, AMSTERDAM, Netherlands; [5]Novartis Pharmaceuticals, EAST HANOVER, USA; [6]University of Bologna Institute of Hematology and Medical Oncology Seragnoli, BOLOGNA, Italy; [7]MD Anderson Cancer Center, HOUSTON, USA

Background. Nilotinib, a potent and highly selective BCR-ABL inhibitor, has been approved in several countries including the US and Europe for the treatment of patients (pts) with Philadelphia chromosome-positive chronic myelogenous leukaemia (Ph^+ CML) in chronic phase (CP) and accelerated phase (AP) who are resistant or intolerant to prior therapy including imatinib. *Methods*. This phase 2, open-label study evaluated the efficacy and safety of nilotinib in pts with Ph^+ CML-AP with imatinib resistance or intolerance. Primary and key secondary endpoints were rate of confirmed haematological response (HR) and major cytogenetic response (MCyR), respectively. Nilotinib was started at 400 mg twice daily (BID) and escalated to 600 mg BID for inadequate responses, in the absence of safety concerns. *Results.* Included in the analysis were 136 pts who received at least 1 dose of nilotinib. Of these 136 pts, 109 (80%) were imatinib-resistant and 27 (20%) were imatinib-intolerant. Median age was 58 (22-82) yrs; median dose intensity was 781 mg/day; median treatment duration was 7 months. Of 129 pts, confirmed HR occurred in 69 (54%) and complete HR occurred in 34 (26%). In imatinib-resistant and -intolerant pts, confirmed HR occurred in 55/104 pts (53%) and 14/25 (56%), respectively. A majority of responding pts (82%) maintained HR for at least 12 months. MCyR occurred in 40/129 pts (31%) and 24/129 pts (19%) had complete CyR. Of imatinib-resistant pts, 30/104 pts (29%) had a MCyR, and 10/25 imatinib-intolerant pts (40%) had a MCyR. At 12 months, the estimated overall survival rate was 81%. At data cutoff, treatment was ongoing for 57/136 pts (42%). The most common grade 3/4 laboratory abnormalities were thrombocytopenia (41%), neutropenia (39%), anaemia (25%), and asymptomatic elevated serum lipase (16%). Grade 3/4 non-haematological AEs were rare and included nausea, fatigue, headache, and diarrhoea. There was no occurrence of QTcF prolongation >500 msec from baseline. Grade 3/4 fluid retention was rare, occurring in <1% of all pts. Grade 3/4 rate for any bleeding events is 2.2%. There was minimal nilotinib cross-intolerance in patients intolerant to prior imatinib treatment. *Conclusions*. The results of this study confirm that nilotinib induces significant and durable responses in CML-AP pts with imatinib resistance or intolerance. Nilotinib is well tolerated, with minimal occurrence of grade 3/4 AEs, and is an effective treatment option for this advanced CML population.

0119

DASATINIB IS ASSOCIATED WITH DURABLE TREATMENT RESPONSES IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA: LONG-TERM FOLLOW-UP FROM THE PHASE I TRIAL (CA180002)

J. Cortes,[1] C.L. Sawyers,[2] H.M. Kantarjian,[1] N.P. Shah,[3] R. Paquette,[4] E. Bleickardt,[5] P. Paliwal,[5] M. Talpaz[6]

[1]MD Anderson Cancer Center, HOUSTON; [2]Memorial Sloan-Kettering Cancer Center, NEW YORK; [3]University of California San Francisco School of Medicine, SAN FRANCISCO; [4]UCLA, LOS ANGELES; [5]Bristol-Myers Squibb, WALLINGFORD; [6]University of Michigan, ANN ARBOR, USA

Background. Dasatinib is a potent BCR-ABL inhibitor, approximately 325-fold more potent than imatinib and 16-fold more potent than nilotinib for *in vitro* kinase inhibition. Dasatinib was approved for the treatment of imatinib-resistant/-intolerant chronic myeloid leukemia (CML) in any phase or Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ ALL) because of efficacy observed during a phase I trial (CA180002) and a series of phase II studies (the START program). Long-term follow-up is now available for patients with CML in chronic phase (CP) treated during the 002 study. *Aims*. To investigate the durability of dasatinib treatment responses and long-term tolerability of patients treated in a phase I study. Data have been assessed after a minimum follow-up of 27 months. *Methods*. Patients with CML-CP who had experienced hematologic resistance/relapse on imatinib or who had discontinued imatinib following toxicity were enrolled from November 2003 to April 2005. Dasatinib was administered at 15-180 mg/d on a QD or BID schedule according to a dose-escalation protocol, with most patients initially treated on a 5 d on/ 2 d off schedule (dasatinib 100 mg QD is now the approved dose for patients with CML-CP following imatinib resistance or intolerance). In addition to safety assessments, rates of complete hematologic response (CHR) or major/complete cytogenetic response (MCyR/CCyR), and duration of progression-free survival (PFS) or overall survival were evaluated. *Results*. Of 45 patients with late CML-CP, 91% had received prior interferon-α, 62% had received imatinib at a dose >600 mg/d, and 58% had received imatinib for >3 years. Most patients had responded initially to imatinib (CHR 78%, MCyR 42%, CCyR 18%), 80% eventually became imatinib resistant (20% intolerant), and 73% had baseline BCR-ABL mutations. Dasatinib was administered QD to 22 patients for a median of 31 months (range 3.6-38.5) and BID to 23 patients for median of 23 months (range 0.5-36.1). Response rates were similar for QD *vs* BID treatment (CHR: 95.5% *vs* 87.0%; MCyR 45.5% *vs* 60.9%; CCyR 45.5% *vs* 43.5%). Across all patients, median durations of CHR and MCyR were not reached (71% of responding patients had maintained MCyR at 33 months), and at 36 months, the PFS rate was 64% and the overall survival rate was 82%. In a landmark analysis, achieving a MCyR at any time on dasatinib was associated with higher 36-month PFS (87% *vs* 37%) and overall survival (95% *vs* 66%). At last follow-up, 62% of patients remained on dasatinib; 18% discontinued following disease progression, only 1 patient (2%) discontinued for toxicity, and 11% died. Grade 3/4 neutropenia and thrombocytopenia were each noted in 47% of patients. Nonhematologic side effects were typically mild to moderate. The most common grade 3/4 nonhematologic side effects were pleural effusion (11%), gastrointestinal bleeding (7%), and dyspnea or pulmonary edema (4%). *Summary and Conclusions*. Long-term follow-up from this early study confirms that dasatinib is associated with high rates of hematologic and cytogenetic responses in CML-CP following imatinib failure. Dasatinib treatment responses were durable and translated into favorable PFS and overall survival. Side effects rarely led to treatment discontinuation.

0120

INTERIM RESULTS FROM A PHASE I CLINICAL TRIAL OF THE BCR-ABL INHIBITOR XL228 IN DRUG-RESISTANT PH^+ LEUKEMIAS

P. Shah,[1] E. Asatiani,[2] J. Cortes,[3] R. L. Paquette,[4] J. Pinilla-Ibarz,[5] C. Kasap,[1] L. A. Bui,[6] Y. Yaron,[6] D.O. Clary,[6] M. Talpaz[7]

[1]UCSF, SAN FRANCISCO; [2]Georgetown University, WASHINGTON, D. C.; [3]MD Anderson Cancer Center, HOUSTON; [4]UCLA, LOS ANGELES; [5]Moffitt Cancer Center, TAMPA; [6]Exelixis, Inc., SOUTH SAN FRANCISCO; [7]University of Michigan, ANN ARBOR, USA

Background. Treatment of the Philadelphia chromosome-positive leukemias (CML and Ph^+-ALL) has been revolutionized through the development of inhibitors of the oncoprotein Bcr-Abl, a drug class pioneered by imatinib. Although long term remissions are commonly achieved with this agent, relapses occur due to several mechanisms, including point mutations in the BCR-ABL gene and emergence of drug-resistant clones. While the second generation Abl inhibitors dasatinib and nilotinib have been effective in treating many patients who have become resistant to imatinib, there are certain mutated forms of Bcr-Abl which are refractory to all three approved inhibitors, notably the gatekeeper substitution T315I. In patients who relapse after responding to dasatinib treatment, a T315I-harboring clone is commonly found. Patients found to harbor this mutation have very poor response rates to the approved Abl kinase inhibitors and a poor prognosis. XL228 is a protein kinase inhibitor with potent activity against wild-type and T315I isoforms of Bcr-Abl (wild type Abl, Ki=5 nM; T315I, Ki=1.4 nM) with additional activity against IGF1R and Src. XL228 blocks downstream signaling from Bcr-Abl T315I in cell lines and modulates pCrkL levels in mouse K562 xenografts consistent with inhibitory activity against Bcr-Abl *in vivo*. Based on these data, we have initiated a Phase I clinical trial in patients with CML or Ph^+-ALL who are resistant or intolerant to standard therapies. *Aims*. The primary objective is to determine the safety, tolerability, and maximum tolerated dose of XL228 in patients with chronic, accelerated or blast phase CML, or Ph^+-ALL. The secondary and exploratory objectives include evaluating the pharmacokinetics of XL228, pharmacodynamic correlates of drug treatment, the safety and tolerability of longer term dosing, and preliminary evaluation of efficacy. *Methods*. XL228 is administered as a weekly infusion. Three cohorts (11 subjects) have been enrolled and evaluated as of abstract submission. *Results*. When dosed at 0.45, 0.9, and 1.8 mg/kg, XL228 has proven to be well-tolerated with no dose-limiting toxicities or drug-related severe

adverse events reported to date. Preliminary, unaudited safety data include the following Grade 1 adverse events which may be drug related: warmness and pain at the infusion site, fatigue, nausea, and peripheral neuropathy (1 patient). At this time, patients currently on study have been dosed for up to 6 months. Pharmacokinetic analysis of the first cohorts has demonstrated an approximately dose-proportional exposure, with a mean terminal half life of 15 to 38 hours. The mean volume of distribution ranges between 14.2 and 15.4 L/kg, suggesting XL228 distributes widely into the tissues. Weekly dosing results in low to moderate accumulation. There are preliminary indications of pharmacodynamic activity based on transient shifts in phospho-CrkL determined through flow analyses of patient peripheral blood samples. While several patients have been classified as having stable disease, no significant decreases in BCR-ABL qPCR or white count have been measured as of abstract submission. Dose escalation is continuing. *Summary*. XL228 is a promising agent for treating drug-resistant CML and Ph^+-ALL, including patients harboring the T315I gatekeeper mutation. Clinical evaluation of XL228 is ongoing.

0121

EFFICACY OF NILOTINIB IN PATIENTS (PTS) WITH NEWLY DIAGNOSED, PREVIOUSLY UNTREATED PHILADELPHIA CHROMOSOME (PH)-POSITIVE CHRONIC MYELOGENOUS LEUKEMIA IN EARLY CHRONIC PHASE (CML-CP)

J. Cortes,[1] D. Jones,[1] S. O'Brien,[1] A. Ferrajoli,[1] G. Borhtakur,[1] J. Burger,[1] W. Wierda,[1] G. Garcia-Manero,[1] L. Letvak,[2] H. Kantarjian[1]

[1]MD Anderson Cancer Center, HOUSTON, USA; [2]Novartis, EAST HANOVER, USA

Background. Nilotinib is an oral tyrosine kinase inhibitor with increased selectivity against Bcr-Abl approximately 30-fold more potent than imatinib effective in CML after imatinib failure. We initiated a phase II study to evaluate the efficacy of nilotinib as 1st line therapy in pts with newly diagnosed CML-CP. *Aims*, To investigate the efficacy and safety of nilotinib as first-line therapy in CML-CP. *Methods*. The primary objective was to estimate the proportion of pts attaining major molecular response at 12 months (mo). Pts with untreated CML-CP were eligible and received nilotinib 400mg twice daily. *Results*. Thirty-five pts have been treated for a median of 6.5 months (mo). The median age was 47 years (yrs). At 6 months, 100% of patients reaching this time point (n=20) have achieved a complete cytogenetic response [CCyR]. The rate of complete cytogenetic response [CCyR] at 3, 6 and 12 mo compares favorably to those observed in historical controls treated with imatinib 400 mg or 800 mg daily (Table 1).

Table 1

Months on therapy	Percent with CCyR (No. evaluable)			p value
	Nilotinib	Imatinib 400mg	Imatinib 800mg	
3	96 (31)	37 (49)	62 (202)	< 0.0001
6	100 (20)	54 (48)	82 (199)	< 0.0001
12	100 (11)	65 (48)	86 (197)	0.0007

Major molecular response (MMR) was observed in 13% at 3 mo, 45% at 6 mo, and 45% at 12 mo. Grade 3-4 thrombocytopenia was observed in 6% and neutropenia in 3%. Other grade 3-4 adverse events included elevation of lipase in 9% and bilirubin in 9%. Fifteen (43%) pts had transient treatment interruptions (median 11 days) and 14 had dose reductions. The actual median dose is 800 mg daily. Thirty-two patients continue on study. *Conclusion*. Nilotinib 400 mg twice induces a CCyR in nearly all patients as early as 3 months after the start of therapy with a favorable toxicity profile. Accrual is ongoing.

0122

DASATINIB COMPARED WITH HIGH-DOSE IMATINIB IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE (CML-CP) AFTER FAILURE OF STANDARD-DOSE IMATINIB - A TWO-YEAR UPDATE OF THE START-R STUDY

P. Rousselot,[1] S. Corm,[2] R. Paquette,[3] E. Bleickardt,[4] D. Dejardin,[4] H.M. Kantarjian,[5] D. Niederwieser[6]

[1]Hôpital Mignot, VERSAILLES, France; [2]Hôpital Huriez, LILLE, France; [3]UCLA, LOS ANGELES, USA; [4]Bristol-Myers Squibb, WALLINGFORD, USA; [5]MD Anderson Cancer Center, HOUSTON, USA; [6]University of Leipzig, LEIPZIG, Germany

Background. Resistance to standard-dose imatinib and subsequent treatment failure is a well-established problem that affects many patients with CML-CP. High-dose imatinib may be a therapeutic option in such cases. Dasatinib may override multiple mechanisms of imatinib resistance. It is 325-fold more potent against native BCR-ABL than imatinib *in vitro*, and is active against imatinib-resistant mutations with the exception of T315I. Dasatinib has also demonstrated efficacy in patients with imatinib-resistant CML. *Aims*. To evaluate the efficacy and safety of dasatinib and high-dose imatinib in patients with CML-CP after failure of standard-dose imatinib with a minimum follow-up of 24 months. *Methods*. START-R is an international, randomized, phase II study. In total, 150 patients with CML-CP who failed imatinib 400-600 mg/d received either dasatinib 70 mg BID (n=101) or imatinib 800 mg/d (n=49). The primary endpoint was major cytogenetic response (MCyR) at 12 weeks. Patients without MCyR at 12 weeks, those who experienced confirmed progression, or those with intolerance despite dose reduction were permitted to cross over to the other study therapy. Doses of dasatinib could be escalated to 90 mg BID for patients with inadequate responses at 12 weeks or progression. The definition for progression was: increasing WBC count, loss of complete hematologic response/MCyR, confirmed accelerated/blast phase disease, or death. Data are now presented for a minimum follow-up (LPFV to database closure) of 24 months. *Results*. At 12 weeks, MCyR rates were significantly higher for patients receiving dasatinib than for those receiving high-dose imatinib (36% *vs* 29%; p=0.4025). At last follow-up, MCyRs had been achieved with dasatinib *vs* high-dose imatinib treatment by 53% *vs* 33% (p=0.017), with the difference being attributable to rates of complete cytogenetic response (44% *vs* 18%; p=0.0025). Moreover, MCyRs were more durable for dasatinib: after 18 months, 90% of dasatinib-treated patients maintained MCyRs, compared with 74% of imatinib-treated patients. At 24 months, dasatinib was also associated with significantly higher major molecular response rates (29% *vs* 12%; p=0.028), improved time to treatment failure (patients without failure at 24 months: 59% *vs* 18% p<0.0001), and extended progression-free survival (PFS rates at 24 months; 86% *vs* 65%; p=0.0012). The PFS superiority of dasatinib over high-dose imatinib was apparent for patients receiving prior imatinib both at 400 mg/d (p=0.0562) and 600 mg/d (p=0.0033). Grade 3-4 non-hematologic adverse-event rates were minimal for both treatment groups. Grade 3-4 cytopenia was more common in the dasatinib arm. Rates of toxicity-related treatment discontinuation were similar between treatments (dasatinib 22% *vs* imatinib 20%). *Conclusions*. These extended, 24-month follow-up data support earlier START-R reports: the overall benefit-risk assessment favors dasatinib over high-dose imatinib in patients with CML-CP patients who failed standard-dose imatinib. The current labeled dose of 100 mg QD was approved based on a subsequent phase III, dose-optimization study which showed comparable efficacy with improved tolerability compared with 70 mg BID.

0123

DATA FROM A MULTICENTER OPEN LABEL STUDY OF SUBCUTANEOUS (SC) OMACETAXINE MEPESUCCINATE (OMA) IN IMATINIB (IM)-RESISTANT CHRONIC MYELOID LEUKEMIA (CML) PATIENTS (PTS) WITH THE T315I MUTATION

J. Cortes,[1] H.J. Khoury,[2] S. Corm,[3] F.E. Nicolini,[4] A.C. Benichou,[5] A.R. Craig,[6] E. Humphriss,[6] H. Kantarjian[1]

[1]MD Anderson Cancer Center, HOUSTON, USA; [2]Emory University School of Medicine, ATLANTA, USA; [3]CHRU Hôpital Claude Huriez, LILLE, France; [4]CHU Hôpital Edouard Herriot, LYON, France; [5]Stragen France, LYON, France; [6]ChemGenex, MENLO PARK, USA

Background. Omacetaxine (homoharringtonine, HHT) is clinically active against Ph^+ CML, with a mechanism of action independent of

tyrosine kinase (TK) inhibition. Currently available TK inhibitors (TKIs) have not demonstrated activity in CML patients with T315I mutation. *Aims.* We are evaluating the safety and efficacy of OMA in Pts with IM-resistant T315I$^+$ Ph$^+$ CML. *Methods*. Eligible Pts- adult CML with confirmed T315I mutation following imatinib failure. All patients must provide informed consent to participate. Induction schedule: 1.25 mg/m^2 OMA SC twice daily (BID) for 14 days every 28 days until complete hematologic response (CHR) or hematologic improvement. Maintenance schedule: 1.25 mg/m^2 OMA SC BID for 7 days every 28 days, for up to 24 mos. *Results*. To date, 33 Pts have been enrolled, 87% having failed multiple prior TKIs; 19 in chronic phase (CP), 7 in accelerated phase (AP) and 7 in myeloid blast phase (BP). Median age: 58 yrs (19-83), median disease duration: 58 mos. (5-285). An independent data monitoring committee composed of 2 medical doctors and a PHD biostatistician has been assembled to evaluate all responses reported in the study. This committee has completed the first adjudication of the initial 10 CP patients enrolled in the study. Overall response in these 10 patients demonstrates a CHR rate of 90% and a complete cytogenetic response rate of 20%. In addition, 1 patient achieved a minor cytogenetic response. The median time to response was 2 mos. (range 1-5) and the median duration of response for both hematologic and cytogenetic response is currently 6 mos. (range 1-12+). All responding patients (N=9) remain active in maintenance OMA treatment. Safety data are available on 23 patients enrolled in all disease phases and OMA has been well tolerated with the primary toxicity being myelosuppresion (managed with adjustments to the number of dosing days per cycle). Incidence of treatment emergent grade 3/4 events in these patients include: thrombocytopenia 61%, neutropenia 44%, anemia 39%, febrile neutropenia 22% and pancytopenia 13%. Mild injection site pain (23%) and erythema (13%) have also been reported. Grade 3-4 treatment emergent non hematologic events have been infrequent with individual events occurring in less than 5% (1 patient/event) of patients. *Conclusions*. OMA therapy in T315I+ IM-resistant CML is well tolerated and is resulting in durable CHRs and cytogenetic responses in the initial 10 CP patients enrolled as determined by an independent Data Monitoring Committee.

0124

CHRONIC MYELOID LEUKEMIA IN BLAST CRISIS TREATED WITH IMATINIB 600 MG: OUTCOME OF THE PATIENTS ALIVE AFTER A 6-YEAR FOLLOW-UP

F. Palandri,[1] F. Castagnetti,[2] N. Testoni,[2] S. Bassi,[3] M. Breccia,[4] R. Varaldo,[5] A. Liberati,[5] G. Specchia,[5] E. Zuffa,[5] G. Rege-Cambrin,[6] M. Bocchia,[5] G. Saglio,[6] F. Pane,[7] G. Martinelli,[2] M. Baccarani,[2] G. Rosti[2]

[1]Department of Hematology Seragnoli, BOLOGNA; [2]Department of Hematology Seragnoli, BOLOGNA; [3]Division of Hematology, AIEOP, MILANO; [4]Department of Cellular Biotechnology and Hematology, University La Sapienza, ROMA; [5]Division of Hematology, GENOVA; [6]Department of Clinical and Biological Science, University of Turin at Orbassano, TORINO; [7]CEINGE Biotecnologie Avanzate and Department of Biochemistry and Medical Biotech, NAPOLI, Italy

Background. Management of Blast crisis Chronic Myeloid Leukemia (BC-CML) is the most challenging entity in the treatment of chronic myeloproliferative disorders. In 2000, the introduction of Imatinib mesylate (IM) has opened a new option in the treatment of BC-CML. Early results have shown the superiority of IM compared to conventional chemotherapy; however, the long-term outcome of these patients remains to be clarified. *Aims.* The GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) CML Working Party conducted a phase II, multi-institutional prospective study (CML/003) to investigate the long-term effects of IM in BC-CML patients, treated with IM 600 mg daily. Ninety-two patients were enrolled. After a 6-year follow-up, we assessed the IM long-term efficacy, response duration and survival, and we characterized the prognostic factors associated with a favourable outcome. *Methods*. Patients were monitored for hematologic and cytogenetic response at 1-3 months intervals. A complete hematologic response (CHR) required the normalization of platelet and white cell differential count and absence of extramedullary involvement. The definition of return to chronic phase (RTC) required less than 15% blasts and less than 30% blasts plus promyelocytes in blood or bone marrow and less than 20% peripheral basophils. Cytogenetic analysis was performed with standard banding techniques and the response was rated as usual. *Results*. Forty-six out of 92 patients (50%) had a sustained RTC, and 24 patients (26%) achieved a CHR. RTC was subsequently lost by 22 patients, for a median duration of the second CP of 11 months (range 1-67). Sixteen patients lost the CHR, for a median duration of the CHR of 6 months (range 1-43). Sixteen patients (17%) had a cytogenetic response (9 complete, 1 partial, and 6 minor or minimal). CCgR was subsequently lost by all but 2 pts after 2 to 12 mos from its first achievement, for a median CCgR duration of 7 months. The Kaplan-Meier median survival time was 7 months, and the survival rates were 53% at 6 months, 29% at 12 months, 23% at 18 months and 11% at 36 months; for the 10 patients who achieved a MCgR, OS was significantly better (p=0.001) (Figure 1). After a median observation time of 66 months, 7 (8%) patients are alive: 3 patients are on IM treatment (1 in CHR, 1 in partial CgR and 1 in CCgR). Three patients are in complete remission after allogeneic transplant. One patient is alive in BC, after failure of a second-generation TKI. *Conclusion*. We confirm that IM as monotherapy was valuable and safe in the short-term, but relapse rate was high and the longer term clinical outcome was not significantly influenced.

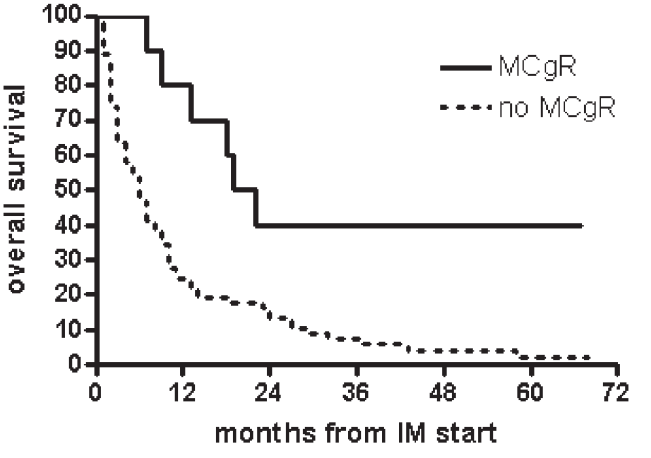

Figure 1. OS according to cytogenetic response.

0125

IMATINIB 600 MG ACCELERATED PHASE CHRONIC MYELOID LEUKEMIA INDUCES DURABLE CYTOGENETIC RESPONSES IN THE LONG-TERM: THE GIMEMA CML WORKING PARTY EXPERIENCE AFTER A 7-YEAR FOLLOW-UP

F. Palandri,[1] F. Castagnetti,[2] N. Testoni,[2] G. Alimena,[3] G. Rege-Cambrin,[4] M. Breccia,[3] G. Specchia,[5] B. Bruno,[5] M. Miglino,[5] F. Levato,[5] G. Saglio,[4] F. Pane,[6] G. Martinelli,[2] M. Baccarani,[2] G. Rosti[2]

[1]Department of Hematology Seragnoli, BOLOGNA; [2]Department of Hematology Seragnoli, BOLOGNA; [3]Department of Cellular Biotechnology and Hematology, University La Sapienza, ROMA; [4]Department of Clinical and Biological Science, University of Turin at Orbassano, TORINO; [5]Division of Hematology, BARI; [6]CEINGE Biotecnologie Avanzate and Department of Biochemistry and Medical Biotech, NAPOLI, Italy

Background. Imatinib mesylate (IM), a targeted inhibitor of the BCR-ABL tyrosine-kinase, is the standard of care for chronic myeloid leukemia (CML). In patients with CML in accelerated phase (AP-CML), the advent of IM significantly increased survival. However, few long-term data on the outcome of these patients based on large, prospective and controlled trials are available. *Aims*. The GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) CML Working Party is conducting a phase II, multi-institutional prospective study (CML/003) to investigate the long-term effects of IM in AP-CML patients, treated with imatinib 600 mg daily. One hundred and eleven patients were enrolled; median follow-up of the 41 living patients is 82 months (range, 73-87). *Methods*. Patients were monitored for hematologic and cytogenetic response at 1-3 months intervals. A complete hematologic response (CHR) required the normalization of platelet and white cell differential count and absence of extramedullary involvement. The definition of return to chronic phase (RTC) required less than 15% blasts and less than 30% blasts plus promyelocytes in blood or bone marrow and less than 20% peripheral basophils. Cytogenetic analysis was performed with standard banding techniques. *Results*. In 107 patients (96%) was observed a RTC and 79 patients (71%) achieved also a CHR. Cumulative best rates of major cytogenetic response (MCgR) and complete cytogenetic response (CCgR) were 30% and 21%, respectively. CCgR was subsequently lost by 6 patients after 3 to 36 months from its first achievement (median,

10 months). A total of 90 patients (81%) discontinued IM, after a median time of 25 months (range, 1-86); only 7 patients (6%) discontinued IM because of adverse events. At last follow-up, 4 patients were alive in complete remission after allogeneic transplant, 16 patients have switched to second generation tyrosine kinase inhibitor and 21 patients were alive on IM therapy (13 in complete, 5 in partial, 1 in minor and 2 in null cytogenetic response). No late toxicities have been observed. The estimated rate of overall survival (OS) at 7 years was 43%, and was associated with the achievement of a MCgR (Figure 1). *Conclusion*. After a 7-year follow-up, IM continues to induce durable responses in patients with AP CML, with negligible toxicity.

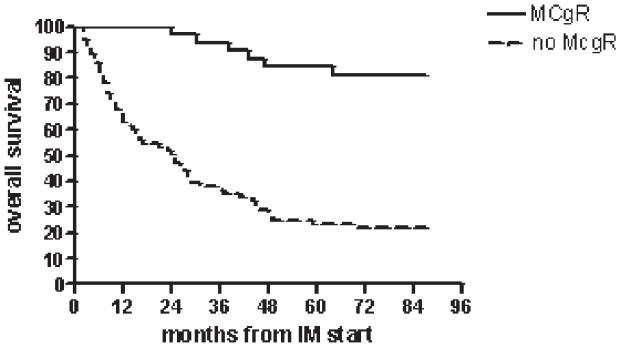

Figure 1. OS according to cytogenetic response

0126

USE OF IMATINIB IN CHRONIC PHASE CML IN CLINICAL PRACTICE: THE UNIC STUDY

J.L. Steegmann,[1] M. Michallet,[2] E. Morra,[3] D. Marin,[4] G. Ossenkoppele,[5] G. Verhoef,[6] T. Kühr,[7] M. Björeman,[8] L. Van Bree,[9] K. Cerri[9]

[1]Hospital de La Princesa, MADRID, Spain; [2]Hôpital Edouard Herriot, LYON, France; [3]Ospedale di Niguarda Ca' Granda, MILAN, Italy; [4]Hammersmith Hospital, LONDON, UK; [5]VU University Medical Centre, AMSTERDAM, Netherlands; [6]University Hospital Leuven, LEUVEN, Belgium; [7]Klinikum Kreuzschwestern Wels, WELS, Austria; [8]University Hospital Örebro, ÖREBRO, Sweden; [9]Bristol-Myers Squibb International, BRAINE L'ALLEUD, Belgium

Background. Imatinib mesilate is widely used to treat chronic myeloid leukaemia (CML). *Aims*. The Unmet Needs in CML (UNIC) study aimed to address knowledge gaps on how CML patients are treated and monitored in clinical practice. Here, we present data on the use of imatinib in patients with chronic phase CML. *Methods*. UNIC was a cross-sectional study, with retrospective chart review of patients currently treated for CML or Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph^+ALL) across 8 European countries (Austria, Belgium, France, Italy, The Netherlands, Spain, Sweden, UK). Patients were recruited from September 2006 to March 2007. The study was designed to estimate the proportion of patients ever treated with imatinib and those imatinib-treated patients who have experienced imatinib resistance and/or intolerance (primary objectives). A secondary aim was to describe dosing patterns. A registry was collected of potentially eligible patients - those aged ≥18 years and treated for CML/Ph^+ALL at the participating centres (academic, non-academic, private clinic or other). Case Report Forms (CRFs) were completed for eligible patients until the recruitment target was reached. Data were collected at the most recent visit and retrospectively through clinical chart review. *Results*. CRFs were analysable for 1492 chronic phase CML patients, of whom 97% had received imatinib. Patients were defined as imatinib resistant/intolerant if resistance/toxicity led to a change in, or discontinuation of, imatinib use, as reported in their medical chart. Of the imatinib-treated chronic phase CML patients, 44.6% were imatinib resistant and/or intolerant at some time since imatinib initiation. A total of 20% of patients stopped and did not restart imatinib during the study period. Furthermore, 23% of patients stopped imatinib temporarily due to toxicity (median duration 22 days), of whom ~30% had >1 such interruption. In all, 41% (563/1366) of chronic phase CML patients had a dose increase and 33% (441/1352) had a dose decrease. The most common imatinib dose increase was from 400 mg to 600 mg (33%); the most common dose decrease was from 400 mg to 300 mg (39%). 16% of increases and 14% of decreases were both to and from imatinib doses <400 mg. Of the 520 patients with ≥1 dose increase, 17.5% were imatinib resistant just prior to dose change, of whom 63% were resistant at last observation. The median last imatinib dose was higher in imatinib-resistant patients (600 mg; n=149) than non-resistant patients (400 mg; n=1205). Of the imatinib-resistant patients, 41% had received doses≤400 mg. Of the patients with imatinib dose increases to 600 mg and 800 mg, 22% (62/284) and 32% (36/112), respectively, had a toxicity following the dose increase; and 88% (173/197) and 96% (64/67), respectively, of those without a cytogenetic response before the dose increase did not have a cytogenetic response after the increase. Summary/conclusions. A notable proportion of imatinib-treated chronic phase CML patients in this large observational study needed dose modification, where 41% had a dose increase and 33% had a dose decrease. Imatinib-resistant patients received higher imatinib doses than non-resistant patients.

0127

EXPRESSION OF H-OCT1 PREDICTS FOR ACHIEVEMENT OF CCYR IN IMATINIB TREATED PATIENTS WHILE THE LEVEL OF PHOSPHO-CRKL INHIBITION IN CD34 POSITIVE CELLS SEEMS TO BE OF LITTLE PROGNOSTIC VALUE

S. Sorouri Khorashad, S. Wagner, D. Marin, A.G. Reid, D. Milojkovic, S. Willimott, G. Gerrard, L. Foroni, J.M. Goldman, J.F. Apperley

Imperial College of London, LONDON, UK

Background. About 15% of the patients who are treated with IM in chronic phase as first line treatment do not achieve complete cytogenetic response (CCyR). The mechanisms of failure to achieve CCyR in the majority of cases are unknown. Identification at diagnosis of patients who might subsequently fail to achieve CCyR would provide a considerable aid to clinical management. *Aims*. It has been suggested that measuring response to IM in CD34 cells from pre-IM samples might provide some information about patients' future response to therapy. We therefore assessed two such approaches for their predictive value: 1) measurement of the level of inhibition of Crkl phosphorylation (p-Crkl) by IM in CD34 positive cells; and 2) the level of h-OCT1 expression. Phosphorylation of the adaptor protein Crkl, which is a direct substrate of BCR-ABL, is known to be lower in patients responding to imatinib mesylate, and may therefore provide an in-vitro method of assessing likelihood of response. hOCT1 actively transports imatinib into cells, suggesting that patients with low baseline expression of hOCT1 may be unable to achieve adequate intracellular concentrations of imatinib, and hence fail to achieve a cytogenetic response. *Methods*. We analysed the level of P-Crkl inhibition by IM in CD34 positive cells from pre-IM samples and the level of h-OCT1 expression in 25 patients who had been treated with IM as first line therapy, 10 of whom failed to achieve CCyR during therapy with IM. Separated CD34 cells obtained before start of IM were cultured with and without 5 μM IM for 16 hours. The P-Crkl in the treated culture was calculated as a percentage of the untreated using flow cytometry. H-OCT1 transcript was measured by real time PCR and expressed as a ratio to the control gene which was G6PD in this study. *Results*. P-Crkl reduction was not significantly different in the patients who achieved CCyR from those who did not (p=0.53). Clinical response was not significantly different between the patients who had ≥50%reduction in P-Crkl and those who did not (p=0.97). The expression level of h-OCT1 in diagnostic samples was significantly associated with time to achievement of CCyR. The patients who had a h-OCT1/G6PD ratio >10 had RR of 4.2 to achieve CCyR than those for whom this ratio was less than 10 (p=0.027). Interestingly, there was no correlation between the inhibition level of P-Crkl and the level of h-OCT1 (p=0.86). This observation suggests that the level of h-OCT1 expression may not influence inhibition of P-Crkl by 5 μM IM. *Summary*. Level of *in vitro* inhibition of p-Crkl in CD34 positive cells by IM does not appear to predict for future response, however, the expression level of h-OCT1 shows a significant correlation with time to CCyR and warrants further investigation as a prognostic indicator.

0128

DASATINIB LACK OF CROSS INTOLERANCE TO IMATINIB IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE (CML-CP) WHO ARE INTOLERANT TO IMATINIB - A RETROSPECTIVE SAFETY ANALYSIS

F. Guilhot,[1] S.L. Goldberg,[2] R.M. Stone,[3] M. Mauro,[4] Y. Matloub,[5] T.T. Chen,[5] H.J. Khoury[6]

[1]CHU de Poitiers, POITIERS, France; [2]Hackensack University Medical Center, HACKENSACK, USA; [3]Dana-Farber Cancer Institute, BOSTON, USA; [4]Oregon Health & Science University, PORTLAND, USA; [5]Bristol-Myers Squibb, WALLINGFORD, USA; [6]Emory University School of Medicine, ATLANTA, USA

Background. Cross intolerance may be a barrier in the second-line treatment of imatinib-intolerant patients with CML-CP. Dasatinib is a BCR-ABL inhibitor structurally unrelated to imatinib; it is effective in patients with CML-CP intolerant to imatinib and is generally well tolerated. The most common adverse events of imatinib treatment are fluid retention, gastrointestinal intolerance, and cytopenias. *Aims*. To assess whether the type of intolerance to imatinib (hematologic or non-hematologic toxicity) predicts the safety of dasatinib. *Methods*. Pooled data from two multicenter studies, one phase II and one phase III, in patients with CML-CP were analyzed. Imatinib intolerance was defined as grade ≥3 non-hematologic toxicity or any grade 4 hematologic toxicity lasting >7 days. Dasatinib was administered according to one of four schedules: 70 mg BID, 100 mg QD (the approved dosing schedule), 50 mg BID, and 140 mg QD. Median duration of dasatinib therapy was 11.96 months (0.03-30.06 months). Cross intolerance between imatinib and dasatinib was evaluated using discontinuations due to NCI-CTAE grade 3-4 adverse events. Evidence for a correlation between fluid retention on imatinib and subsequent pleural effusions on dasatinib was examined. *Results*. In total, 271 patients with imatinib-intolerant CML-CP were eligible for analysis. The duration of imatinib therapy was <1 year in 58% of patients. Imatinib intolerance was related to hematologic toxicity in 46 patients and non-hematologic toxicity in 225 patients. The exact cause of imatinib intolerance related to non-hematologic toxicity was defined in 210 patients (Table 1).

Table 1.

Adverse events causing intolerance	No. of imatinib-intolerant pts	No. of dasatinib-intolerant pts	No. of pts discontinuing dasatinib
Rash	87	3	1
Hepatotoxicity	48	0	0
Myalgia/arthralgia	22	1	1
GI toxicities	15	2	0
Fluid retention	10	0	0
Pulmonary toxicities	10	0	0
Bone pain	5	0	0
Others (1 or 2 pts each)	13	3*	0
TOTAL	**210**	**9**	**2**

*Fatigue, headache, and cardiac toxicity

Nine (4%) patients experienced the same grade 3-4 non-hematologic toxicity on dasatinib as on imatinib, and only two (1%) discontinued dasatinib. Seven patients continued on dasatinib following dose reduction. Ten patients had fluid retention on imatinib, and none developed pleural effusion on dasatinib. Among the 46 patients who had discontinued imatinib due to hematologic toxicity, six (13%) discontinued dasatinib due to the same hematologic toxicity. *Conclusions*. In this large population of imatinib-intolerant patients with CML-CP, there was no evidence of cross intolerance between imatinib and dasatinib, suggesting that imatinib-intolerant patients may be successfully switched to dasatinib without being at risk of experiencing similar grade 3-4 toxicities. There was also no evidence to support a correlation between fluid retention with imatinib and pleural effusions with dasatinib.

0129

HAPLOIDENTICAL BLOOD AND MARROW TRANSPLANTATION IN ADVANCED CHRONIC MYELOID LEUKEMIA: A SINGLE-CENTER REPORT OF 35 PATIENTS

Z. Zhao, T. Wu, J.B. Wang, X.Y. Cao, Y.M. Ying, D.P. Lu

Beijing Daopei Hospital, BEIJING, China

Background. Chronic myeloid leukemia (CML) has substantially improved survival with the application of imatinib, however, the patients with advanced CML such as in accelerated phase (AP) or blast crisis (BC) have dismal prognosis even in the era of tyrosine kinase inhibitors. Allogeneic hematopoietic stem cell transplantation is still the only way to cure the patients with advanced CML. HLA mismatched / haploidentical blood and marrow transplantation (haplo-BMT) from family donor is a feasible alternative option for patients with hematological malignancies who need transplant but lack of matched either sibling or unrelated donor (*Lu DP et al. Blood 2006; 107:3065*). *Aims*. In current clinical study, the efficacy of haplo-BMT in the treatment of advanced CML was investigated. *Methods.* From November 2002 to October 2007, thirty-five patients with advanced CML without matched either sibling or unrelated donors received haplo-BMT. All patients or their guardians signed consent forms approved by the institutional review board. Eleven patients achieved the second or the third CP after treatment with imatinib or chemotherapy or both before pre-conditioning, but there were thirteen cases in AP and 11 patients in BC at the time of transplantation. The median age was 29 years (range 8 to 51 years). The regimens for pre-conditioning and GVHD prophylaxis have been published previously. G-CSF primed both bone marrow and peripheral blood were employed as the grafts. The median follow-up time among living patients was 25.5 months (range, 5 to 57 months). *Results.* The cases of HLA-antigen mismatched between donor and recipient as 1, 2, and 3 antigens were 1, 12, and 22 respectively. The mean mononuclear cells and $CD34^+$ cells were $7.19\pm1.37\times10^8$/kg and $2.54\pm1.50\times10^7$/kg. All but one patient achieved hematopoietic reconstitution. The median days for neutrophil and platelet engraftment were 13 (range, 8-22) and 17 (range, 9-151), respectively. Hyperacute GVHD occurred in 28.6% patients. The cumulative incidence of grade II to IV acute GVHD was 47.8%. Among 27 patients with survival longer than 100 days, 16 of them had chronic GVHD. Two-year cumulative overall survival rates were 75.0%, 26.6% in patients with CML-CP2 or CP3, CML-AP or BC, respectively (p<0.01). *Conclusions*. Haploidentical BMT is a feasible therapeutic mean for patients with advanced CML if they have no matched donor available. It is better to perform haplo-BMT at CML-CP2 or CP3 other than CML-AP or BC.

0130

BLASTS, EOSINOPHILS, SPLEEN ENLARGEMENT, AND CONCOMITANT DISEASE PROVIDE INDEPENDENT SIGNIFICANT PROGNOSTIC INFORMATION ON THE PROBABILTY TO LIVE MORE THAN NINE YEARS WITH CONSERVATIVE DRUG TREATMENT

M. Pfirrmann,[1] F. Waibel,[2] M. Lauseker,[1] S. Saussele,[2] M. Rohrbacher,[2] A. Leitner,[2] J. Hasford,[1] R. Hehlmann,[2] A. Hochhaus[2]

[1]Ludwig-Maximilians-University, MUNICH; [2]Medizinische Fakultät Mannheim der Universität Heidelberg, MANNHEIM, Germany

Background. 1477 newly diagnosed patients with chronic myeloid leukemia (CML) in chronic phase were recruited for German CML studies I, II and III between 1983-2001. Most patients (n=1094, 74%) received drug treatment as first-line therapy. 383 patients received allogeneic stem cell transplantation in first chronic phase and are not considered here. *Aims.* Based on a median observation time of 9.6 years for 238 living patients (22% of 1094) for the drug-treated patients, we sought to investigate which factors evaluated at diagnosis provide prognostic information with regard to survival beyond nine years after registration in one of the trials. *Methods*. To be able to determine the final outcome (dead or alive nine years after registration), living patients who were observed for <9 years were excluded (n=95). The prognostic influence of the candidate variables on the final outcome was examined by conditional logistic regression with *at least nine year survival* as response. Responders and non-responders were matched by allocated drug treatment (busulfan (BU), hydroxyurea (HU), interferon-α (IFN), combination therapy IFN+HU), sex, and age group (<50, 50-64, >64 years). To avoid too small strata, further 38 patients allocated to intensive chemotherapy were not considered. *Results.* The 961 patients of the final analysis sample comprised 684 patients randomised within the trials CML I and II: 164 patients were randomised to BU, 255 to HU, 102 to IFN, and 163 to

IFN+HU. The remaining 277 patients were part of the CML study III and allocated to receive IFN+HU as first-line therapy. Only drug treatment but not trial origin had an influence on survival. After nine years, 177 patients were still alive (18%): 12 (7%) allocated to BU, 31 (12%) to HU, 19 (19%) to IFN, and 115 (26%) to IFN+HU. Of 130 patients (73% of 177) who were still alive at last evaluation, 62 (48%) received second line imatinib therapy, 30 (23%) IFN, 22 (17%) HU, 11 (8%) no therapy, 2 (2%) other therapy, and for 3 (2%) therapy was unknown. With a significance level of 0.05, conditional logistic regression identified white blood cell count, the proportions of basophils, blasts, eosinophils, and erythroblasts in peripheral blood, hemoglobin, lactate dehydrogenase, spleen size, weight loss, and Karnofsky index to have a significant univariate association with survival outcome. In addition to these variables, the univariately non-significant parameters fever, general ill-feeling, concomitant disease, platelet count, and proportions of myelocytes and promyelocytes in peripheral blood qualified for multiple analyses. A backward and forward selection strategy yielded a final model with four independent variables: blasts, eosinophils, spleen enlargement, and concomitant disease. Apart from the latter one, these variables were also part of the Hasford score (*Hasford et al., JNCI 1998; 90:850-8*). *Conclusions*. Matched for allocated first-line treatment, sex, and age group, our results indicate that blasts, eosinophils, spleen enlargement, and concomitant disease provide independent, statistically significant prognostic information on the probability to live beyond nine years with conservative drug treatment.

0131

MEASUREMENT OF IMATINIB AND ITS METABOLITE, CGP-74588, IN TREATED CHRONIC MYELOID LEUKAEMIA PATEINTS BY HPLC

A. Davies, A. Hayes, K. Knight, S.J. Watmough, M. Pirmohamed, R.E. Clark

University of Liverpool, LIVERPOOL, UK

Background. Recent data have indicated that plasma imatinib levels might correlate with clinical outcome in chronic myeloid leukaemia (CML). Imatinib is mainly metabolised by the cytochrome P450 isoform (CYP3A4) to produce the N-desmethyl metabolite CGP-74588. Although equipotent, CGP-74588 has a longer half-life than imatinib, and may have longer lasting effects. A variety of concomitant medications and foodstuffs can either inhibit or induce drug metabolism pathways, in particular CYP3A4, and thereby alter the level of the interacted drug. It is therefore possible that plasma levels of CGP-74588, as well as imatinib, correlate with clinical outcome. *Methods*. We have developed a high performance liquid chromatography (HPLC) technique that will assay both imatinib and CGP-74588 simultaneously, using clozapine as the internal standard. Solid phase extraction (SPE) was used to prepare the plasma samples (200 μL). The samples were separated by UV-HPLC through a Gemini C6-Phenyl (150 mm x 4.6 mm ID; 5 μm) column (Phenomenex) under isocratic mobile phase conditions of MeOH:50mM ammonium acetate (65:35, v/v) at a flow rate of 1 mL/min and detected at 260 nm. *Results*. Complete separation of the imatinib and CGP-74588 peaks was observed with retention times of 5.5 and 3.7 minutes, respectively, during a run time of 17 minutes. Mean extraction recovery of imatinib and CGP-74588 are 89% and 96% respectively. Calibration curves are linear (r^2=0.9999) between 12,000 and 750 ng/mL. The limit of quantification (LOQ) is 100 ng/mL (<15% bias). Intra-day precision is demonstrated with CVs at or below 2% and 4.5% for imatinib and CGP-74588 respectively. The method is reproducible with mean inter-day CVs of less than 3.5% and 3% for imatinib and CGP-74588 respectively. Analysis of clinical samples has demonstrated contrasting plasma imatinib levels 22-26 hours after identical doses, with subsequent clinical outcome being more favourable with higher plasma levels. In addition, the ratio of imatinib to CGP-74588 was not constant, and may relate in part to the time since last dose of imatinib, and CYP3A4 activity. As predicted, levels of both imatinib and CGP-74588 are higher in samples 11-13 hours post dose than 22-26 hours post dose, and in patients receiving 600 or 800 mg daily doses compared to 400 mg daily dosing. Expansion of these findings with a wider dataset is currently in progress. *Summary*. The present entirely HPLC based method to assay both imatinib and CGP-74588 avoids the need for mass spectrometry and for radioactive laboratory standards, and can be used to monitor individual patient responses to imatinib and improve the prediction of clinical outcome in CML.

0132

COMBINED THERAPY WITH IMATINIB FOR INDUCTION AND INTERFERON AS CONSOLIDATION INDUCES A SUSTAINED CYTOGENETIC REMISSION THAT FACILITATES DISCONTINUATION OF ANTI LEUKEMIC THERAPY IN CHRONIC MYELOGENOUS LEUKEMIA

I. Hardan,[1] N. Amariglio,[1] L. Trachtenbrot,[1] A. Simon,[1] A. Shimoni,[1] I. Levi,[2] M. Koren- Michowitz,[1] Y. Binenbaum,[1] G. Rechavi,[1] A. Nagler[1]

[1]Sheba Medical Center, TEL HASHOMER; [2]Soroka Medical Center, BEER SHEVA, Israel

Imatinib Mesylate (IM, Gleevec) is capable of induction of cytogenetic and molecular response in a substantial proportion of newly diagnosed and previously treated chronic myelogenous leukemia (CML) patients. Discontinuation of therapy results in an early disease progression in most cases due to IM reduced activity on immature Ph+ progenitors. IM is therefore needed for lifelong, while the duration of response to tyrosine kinase inhibibitors and the adverse effects of long term use of these agents are yet unknown. Alpha Interferon (IFN) is capable of controlling immature Ph^+ progenitors, mainly in the set up of minimal residual disease (MRD), by mounting an autologous anti leukemic activity mediated by CML specific T cell clones. Therefore, unlike IM, IFN therapy discontinuation in responding patients often does not result in disease progression. *Aims*. We conduct a prospective clinical trial aims to study the efficacy of further eliminating residual Ph^+ progenitor cells in patients with IM induced complete cytogenetic remission (CCyR) and it's potential role in facilitating discontinuation of (and freedom from the need of) anti-leukemic therapy. *Methods*. Eligibility includes more than one year of documented CCyR on IM therapy and lack of history of IFN intolerance. PEG-IFN2α (Pegasys) was added to the IM therapy at enrolment for 12 month. IM therapy was discontinued after 9 month of IFN administration. Thus, 12 month after enrolment patients remain off anti leukemic therapy. Twelve patients (9 m, 3 f ; median age 47y, range 32-61) were enrolled. Median CML duration at enrolment was 61 month (range 24-108). Six patients were previously treated with IFN; one patient had a previous autologous and one an allogeneic BMT. Three patients had a previous documentation of accelerated phase CML. Hasford score at diagnosis was low in seven patients and intermediate/high in 3 and 2 patients respectively. Median duration of IM therapy was 54m (range 21-72). At enrolment seven patients had a major molecular response (MMR) in BM cells including one with undetectable BCR-ABL transcript (molecular remission, CMR). *Results*. One patient discontinued IFN therapy after 4 weeks due to side effects. 11 patients received 90-180 mg of PEG-IFN/week according to blood counts for 52 weeks and discontinued IM therapy 39 weeks after initiation of IFN therapy. Nine Of the 11 patients improved their molecular response (either to MMR or to CMR) during the IFN therapy (one remained in CMR and one in MMR). At the day of IM discontinuation 7 of 11 patients had a documented molecular remission (CMR). With a median follow up of 9 month from discontinuation of IM therapy (range 3-16m) two of 11 patients relapsed (at 8 and 9 month after IM discontinuation, and regained their base line response with IM therapy) while nine of the 11 patients remain in CCR off therapy. Four patients are >15 month from IM discontinuation (>12 month off all therapy). These four patients are in a sustained cytogenetic remission (two with CMR and two with MMR). *Conclusions*. An adition of one year of consolidation therapy with IFN can facilitate discontinuation of Imatinib (and all anti leukemic) therapy in patients with CML in cytogenetic remission. A longer follow-up and larger experience are needed to determine the off-therapy duration of remission and to define the sub-population of patients that may benefit from this strategy.

0133

TREATMENT OF PHILADELPHIA-POSITIVE CHRONIC MYELOID LEUKEMIA WITH IMATINIB: INTRODUCING THE CONCEPT OF STABLE MOLECULAR RESPONSE

F. Palandri,[1] I. Iacobucci,[2] S. Soverini,[2] F. Castagnetti,[2] A. Poerio,[2] N. Testoni,[2] G. Alimena,[3] M. Breccia,[3] G. Rege-Cambrin,[4] M. Tiribelli,[5] R. Varaldo,[5] E. Abruzzese,[5] B. Bruno,[5] F. Pane,[6] G. Saglio,[4] G. Martinelli,[2] M. Baccarani,[2] G. Rosti[2]

[1]Department of Hematology Seragnoli, BOLOGNA; [2]Department of Hematology Seragnoli, BOLOGNA; [3]Department of Cellular Biotechnology and Hematology, University La Sapienza, ROMA; [4]Department of Clinical and Biological Science, University of Turin at Orbassano, TORINO; [5]Division of Hematology, UDINE; [6]CEINGE Biotecnologie Avanzate and Department of Biochemistry and Medical Biotech, NAPOLI, Italy

Background. The achievement of a major molecular response (MMolR) at 12 months is a surrogate marker of progression to accelerated/blast phase free survival (PFS) in chronic myeloid leukemia (CML) patients treated with imatinib (IM). *Aims*. We evaluated the prognostic value of the long-term prospective evolution of the MolR based on a retrospective analysis of 130 late chronic phase patients, who achieved a complete cytogenetic response (CCgR) with IM 400 mg. *Methods*. Two hundred and seventy-seven patients were treated with IM 400 mg daily after Interferon-α (IFN-α) failure, and 153 (53%) patients obtained a CCgR. We have selected for this analysis 130 out of 153 patients (85%), on the basis of the following criteria: 1) a CCgR confirmed at least twice; 2) at least 3 molecular tests performed after achieving the CCgR (median number of evaluable molecular tests: 5; range: 3-8). Patients were monitored for cytogenetic and MolR every 6 months. Cytogenetic analysis was performed with conventional methods. MolR was assessed on peripheral blood by quantitative PCR (RQ-PCR, TaqMan) and results were expressed as a ratio of BCR-ABL:ABL %. MMolR was defined as a ratio BCR-ABL/ABL % less than 0.05; a complete MolR (CMolR) was defined as undetectable BCR-ABL transcript levels by RQ-PCR confirmed by nested PCR. *Results*. In 71 patients (55%) MolR was always major (stable MMolR); in 19 (15%) MolR was occasionally less than major (unstable MMolR) and in 40 patients (30%) MMolR was never achieved (never MMolR). Patients with stable MMolR had longer CCgR duration and better PFS compared to patients with absent or unstable MMolR. The achievement of a MMolR, if maintained continuously, conferred a marked long-term stability to the CCgR (rate of CCgR loss: 4%): the probability of remaining in CCgR after 6 years was calculated by the Kaplan-Meyer method (Figure 1) and was 95% for patients in stable MMolR and 67% for patients with unstable or never MMolR (p<0.0001, log-rank test).

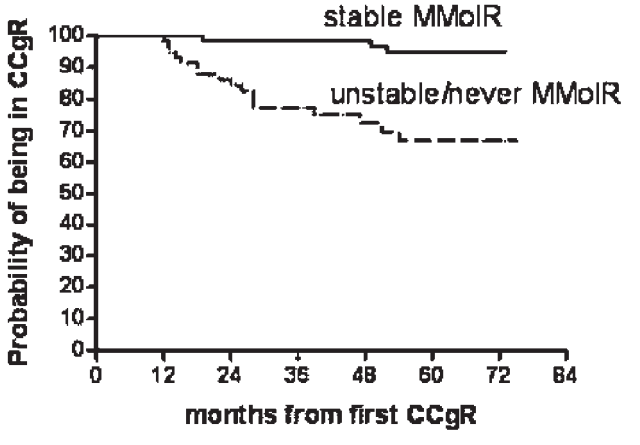

Figure 1. CCgR duration according to RQ-PCR categories.

Patients with stable MMolR have a significantly lower risk of losing the CCgR than patients with unstable (4% *vs* 21%, p=0.03) and never MMolR (4% *vs* 33%, p<0.0001). Finally, if a MMolR is not maintained continuously, the risk of losing the CCgR is higher but not significantly than if it is never achieved (33% *vs* 21% p=0.5). *Conclusion*. These data confirm that achieving a MMolR is prognostically important but point out that the prognostic value of achieving a MMolR is greater if the response is confirmed and stable.

0134

EXPANDING NILOTINIB ACCESS IN CLINICAL TRIALS (ENACT) STUDY IN ADULT PATIENTS WITH IMATINIB-RESISTANT OR INTOLERANT CHRONIC MYELOID LEUKEMIA (CML): UPDATED SAFETY ANALYSIS

F. Nicolini,[1] G. Alimena,[2] Z. Shen,[3] H.-K. Al-Ali,[4] A. Turkina,[5] G. Smith,[6] R. Pasquini,[7] S. Jootar,[8] Y. Hsu,[9] M.L. Veronese,[9] B. Powell[10]

[1]Hôpital Edouard Herriot, LYON, France; [2]Cellular Biotechnology and Hematology, University La Sapienza, ROME, Italy; [3]Rui Jin Hospital, Shanghai Jiao Tong University School of Medicine, SHANGHAI, China; [4]Medizinische Klinik und Poliklinik II, Universitat Leipzig, LEIPZIG, Germany; [5]Hematological Scientific Center, MOSCOW, Russian Federation; [6]Leeds Teaching Hospital, LEEDS, UK; [7]Universidade Federal do Paraná, CURITIBA, Brazil; [8]Medicine, Mahidol University, SALAYA, Thailand; [9]Novartis Pharmaceuticals, EAST HANOVER, USA; [10]Comprehensive Cancer Center of Wake Forest University Baptist Medical Center, WINSTON-SALEM, USA

Background. Nilotinib, a potent and highly selective BCR-ABL inhibitor, has been approved in several countries including the US and Europe for the treatment of patients (pts) with Philadelphia chromosome-positive chronic myelogenous leukemia (Ph^+ CML) in chronic phase (CP) and accelerated phase (AP) who are resistant or intolerant to prior therapy including imatinib. The ENACT study, which is an open label, multicenter, ongoing study, was initiated to provide expanded access to nilotinib and obtain additional safety information in pts with imatinib-resistant or -intolerant CML- chronic phase (CP), -accelerated phase (AP), or -blast crisis (BC). *Methods*. Pts received nilotinib 400 mg twice daily (BID). Dose escalation was not permitted. Pts who required dose reduction to 400 mg once daily due to toxicity were allowed to have a dose re-escalation to 400 mg BID after resolution of the adverse events (AEs) ≤grade 1, lack of response, or persistent disease at the investigator's discretion. Efficacy assessment was not an objective of this study. *Results*. To date, more than 1600 patients have been enrolled, with results for the first 1056 pts (CP, n=818; AP, n=120; BC, n=118) included for this analysis. Median age of pts was 53 years, and 69% were imatinib-resistant. AE data are available for 974 pts. At data cut-off (June 30, 2007), 710 pts (67%) were continuing on nilotinib. Median duration of nilotinib exposure was 124 (1-522) days for CP pts, 90 (10-361) days for AP pts, and 54 (1-354) days for BC pts; median dose intensity was 800, 783, and 785 mg/day, respectively. The main reasons for discontinuation were inadequate responses (13%), and AEs (11%). The majority of AEs were hematologic, with the most common grade 3/4 hematologic toxicities being thrombocytopenia (22%) and neutropenia (13%). Non-hematologic AEs were mostly mild to moderate and included headache, rash, and nausea. Deaths were reported for 28 (3%) pts, and occurred more frequently among those with BC (n=15). A low incidence of QT prolongation (QTcF > 500 msec, n=2, 0.2%) was observed overall. *Conclusion*. This updated analysis of a large expanded access study further demonstrates that nilotinib is generally well tolerated in heavily pretreated pts in all phases of CML with safety profile reported in ENACT being similar to that observed in the pivotal phase II registration study.

Chronic myeloid leukemia / Myeloproliferative disorders

0135

DEVELOPMENT OF PROTOTYPE REFERENCE MATERIALS FOR BCR-ABL QUANTITATIVE RT-PCR

C.P. Cross,[1] A. Hochhaus,[2] M.C. Mueller,[2] G. Saglio,[3] S. Branford,[4] T.P. Hughes,[4] J. Gabert,[5] Y.L. Wang,[6] P. Metcalfe,[7] H.E. White[8]

[1]University of Southampton, SALISBURY, UK; [2]III Medizinische Klinik, MANNHEIM, Germany; [3]University of Turin, TURIN, Italy; [4]IMVS, ADELAIDE, Australia; [5]Université de la Méditerranée, MARSEILLES, France; [6]Weill Medical College of Cornell University, NEW YORK, USA; [7]NIBSC, SOUTH MIMMS, UK; [8]NGRL Wessex, SALISBURY, UK

Background. RQ-PCR is used routinely to quantify levels of BCR-ABL mRNA and thereby monitor the response of CML patients to treatment. Despite efforts to establish standardised protocols, there is still substantial variation worldwide in the way RQ-PCR for BCR-ABL is undertaken, what control gene is used for normalisation and how results are reported. An international scale (IS) has been established that is anchored to two key points defined in the IRIS trial: a common baseline (100% BCR-ABLIS) and major molecular response (0.1% BCR-ABLIS). Definition of the IS currently relies on relating results directly or indirectly to the Adelaide international reference laboratory. A more robust definition of the IS requires the development of internationally accredited reference reagents. *Aims*. To produce and assess the use of freeze dried cell lines and armored RNA constructs as candidate reference materials for the standardisation of BCR-ABL RQ-PCR protocols. *Methods*. Development of materials was co-ordinated by the National Genetics Reference Laboratory (Wessex) in conjunction with the National Institute of Biological Standards and Controls and Asuragen Inc. Field trial evaluation of the reagents was performed by 33 laboratories worldwide. (i) Freeze dried cell lines. In an initial assessment of 30 haemopoietic cell lines, HL60 and KG1 were found to express relative levels of BCR, ABL and GUSB (the three principal control genes used for normalisation of RQ-PCR results) that were closest to normal leukocytes and were therefore selected for further analysis and validation. Reference standards were prepared by diluting K562 cells (b3a2 BCR-ABL expressing cell line) into HL60 and KG1. Four levels of BCR-ABL in each negative cell line were produced and freeze dried at 3×10^6 cells/vial. The performance of the freeze dried cells was assessed by an international field trial (June - October 2007) that involved 14 laboratories (7 EU, 4 USA, 3 Asia/Australasia) using 7 different protocols and 9 different RQ-PCR platforms. (ii) Armored RNAs were produced for b2a2 and b3a2 BCR-ABL, plus BCR, ABL and GUSB as control genes and their performance was assessed in an international field trial (October-November 2007) that involved 29 laboratories (22 EU, 3 USA, 4 Asia/Australasia). Results RNA (median 30 μg/vial) was successfully extracted from freeze dried cell mixtures shipped worldwide at ambient temperature. The HL60/K562 and KG1/K562 cell mixes performed equally well producing coefficients of variation within and between laboratories that were comparable to those seen with patient samples. The aRNA trial showed good comparability of normalised %BCR-ABL/control gene results between laboratories. The reagents worked very well when directly heat lysed prior to cDNA synthesis but further modifications are required to ensure adequate yields following RNA extraction. *Summary*. Both freeze dried cells and armoured RNAs appear to be suitable for development of BCR-ABL reference reagents. We are planning large scale production of the freeze dried cell lines as 'higher order' internationally accredited reference materials. The armoured RNAs will undergo a further round of field trial evaluation with the aim of establishing them as secondary reference reagents.

0136

DASATINIB EARLY INTERVENTION AT CYTOGENETIC RATHER THAN HEMATOLOGIC RESISTANCE TO IMATINIB IS ASSOCIATED WITH MORE FAVORABLE RESPONSES

H. Kantarjian,[1] A. Quintas-Cardama,[1] S. O'Brien,[1] F. Ravandi,[1] G. Borthakur,[1] Y. Matloub,[2] D. Liu,[2] T.T. Chen,[2] J. Cortes[1]

[1]MD Anderson Cancer Center, HOUSTON; [2]Bristol-Myers Squibb, WALLINGFORD, USA

Background. In patients with chronic myeloid leukemia (CML) in chronic-phase (CP), development of resistance or intolerance to imatinib, or progression to advanced-phase disease, is associated with poor clinical outcome. Dasatinib, a BCR-ABL inhibitor that is 325-fold more potent than imatinib and 16-fold more potent than nilotinib *in vitro*, is an effective treatment for patients with CML in any phase or Philadelphia chromosome-positive acute lymphoblastic leukemia following imatinib resistance or intolerance. Treatment with dasatinib or other second-generation tyrosine kinase inhibitors is more effective when administered during CML-CP compared with during advanced-phase disease. Importantly, treatment after cytogenetic relapse on imatinib rather than after hematologic relapse, i.e. earlier intervention, is an independent prognostic factor for survival. *Aims*. To further assess the impact of early intervention with dasatinib following imatinib failure, clinical trial data in patients with imatinib-resistant CML-CP have been analyzed. The primary objective was to determine whether dasatinib treatment was associated with more favorable outcomes when administered following loss of major cytogenetic response (MCyR) on imatinib rather than following loss of both MCyR and complete hematologic response (CHR). *Methods*. Data from three phase II/III dasatinib clinical studies in imatinib-resistant CML-CP were analyzed retrospectively: CA180-013 (70 mg BID), CA180-017 (70 mg BID), and CA180-034 (100mg QD, 50 mg BID, 140 mg QD, 70 mg BID) (dasatinib 100 mg QD is now the approved dose for patients with CML-CP following imatinib failure). Assessments included complete cytogenetic response (CCyR) rate and progression-free survival (PFS). *Results*. The analysis identified 151 patients who received dasatinib treatment after confirmed loss of MCyR and 33 patients who received dasatinib after loss of both MCyR and CHR (n=33) during imatinib treatment. More than half of patients had received imatinib doses >600 mg/d and more than 65% had received imatinib for >3 years. Following dasatinib treatment, higher CCyR rates were achieved in patients treated after loss of MCyR (72%) than in those treated after loss of both MCyR and CHR (42%) (Table 1). Patients treated after loss of MCyR also had prolonged PFS (median PFS not reached) compared with those treated after loss of both MCyR and CHR (median PFS 18.5 months), with 24-month PFS rates of 89% and 29%, respectively. A third group of patients was identified who had confirmed loss of CHR (n=146). Among these patients, one quarter had also achieved MCyR but loss of MCyR was not recorded. Dasatinib was also effective after loss of CHR on imatinib, with a CCyR rate of 35% and a 24-month PFS rate of 64% (median PFS not reached) in this group. *Summary and Conclusions*. Dasatinib is an effective treatment for patients with CML-CP who have experienced imatinib failure. Response rates and PFS were improved when dasatinib was administered early after imatinib failure, i.e. after loss of MCyR rather than after loss of both MCyR and CHR. To increase treatment benefits associated with dasatinib, early intervention (at loss of MCyR) is recommended and close monitoring of response during imatinib treatment is required to identify when resistance first develops.

Table 1. Achievement of CCyR by patients treated dasatinib after loss of response to prior imatinib.

	Loss of MCyR		Loss of MCyR and CHR		Loss of CHR	
	n/N	%	n/N	%	n/N	%
All patients	108/151	72	14/33	42	51/146	35
70 mg BID	87/123	71	13/32	41	49/132	37
100 mg QD*	21/28	75	1/1	100	2/14	14
Study-specific data						
CA180-013	36/47	77	6/17	35	30/71	42
CA180-017	12/19	63	1/2	50	6/22	27
CA180-034						
50 mg BID	11/17	65	1/2	50	3/12	25
70 mg BID	19/23	83	3/6	50	2/11	18
100 mg QD	21/28	75	1/1	100	2/14	14
140 mg QD	9/17	53	2/5	40	8/16	50

*From study CA180-034

0137

RESPONSE TO NILOTINIB IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS IN CHRONIC PHASE (CML-CP) ACCORDING TO BCR-ABL MUTATIONS AT BASELINE

J. Radich,[1] D.-W. Kim,[2] G. Martinelli,[3] S. Soverini,[3] S. Branford,[4] M. Mueller,[5] A. Haque,[6] Y. Shou,[6] A. Hochhaus,[5] T. Hughes,[4] G. Saglio[7]

[1]Fred Hutchinson Cancer Research Center, SEATTLE, USA; [2]Catholic University of Korea, SEOUL, South-Korea; [3]University of Bologna, BOLOGNA, Italy; [4]Hanson Institute Centre for Cancer, ADELAIDE, Australia; [5]Medizinische Klinik III, MANNHEIM, Germany; [6]Novartis Pharmaceuticals, EAST HANOVER, USA; [7]University of Turin, TORINO, Italy

Background. Nilotinib (Tasigna®) is a potent and highly-selective BCR-ABL inhibitor approved in the US and Europe for the treatment of patients (pts) with Philadelphia chromosome-positive (Ph^+) chronic myeloid leukemia (CML) in chronic phase (CP) and accelerated phase (AP) who are resistant or intolerant to prior therapy including imatinib. *Methods*. This subanalysis of a phase 2 study of nilotinib in imatinib-resistant or-intolerant CML-CP pts assessed the occurrence of baseline BCR-ABL mutations and their impact on treatment outcome following 12 months of therapy. The BCR-ABL kinase domain (amino acid 230-490) was amplified and mutations identified by direct sequencing that allowed for detection of > 20% minor alleles. *Results*. Of the 275 pts with mutation data available, 113 (41%) had BCR-ABL mutations at initiation of nilotinib therapy. Amongst imatinib-resistant patients, the frequency of mutations at initiation was 56%. After 12 months of therapy, major cytogenetic response (MCyR) was achieved in 60%, complete cytogenetic response (CCyR) in 42%, and major molecular response (MMR) in 25% of pts without baseline mutations, *vs* 50%, 32%, and 21% of pts with mutations, respectively, among the imatinib-resistant pts. Response rates in pts with mutations of ≤150 nM cellular IC50 (*M244V, L248V, G250E, Q252H, E275K, D276G, F317L, M351T, E355A, E355G, L387F, F486S*) and with mutations of unknown IC50 values had comparable response rates to those for pts without baseline mutations: MCyR were 62% and 58%, respectively, and CCyR were 40% and 42%, respectively. For patients (n=26) with less sensitive BCR-ABL mutations (*in vitro* cellular IC50 > 150 nM), MCyR was achieved in 1 of 8 pts (13%) with Y253H, 3 of 8 pts (38%) with E255K/V, and 1 of 10 pts (10%) with F359C/V. None of these patients achieved CCyR. *Conclusions*. A significant proportion of imatinib-resistant pts with BCR-ABL mutations at baseline respond to imatinib. Pts with sensitive BCR-ABL mutations exhibited response rates on nilotinib therapy comparable to those for patients with no baseline mutations. Less sensitive mutations represented <15% of the cohort and may be associated with less favorable responses. Larger cohort and longer follow-up are required to validate these findings.

0138

EPIDEMIOLOGICAL STUDY ON SURVIVAL OF CHRONIC MYELOID LEUKEMIA (CML) AND DE NOVO PH+ ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS WITH T315I MUTATION - A PRELIMINARY ANALYSIS

D.W. Kim,[1] W. Zhou,[2] G. Martinelli,[3] C. Chuah,[4] F. Yagasaki,[5] I. Dufva,[6] S. Peter,[2] M. Mauro,[7] F. Giles,[8] J. Cortes,[9] J. Pearson,[2] J. Goldman,[10] F. Nicolini[11]

[1]St. Mary's Hospital, The Catholic University of Korea, SEOUL, South-Korea; [2]Department of Epidemiology, Merck Research Laboratories, NORTH WALES, PA, USA; [3]Institute of Hematology and Medical Oncology „Seràgnoli„, University of Bologna, BOLOGNA, Italy; [4]Department of Hematology, Singapore General Hospital, SINGAPORE, Singapore; [5]First Department of Internal Medicine, Saitama Medical School, SAITAMA, Japan; [6]Department of Haematology, Herlev Hospital, University of Copenhagen, HERLEV, Denmark; [7]Oregon Health & Science University, Center for Hematologic Malignancies, PORTLAND, OR, USA; [8]Cancer Therapy & Research Center, Institute for Drug Development, ANTONIO, TX, USA; [9]Department of Leukemia, The University of Texas M.D. Anderson Cancer Center, HOUSTON, TX, USA; [10]Haematology Department, Hammersmith Hospital, Imperial College London, LONDON, UK; [11]Hematology Department, Hôpital Edouard Herriot, LYON, France

Background. T315I mutation is one of the major mechanisms of resistance to Imatinib and 2nd generation tyrosine kinase inhibitors (TKIs) and may be associated with poor survival. *Aims*. The objective of this study was to estimate overall survival (OS) and progression-free survival (PFS) for CML and Ph^+ ALL patients with T315I mutation. *Methods*. This is a retrospective, multi-center epidemiological study. Eligible patients were CML and Ph^+ ALL patients who were identified as possessing T315I mutation between the years 1999 and 2007. The medical records of approximately 200 patients are being abstracted for detailed clinical, treatment, mutation and survival information. Data on 62 patients from Korea, Italy, Singapore, USA, Denmark, and Japan were included in this preliminary analysis. Kaplan-Meier curves were used for survival analysis. Patients who received any new investigational drug were censored at the time it was started. *Results*. For the 62 patients, 24 were female, and the median age at T315I mutation detection was 42 years (range 19-76). Thirty seven patients were Asian, 24 were Caucasian, and 1 was multi-racial. At the time of diagnosis, 48 patients were in chronic phase (CP), 4 in accelerated phase (AP), 4 in myeloid or lymphoid blastic crisis (BC), and 6 were *de novo* Ph^+ ALL. All 6 *de novo* Ph^+ ALL patients were from one site in Italy. Nineteen patients had received IFN and 1 had stem cell transplantation prior to treatment with TKIs. Median time from diagnosis to onset of hematologic or cytogenetic resistance to TKI (using European Leukemia Network criteria) was 23 months (range 3-132), and to first detection of the T315I mutation was 34 months (range 3-164). Twenty-two CP patients progressed to AP or BC at the time of TKI resistance onset (Table 1).

Table 1. Overall survival and progression-free survival of CML and Ph+ ALL patients with T315I mutation.

Starting date: 1st time of TKI resistance*				
Disease phase	**Chronic (N=26)**	**Accelerated (N=15)**	**Blastic** (N=14)**	**Ph+ ALL (N=6)**
Median OS month	53.9	20.1	8.5	8.7
6-month OS rate	88%	92%	50%	75%
Median PFS month	17.0	5.3	3.5	3.2
6-month PFS rate	68%	47%	36%	25%
Starting date: 1st time of T315I mutation detection				
Disease phase	**Chronic (N=22)**	**Accelerated (N=15)**	**Blastic** (N=19)**	**Ph+ ALL (N=5)*****
Median OS month	21.2	Not reached	2.1	Not reached
6-month OS rate	73%	86%	18%	53%
Median PFS month	9.5	Not reached	2.0	0.9
6-month PFS rate	64%	64%	8%	20%

*: One missing data **Myeloid or lymphoid blastic crisis. ***One patient received new investigational drug before T315I mutation and was censored before the starting date.

After TKI resistance onset, 37 patients were on Imatinib, and 47 on 2nd generation TKIs. Fifteen patients were checked for mutation before the onset of TKI resistance, and 24 patients were checked for mutation within 3 months of TKI resistance onset. The median time between TKI resistance and T315I mutation was 7.2 months for CP, 4.4 months for AP, 0.8 month for BC, and 1.2 month for *de novo* Ph^+ ALL. At the 1st time of T315I mutation detection, T315I was the predominant clone in 50 patients, 20 patients also had other mutations (7 patients had P-loop mutations), and 24 patients had developed other mutations before T315I detection. OS and PFS from 1st time of TKI resistance and T315I mutation detection are summarized in Table 1; the median OS follow-up time was 17.5 months (range 0.2-40.6) from T315I mutation. Conclusion. These preliminary results suggest that survival of patients harboring a T315I mutation depended on the disease phase at the onset of TKI resistance or T315I mutation. T315I mutation was usually detected within a short period of time after TKI resistance, especially for patients in the advanced phases. These results will be verified when data collection is completed for the patient cohort.

0139

EFFICACY OF SUBCUTANEOUS HOMOHARRINGTONINE IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS RESISTANT TO TYROSINE KINASE INHIBITORS (TKI) AND HARBORING A BCR-ABL MUTATION

D. Marin,[1] F. Maloisel,[2] L. Legros,[3] F.E. Nicolini,[4] L. Roy,[5] P. Rousselot[6]

[1]Imperial College London, LONDON, UK; [2]CHU Hospice Civil, STRASBOURG, France; [3]CHU Hôpital Archet, NICE, France; [4]CHU Hôpital Edouard Herriot, LYON, France; [5]CHU Hôpital Jean Bernard, POITIERS, France; [6]Hôpital A. Mignot, LE CHESNAY, France

Background. Omacetaxine [OMA, semi-synthetic homoharringtonine] is an alkaloid active on CML cells by inhibiting protein synthesis and inducing the intracellular signals for apoptosis via mitochondrial release of cytochrome C and activation of caspases. *In vitro* studies demonstrated that OMA activity is not affected by the presence of BCR-ABL kinase domain (KD) mutations. *Methods*. We present here a retrospective analysis of the clinical experience acquired in 6 institutions for the SC OMA treatment of CML patients (pts) with at least one BCR-ABL KD mutation and various degrees of resistance to TKI therapy. *Results*. From February 2003 to August 2007, 20 patients fulfilled these criteria. All pts received one or multiple lines TKI therapy (IM 400-800 mg: n=19; dasatinib 140-200 mg: n=5; nilotinib: n=1), 14 patients had also been treated with interferon (IFN - n=7), SCT (n=3), or combined IM+ara-c (n=1), IM+IFN (n=1), IFN+ara-c (n=2). Prior to OMA, baseline characteristics were: mean age 56.9 years (111.9; range 26-74), sex ratio 9F/11M, mean disease duration 72 months (±41, range: 22-148). CML status was: CP with CHR with or without cytogenetic response (n=5; group A), CP without CHR (n=7; group B), AP (n=6; group C), or blast phase (BP - n=2; group D). KD mutation status included 15 pts with a single mutation (*M244V: 3; V244Q: 1; G250E: 1; Q252H: 1; Y253H: 2; T315I: 4; M351T: 1; F359V: 1; E453K: 1*), and 5 pts with combined mutations (*K247R+F317L; T315I+V379I; Y253F+M351T; F311I+T315I; Y253H+T315I*). This series includes so 12 pts harboring pejorative mutations: T315I (n=6), P-loop (n=5), or a combination of both (n=1). SC OMA was administered at 2.5 mg/m^2/day, for 1 to 6 days when combined with IM (n=7: 3 pts from group A and 4 pts from group B), and for up to 14 days when administered as monotherapy. In median, 5 OMA courses were administered (range: 1-20). In group A, all 5 patients (100%) maintained CHR. Of them, 1 pt with previous complete CyR improved his molecular response by 1.26 log reduction, 1 pt with partial CyR achieved complete CyR and improved his molecular response by 1.67 log reduction, and the T315I mutation was no longer detectable after 3 cycles in 2 additional pts. In group B, 5 pts (71%) achieved CHR (P-loop mutation: 2), and 2 pts (29%) did not respond. In group C, 3 pts (50%) achieved CHR, of them 2 pts with the T315I mutation also achieved a CyR (1 complete, 1 minor); 1 pt (17%) with T315I+V379I achieved NEL; and 2 pts (33%) with P-loop mutation did not respond. In group D, the 2 BP pts did not respond (1 T315I, 1 combined T315I+P-loop). Grade 3-4 myelosuppression was reported for 12 pts. OMA was otherwise well tolerated and led to discontinuation of treatment in 1 pt (grade 2 asthenia - combined OMA+IM). Conclusion: OMA is active in BCR-ABL mutated patients and so may be considered as an option for these patients when loosing sensitivity to TKI therapy. Ongoing prospective clinical trials are confirming its role for these patients.

0140

SINGLE NUCLEOTIDE POLYMORPHISMS WITHIN THE ABL KINASE DOMAIN OF CML PATIENTS - PITFALLS IN THE DETECTION OF RELEVANT BCR-ABL MUTATIONS

T. Ernst,[1] M.C. Müller,[2] J. Hoffmann,[2] P. Erben,[2] B. Hanfstein,[2] A. Leitner,[2] R. Hehlmann,[2] A. Hochhaus[2]

[1]Wessex Regional Genetics Laboratory, SALISBURY, UK; [2]III. Medizinische Klinik, Universitätsklinikum Mannheim, MANNHEIM, Germany

Background. The most frequently identified mechanism of acquired imatinib resistance in patients with chronic myeloid leukaemia (CML) is the emergence of point mutations within the BCR-ABL kinase domain. To date, more than 70 different mutations have been described. The BCR-ABL K247R change has been revealed as a rare single nucleotide polymorphism (SNP) occurring likewise in healthy controls and non-hematological cell types. Despite its juxtaposition to the P-loop, biochemical and cellular assays of imatinib and dasatinib sensitivity showed no alteration compared to non-mutated BCR-ABL. *Aims*. Since gene polymorphisms do not automatically necessitate a change in the therapeutic strategy, we systematically analysed if other changes in the BCR-ABL kinase domain should be considered as SNPs rather than acquired mutations. *Methods*. A total of 911 CML patients (501 m, 410 f; median age 58.1 years, range 15.5-85.3) after failure or suboptimal response to imatinib were screened for BCR-ABL kinase domain mutations by denaturing high-performance liquid chromatography (D-HPLC) and direct sequencing. Patients were in chronic phase (n=587), accelerated phase (n=151), myeloid (n=103), or lymphoid blast crisis/ALL (n=70). In comparison to acquired BCR-ABL kinase domain mutations, SNPs also have to occur in normal, untranslocated ABL alleles. Therefore, SNP analysis was based on the search for nucleotide changes in corresponding normal ABL alleles by ABL allele-specific PCR and subsequent D-HPLC/sequencing analysis. Nucleotide changes in normal ABL alleles were confirmed by cloning experiments. *Results*. BCR-ABL kinase domain mutations were detected in 456/911 patients (50%). Eighty-three different mutations affecting 60 amino acids were detected of those six nucleotide changes were uncovered as SNPs (GenBank accession No. U07563): 58758G/A (T240T, n=1), 58778A/G (K247R; n=9), 68708T/G (F311V, n=2), 68722T/G (T315T, n=1), 68736A/G (Y320C, n=1), and 74901A/G (E499E; n=73). A second patient harboring the substitution 68708T/G (F311V) in BCR-ABL alleles did not show any nucleotide change in normal ABL alleles. In this cohort of patients, the 74901G (E499E) change was the most common polymorphism with an allele frequency of 8.0%. The 58778G (K247R) polymorphism had a frequency of 1.0%, whereas the nucleotide changes 58758A, 68722G, and 68736G were detected once only leading to an allele frequency of 0.1% each. *Summary/conclusions*. The vast majority of BCR-ABL kinase domain mutations are acquired mutations and not SNPs. In addition to the previously described K247R polymorphism we uncovered five new SNPs, two of them led to amino acid changes. SNPs could theoretically contribute to primary but not to secondary resistance to tyrosine kinase inhibitors and must therefore be distinguished from acquired mutations. Novel point mutations should be confirmed by analysing the normal ABL alleles to exclude polymorphisms. Pre-existent polymorphisms not representing acquired mutations do not directly require a change in therapeutic strategy, unless there are indications of inadequate response to treatment.

0141

MINIMAL CROSS-INTOLERANCE BETWEEN NILOTINIB AND IMATINIB IN PATIENTS WITH IMATINIB-INTOLERANT CHRONIC MYELOGENOUS LEUKAEMIA (CML) IN CHRONIC PHASE (CP) OR ACCELERATED PHASE (AP)

A. Hochhaus,[1] H.M. Kantarjian,[2] M. Baccarani,[3] P. Le Coutre,[4] A. Haque,[5] N. Gallagher,[5] J. Cortes,[2] F. Giles[6]

[1]Med. Fakultät Mannheim der Universität Heidelberg, MANNHEIM, Germany; [2]MD Anderson Cancer Center, HOUSTON, USA; [3]Institute of Hematology and Medical Oncology Seragnoli, University of Bologna, BOLOGNA, Italy; [4]Campus Virchow, Charité, Humboldtuniversität, BERLIN, Germany; [5]Novartis Pharmaceuticals, EAST HANOVER, USA; [6]University of Texas Health Science Center, SAN ANTONIO, USA

Background. Nilotinib, a potent and highly selective BCR-ABL inhibitor, has been approved in several countries including the US and Europe for the treatment of patients (pts) with Philadelphia chromosome-positive chronic myelogenous leukaemia (Ph+ CML) in chronic phase (CP) and accelerated phase (AP) who are resistant or intolerant to prior therapy including imatinib. The current study examines cross-intolerance between nilotinib and imatinib among CP and AP pts enrolled in the phase 2 registration study of nilotinib. *Methods*. Imatinib-intolerance was defined as no prior major cytogenetic response (MCyR) and discontinuation of imatinib due to Grade 3/4 adverse events (AEs) or persistent (> 1 month) or recurrent (recurred > 3 times) Grade 2 AEs, despite optimal supportive care. Nilotinib-imatinib cross-intolerance was defined as treatment with nilotinib and occurrence (regardless of causality) of Grade 3/4, or persistence or recurrent Grade 2, of the same AE(s) that led to discontinuation of imatinib therapy. The planned starting dose of nilotinib was 400 mg twice daily (BID), which could be escalated to 600 mg BID for lack of response and in the absence of safety concerns. Patients with multiple AEs due to imatinib are counted for each AE. *Results*. 94 CP and 27 AP imatinib-intolerant pts were included in this analysis and the median duration of prior imatinib therapy were 14 and 9 months, respectively. The majority of pts received <600 mg/day of imatinib (66% in CP, 67% in AP). The median duration of nilotinib therapy in this cohort of pts was 13 and 5 months with median dose intensity being 701 and 755 mg/day in CP and AP pts, respectively. Although dose interruption occurred in 70% of CP and 56% of AP pts, the medi-

an percentage of days with dose interruption was only 6% and 7% of the total time on nilotinib, respectively. Of the 57 imatinib-intolerant CP pts with nonhaematological AEs, only 1 discontinued nilotinib due to cross intolerant AE (AST elevation). Of the 29 pts with haematological AEs, 7 discontinued nilotinib (thrombocytopenia). No AP pts discontinued nilotinib due to cross intolerance. Nilotinib was also efficacious in both imatinib-intolerant CML-CP pts (n=59; 63%) and CML-AP pts (n=10; 40%) who achieved MCyR. *Conclusions*. These results are consistent with previously reported findings of minimal nilotinib cross-intolerance in imatinib-intolerant CML-CP and CML-AP pts. Thrombocytopenia emerged as the only imatinib-intolerant laboratory abnormality leading to intolerance that may recur in some pts during nilotinib therapy. In the pivitol phase II registration study, nilotinib has been proven to be effective in Ph^+ CML patients with imatinib-resistance and intolerance. Nilotinib represents an important therapeutic option in CML patients with imatinib-intolerance.

0142

A NEW ASSOCIATION BEETWEN THE ALLELIC JAK2V617F BOURDEN AND THE PLATELET VOLUME IN POLYCYTEMIA VERA (PV) AND ESSENTIAL THROMBOCYTHEMIA (ET)

M. Sobas,[1] M. Pérez-Encinas,[1] C. Quinteiro,[2] T. González,[2] E. Ansoar,[1] S. Ordoñez,[1] J.L. Bello[1]

[1]Hospital Clínico Universitario de Santiago de Compostela, SANTIAGO DE COMPOSTELA; [2]Fundación Galega de Medicina Xenómica, SANTIAGO DE COMPOSTELA, Spain

Background. A differentiation between thrombocytosis caused by a PV, an ET, or a reactive disorder (RD) seems to be easier since the discovering of V617F-JAK2 mutation. However the distinction between an early phase of PV with thrombocytosis and the ET JAK2+ is not possible with the recently published criteria (WHO 2008). The platelet distribution width (PDW) and the mean platelet volume (MPV) are very simple tests useful for the differential diagnosis of thrombocytosis (*Van der Lelie J Clin Pathol 1986*). *Aims*. We evaluated the useful of PDW and MPV in the context of the new criteria diagnostic for PV and ET, in especial to try a better distinction between an early phase of PV and the ET JAK2+. *Methods*. We studied 16 PV and 89 ET patients with a platelet count ≥450×10^9/L diagnosed according the WHO 2008 criteria and followed-up in our Department. We used the JAK2 MutaScreenTMKit (IPSOGEN) for quantification of V617F mutation. MPV and PDW were made in whole blood anticoagulates with edetic acid using the Coulter LH 750 Analyzer. Only patients with a platelet volume analysis previous to treatment with hydroxiurea were included. *Results*. First, we confirmed that MPV and PDW value are higher in PV and ET respect to reactive disorders RD. Comparison of values of MPV and PDW showed a significantly higher PDW (17,75) and MPW (8,73 fl) in PV patients respect to ET patients (PDW (17,11; MPW 7,71 fl). Then we found a higher value of the platelet volume in the PV in comparison with the ET JAK2 +, and when we compared the platelet volume between the ET V617F-JAK2 positive and negative patients. Finally, when we created 3 groups of MPNs: negative with <2% of V617F mutation, between 2-31% and 31-100%, we have observed a statistically significant relation between the allelic ratio of V617F and MPV and PDW in MPNs patients: MPV and PDW increase with higher allelic ratio of V617F mutation. *Conclusions*. We confirm previous reports about the useful of PDW and MPV analysis in differential diagnosis of thrombocytosis. PV patients with thrombocytosis present significantly higher value of PDW and MPV that in ET. We first report the correlation between the JAK^+ status and the PDW and MPV in MPNs, that seem be independent of the platelet count. The study of the combination of *JAK2* status with the platelet volume analysis in a larger cohort of patients could help to do a easy index for the differential diagnosis between the PV and the ET.

Table 1.

	V617F	*Number*	*Mean*	*SO*	*p*
Platelet distribution width	Negative (<2%)	34	16,7794	,99690	,000
	2-31%	56	17,2429	,69252	
	>31%	13	17,7611	,56256	
	Total	103	17,1933	,55593	
Mean distribution volumes	Negative (<2%)	34	7,415	,6861	,000
	2-31%	57	7,904	,6452	
	>31%	18	8,844	,9031	
	Total	109	7,907	,9306	

0143

DECISIONAL FLOW WITH A SCORE SYSTEM TO START PLATELET-LOWERING TREATMENT IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET): LONG-TERM RESULTS

R. Latagliata,[1] A. Rago,[1] A. Spadea,[2] C. Santoro,[1] I. Carmosino,[1] M. Breccia,[1] L. Napoleone,[1] A. Fama,[1] F. Biondo,[1] P. Volpicelli,[1] M.C. Petti,[2] G. Alimena,[1] M.G. Mazzucconi[1]

[1]Hematology- University La Sapienza, ROMA; [2]Hematology, Regina Elena IFO, ROMA, Italy

Background. Treatment of ET is still a matter of debate for the difficulty in deciding between platelet(plt)-lowering agents and a *wait and see* policy with anti-aggregating agents only: a main problem is to identify what patients are *at risk* to develop severe thrombotic events during the course of the disease. *Design and Methods*. We prospectively tested in ET patients at diagnosis with no clear indication to plt-lowering treatment a score system based on the following 6 variables: age, plt level, cardiovascular diseases, previous thrombotic events, smoking, dismetabolic diseases: the score was reassessed in each patient every 3 months during follow-up. *Results.* From 04/92 to 03/98, 168 consecutive adult patients (57 males and 111 females, median age 59.5 yrs, range 20.5 - 84.8, median plt value 1,010×10^9/L, range 587-2,714) with ET according to PVSG criteria were diagnosed at our Institution. Among them, a plt-lowering treatment was started in 32 patients considered *symptomatic* for the presence at diagnosis or within 6 months before of severe haemorrhages, vascular accident or severe disturbances of microcirculation; plt-lowering treatment was also started in all the 33 patients aged > 70 years at diagnosis. The remaining 103 patients were a*symptomatic* and aged <70 years and were classified according to our score system: thirty-two patients with a score ≥4 started plt-lowering treatment with hydroxyurea early after diagnosis. The remaining 71 patients had a score < 4 at diagnosis and received anti-aggregating agents only: of them, 24 (33.8%) started plt-lowering treatment during follow-up (18 for a score increase to ≥4 and 6 for the occurrence of thrombotic complications or symptoms) after a median time from diagnosis of 28 months (range 3-130) while 47 (66.2%) did not start any plt-lowering treatment. Thrombotic complications occurred in 9/103 patients (8.7%) scored at diagnosis: in particular, they occurred in 4/32 patients (12.5%) with score ≥ 4 receiving plt-lowering treatment since diagnosis and in 5/71 patients (7%) with score <4 under anti-aggregating agents only. Overall, 6 out of 103 scored patients died and 27 were lost to follow-up, with a 15-year cumulative survival of 88%. *Summary*. In conclusion, a decisional flow based on present score system appears effective in patients without a clear indication for plt-lowering treatment at diagnosis to discriminate subjects at different risk of thrombotic events and could be useful to decide when a plt-lowering therapy needs to be started.

0144

JAK2 MUTATION AND AN ADDITIONAL THROMBOPHILIC STATE ARE MAJOR PROTHROMBOTIC RISK FACTORS IN MYELOPROLIFERATIONS WITH THROMBOCYTHEMIA - DATA FROM A REGISTRY OF ANAGRELIDE-TREATED PATIENTS

J. Schwarz,[1] M. Penka,[2] M. Doubek,[3] Y. Brychtova,[3] O. Cerna,[4] P. Dulicek,[5] J. Kissova,[2] T. Pavlik[6]

[1]Institute of Hematology and Blood Transf, PRAGUE; [2]Dept. of Clinical Hematology, Masaryk University Hospital, BRNO; [3]Dept. of Internal Medicine/Hematooncology, Masaryk University Hospital, BRNO; [4]Dept. of Clinical Hematology, Charles University Hospital Kralovske Vinohrady, PRAGUE; [5]Dept. of Internal Medicine II. Dept. of Clinical Hematology, Faculty Hospital, HRADEC KRALOVE; [6]Center for Biostatistics and Analyses, Masaryk University, BRNO, Czech Republic

Background aim of the study. We have evaluted prospectively collected data of 336 Czech patients from an international registry of patients treated with anagrelide (ANG) for thrombocythemia in the setting of a myeloproliferative disease (MPD-T). *Patients*. The initial diagnoses (recorded largely according to PVSG criteria) were the following: essential thrombocythemia - 274 (81.5%), idiopathic myelofibrosis - 26 (7.8%), polycythemia vera - 24 (6.7%) and chronic myeloid leukemia - 3 (0.9%). The mean age at diagnosis and at database entry was 47 and 50 years (means: 45.3 and 49.1). The male/female ratio was 37:63%. A vast proportion of patients (80.7%) had been already pretreated with thromboreducing drugs: overall, 67 patients had received ANG, 121 had had interferon-alpha, 223 had been treated with hydroxyurea and 54 patients had received another drug. More than one line of previous thromboreductive therapy was recorded in 146 (43.5%) patients. Follow-

ing registration, 24.1% was receiving another thromboreducing drug in addition to Thromboreductin (ANG brand). The median platelet value was 1015 G/L at diagnosis, and 613 G/L (range 140-3325) at database entry. The treatment goal was achieving 600/400 G/l platelets in low-/high-risk patients. High-risk patients had either previous thrombosis, an additional thrombophilic state (mostly inherited), or JAK2 gene V617F mutation. *Results*. Pretreated and unpretreated patients responded to ANG equally well. In the latter, (n=65; median platelet count of 946 G/Lat day 0), the platelet counts at 3-6-9-12-36 months reached 565-444-451-421-441 G/L, respectively. High-risk patients had lower platelet counts (488-400-387-376-323 G/L; n=118) than good-risk ones (523-441-384-423-455 G/l; n=98) at the given above time points. The mean ANG dose at 3 months was only 1.8 mg/day (median, 1.5 mg/d), whereas onwards, the mean dose was quite stable within 2.2-2.3 mg/day interval (median, 2.0 mg/d). At the time of database entry, 65 (19.3%) patients had a history of a thrombotic event, and 30 (8.9%) had had hemorrhage. During the follow-up after entering the registry, only 11 (3.5%) patients had thrombosis (6 had a major and 5 a minor one). Likewise, there have been very few patients with a bleeding event reported: 2 patients experienced a major and 4 patients a minor event (altogether 1.9% of patients). Only 106 patients had the JAK2 mutation evaluated. Half of them (53 patients) carried the mutation (hetero-/homozygous). Of the 53 patients with the mutation, 21 (39.6%) had thrombosis (before or after registration). Thrombosis was significantly more rare in 53 patients without the mutation, in 9 cases only (17.0%; p=0.017, Fisher´s test, CI 95%). Of 50 evaluable patients with thrombosis, 19 (38.0%) were thrombophilia+, whereas of 147 patients without thrombosis, a thrombophilia+ state was demonstrated only in 31 cases (21.1%; p= 0.024). *Discussion and conclusions*. The database enables analyses of treatment tactics; e.g. it has shown that high-risk patients on ANG do not achieve the desired level of thromboreduction at 3 months. Analysis of thrombotic events confirmed that JAK2 mutation or additional thrombophilic states are major risk factors in MPD-T.

0145

ABSENCE OF THE JAK2 EXON 12 MUTATIONS IN PATIENTS WITH SPLANCHNIC VENOUS THROMBOSIS AND WITHOUT OVERT CHRONIC MYELOPROLIFERATIVE DISORDERS

A. Fiorini, E. Rossi, T. Za, A. Ciminello, P. Chiusolo, S. Sica, G. Leone, V. De Stefano

Institute of Hematology, Catholic University, ROMA, Italy

Background. Thrombosis of major abdominal veins has been reported in 5% to 10% of the patients with polycythemia vera (PV) or essential thrombocythemia (ET). Conversely, molecular hallmarks of chronic myeloproliferative disorders (CMD) can be recognized in a substantial portion of patients with splanchnic venous thrombosis not meeting all the criteria for diagnosis of PV or ET. We have previously reported the presence of the JAK2 V617F mutation in the absence of overt signs of CMD in 21% of overall patients with splanchnic venous thrombosis and in 43% of patients with extrahepatic portal vein thrombosis (*De Stefano et al, J. Thromb. Haemost. 5: 708, 2007*). Recently additional *JAK2* exon 12 mutations have been reported in PV patients *JAK2* V617F-negative (*Scott et al., N. Engl. J. Med. 356: 459, 2007*). *Aims*. The present study is aimed to investigate the prevalence of the JAK2 exon 12 mutations among patients with splanchnic venous thrombosis and without overt CMD. *Methods.* We investigated JAK2 exon 12 mutations in 42 patients (M/F 21/21) with splanchnic venous thrombosis and without overt CMD who had been previously tested for the JAK2 V617F mutation and resulted negative. The median age at the thrombotic event was 44 years (range 18-79). Thrombosis involved the extrahepatic portal vein in 20 patients, the superior mesenteric vein in 14, the hepatic veins in 7, and the splenic vein in 1. DNA samples was obtained from peripheral blood granulocytes. We used allele-specific PCR according to Scott *et al.* for screening JAK2 exon 12 mutations. We tested 41 patients for the F537-K539delinsL mutation, 37 for H538QK539L, 40 for K539L, and 35 for N542-E543del. *Results.* We did not find any mutation of the JAK2 exon 12 in this sample of patients with abdominal thrombosis and JAK2 V617F negative. *Conclusions.* Apparently the *JAK2* exon 12 mutations are not frequently detectable in patients with splanchnic venous thrombosis, unlike the *JAK2* V617F mutation. However, given the rarity of the *JAK2* exon 12 mutations as cause of PV, further multicenter studies on larger samples of patients are needed to confirm this finding.

0146

BLOOD P50 EVALUATION ENHANCES DIAGNOSTIC DEFINITION OF ISOLATED ERYTHROCYTOSIS

E. Rumi,[1] F. Passamonti,[1] L. Pagano,[2] M. Ammirabile,[2] L. Arcaini,[1] C. Elena,[1] A. Flagiello,[3] R. Tedesco,[3] C. Vercellati,[4] A.P. Marcello,[4] D. Pietra,[1] R. Moratti,[5] M. Cazzola,[1] M. Lazzarino[1]

[1]Department of Hematology, Fondazione IRCCS Policlinico San Matteo, Pavia, PAVIA; [2]U.O.C. Microcitemia, Dipartimento Oncoematologia; A.O.R.N.A. Cardarelli, NAPOLI; [3]CEINGE Biotecnologie Avanzate, NAPOLI; [4]Department of Hematology, Fondazione IRCCS Policlinico Mangiagalli Regina Elena, MILANO; [5]Department of Clinical Chemistry, Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy

Background. High oxygen-affinity hemoglobin variants and 2,3-diphosphoglycerate (2,3-DPG) deficiency are inherited diseases generating low tissue oxygen tension and erythropoietin-driven erythrocytosis that compensates for hypoxia. Level of blood p50 is used to recognize these conditions, which are characterized by the presence of isolated erythrocytosis. However, more common diseases share this clinical finding, such as secondary erythrocytosis, polycythemia vera and idiopathic erythrocytosis. *Aims*. In order to enhance diagnostic definition of patients with isolated erythrocytosis, we evaluated venous blood p50 level in 102 consecutive patients with isolated erythrocytosis. *Methods*. The blood gas analyzer used (Radiometer ABL 800 Flex) was equipped with the FLEXQ module to automatically identify p50 value (normal range: 25 to 29 mmHg). Besides p50 evaluation, the initial diagnostic work-up included blood cell count, arterial oxygen saturation, serum erythropoietin measurement, screening for JAK2 mutations. WHO criteria were applied for polycythemia vera. Diagnosis of idiopathic erythrocytosis implied exclusion of secondary erythrocytosis and polycythemia vera. Patients with low level of p50 were further studied for high oxygen-affinity hemoglobin variants and 2,3-diphosphoglycerate (2,3-DPG) deficiency. Samples for molecular analysis were obtained after patient provided written informed consent. *Results*. Seven patients had relative erythrocytosis. Among 95 patients with absolute erythrocytosis, 4 (4.2%) had decreased p50 level. The extended study of family members revealed a familial inheritance. These patients were investigated for hemoglobin variants and deficiency of 2,3-DPG. Family 1 had Hemoglobin Malmö; Family 2 had Hemoglobin San Diego. In Family 3 the proband had a new high oxygen-affinity hemoglobin variant (Hemoglobin Safi) resulting from the transversion C'A at codon 81 of the β2-globin gene. In Family 4 a deficiency of 2,3 diphosphoglycerate mutase was found. Within the 91 patients with normal p50 values, 46 (51%) had secondary erythrocytosis, 13 (14%) polycythemia vera, and 32 (35%) idiopathic erythrocytosis. *Conclusions*. This study suggests to investigate blood p50 level in patients with isolated erythrocytosis.

0147

TREATMENT RESPONSE AND COMPLICATIONS OF PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA RECEIVING HYDROXYUREA

D. Sotiropoulos,[1] A. Papalexandri,[1] P. Tsirigotis,[2] A. Pouli,[3] P. Kyriazopoulos,[4] M. Protopapa,[5] I. Dervenoulas,[2] A. Fassas,[1] A. Anagnostopoulos[1]

[1]G Papanikolaou Hospital, THESSALONIKI; [2]Attikon Hospital, ATHENS; [3]St. SAVVAS, ATHENS; [4]Patision hospital, ATHENS; [5]Hospital of Serres, SERRES, Greece

Essential thrombocythemia (ET) is a myeloproliferative disorder characterized by persistent thrombocytosis. The frequency of JAK2V617F mutation is estimated at approximately 50% of the patients. Thrombotic episodes and bleeding complications are the most frequent events. Secondary malignancies can also occur. Hydroxyurea, anagrelide and interferon-a are available treatments. We studied retrospectively 197 patients, 79 male and 118 female with a median age of 58 years (19-85). Thrombosis at diagnosis presented in 33/197 patients and hemorrhage in 8/197. Median platelet count was 990*106/L (502-2500*106/L). Splenomegaly was present in 32 patients and fibrosis in 44.The majority of the patients received hydroxyurea (HU) as front-line therapy (125/197). 21 patients received anagrelide and 20 interferon-a, 4 received a combined therapy with HU and interferon-a while 27 patients received no therapy. *JAK2V617F* mutation was present in 37/72 patients (51%). There was no difference in age at diagnosis between V617F-positive and V617F-negative patients. An elevated WBC count was observed in V617F-positive patients at diagnosis (p=0,015). Hemoglobin level and platelet count were similar in the two groups of patients. The WBC and

PLT count was not correlated with thrombosis or bleeding at diagnosis. Of the patients receiving hydroxyurea, 94% had a response, a significantly higher percentage compared to the other therapy groups (p<0,001) .Response was considered when a patient didn't need a second-line therapy in a not otherwise refractory disease. Response at six months (PLT<600.000×10^6/L) was more frequently seen with HU compared with anagrelide (p<0,05). A quick response did not reduce the incidence of thrombotic or bleeding complications. No difference between thrombotic complications (6/66 patients, 9.01%) or hemorrhage (4/66 patients, 6.06%) was observed between the V617F-positive and V617F-negative patients. Secondary malignancies were observed in 6/197 patients (0,03%) with no correlation to HU therapy. In conclusion, our study showed a significant difference between the HU group of patients and those who did not receive HU as front line therapy with regard to therapy response. A response at six months did not affect the incidence of complications. There was no difference in hemoglobin level at diagnosis in V617F-positive and V617F-negative patients but a higher WBC count was observed in the V617F-positive group of patients.

0148

OCCULT T-CELL CLONES OCCUR IN THE MAJORITY OF PATIENTS WITH SUSTAINED AND UNEXPLAINED HYPEREOSINOPHILIA

G. Helbig,[1] M. Majewski,[2] J. Grzegorczyk,[3] J. Dziaczkowska,[1] B. Stella-Holowiecka,[1] J. Wojnar,[1] S. Krzemien[1]

[1]Silesian Medical University, KATOWICE; [2]Institue of Haematology and Transfusion Medicine, WARSAW; [3]Department of Immunology, LODZ, Poland

Background. The T-cell clone, which is thought to be the source of eosinophilopoietic cytokines (mainly IL-5), is identified by clonal rearrangement of the T-cell receptor (TCR) or/and by the presence of an abnormal T-cell immunophenotype. In some cases an unexplained eosinophilia may result from the response to exogenous cytokines produced by T-cells; the entity is termed *lymphoproliferative variant of hypereosinophilic syndrome* (L-HES). Material and methods. The blood samples from 32 patients with non-reactive hypereosinophilia were studied fo TCR rearrangement using the BIOMED-2 multiplex PCR. The FIP1L1-PDGFRA and ETV6-PDGFRB transcripts were detected by RT-PCR. T-cell subpopulation was assesed by flow cytometry. Results. There were 32 patients (16F/16M), at the median age of 54 years (range 17-77). Their median white blood cell (WBC) count was 11.3×10^9/L (range: 5.3-121) with the median eosinophil count of 3.9×10^9/L (range:0.7-99) and the median eosinophil bone marrow infiltration of 30% (range: 14-64). Seventeen patients (53%) demonstrated TCR clonality including TCRB in 16 patients, TCRD in 8 and TCRG in 8. The FIP1L1-PDGFRA fusion transcript was detected in 2 patients (6%), both of them presented TCR clonality. The *ETV6-PDGFRB* fusion protein was found in 2 TCR-positive patients. The immunophenotyping of T-cells revealed an atypical phenotype in 5 cases, all were TCR- positive. 2 out of these 5 patients had an increased lymphocyte count. There were no significant differences in serum levels of IL-4, IL-5 and tryptase between TCR^+ and TCR^- cases. No difference was found in blood eosinophil count, bone marrow eosinophil infiltration, serum IgE and vitamin B12 levels. During the follow-up one patient evolved in overt peripheral T-cell lymphoma and one in T-cell large granular leukemia. Conclusions. The T-cell clone was identified in a majority of studied patients with prolonged eosinophilia. Since the TCR positive cases may evolve in a full-blown T-cell lymphoma, the close monitoring of patients with occult T-cell clone is required.

0149

LOW DOSE SPLENIC IRRADIATION IN MYELOFIBROSIS: OUTCOMES AND TOXICITY OF THREE RADIATION SCHEDULE

M. Federico,[1] P. Guerrieri,[2] A. Russo,[3] G. Cardinale,[4] A. Giordano,[5] R. Lagalla,[6] P. Montemaggi,[2] G. Pagnucco[4]

[1]University of Palermo, PALERMO; [2]Radioterapia Ospedale Oncologico M. Ascoli, PALERMO; [3]Medical Oncology University of Palermo, PALERMO; [4]Ematologia Ospedale Oncologico M. Ascoli, PALERMO; [5]Pathology University of Siena, SIENA; [6]Radiology University of Palermo, PALERMO, Italy

Background. Splenectomy or splenic irradiation (SI) are the sole treatment modality to control drug resistant splenomegaly in patients with Myelofibrosis (MF). Even if splenectomy is associated with 10% risk of operative mortality, a morbidity rate close to 50% and a higher relative risk of blast transformation, it is generally preferred to SI for the longer period of symptoms relief that allows in compare to a single course of radiation. SI, on the opposite, has been considered only in poor surgical candidates with comparable response rates but with a shorter symptoms free interval. Furthermore SI has been associated to a significative incidence of post radiation life-threatening cytopenias. If splenectomy outcomes and complication rates are supported by large retrospective trials, for SI just a few papers have been published mostly on small number of patients with a significative variability among radiation treatments. *Aims*. Although a general agreement to low RT doses emerges from literature, there is not a precise definition of *low doses*. Aim of this work is to assess outcomes and complication rates of splenic irradiation in three cohorts of patients treated with different *low dose* irradiation schedule. Groups have been aggregated on the basis of the mean Normalized Tumor Dose (NTD10), a radiobiologic tool that allows comparing different RT fractionations by normalizing the delivered dose to a standard (2 Gy fraction) treatment.

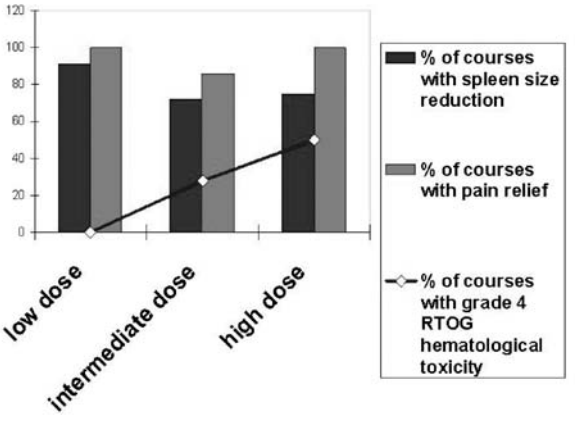

Figure 1. Outcome and toxicity.

Methods. We retrospectively reviewed 14 MF patients (9 M, 5 F) with symptomatic drug resistant splenomegaly and splenic pain; 11 patients (84%) had also constitutional symptoms as night sweets, febricula and an initial state of cachexya. Patients received RT five days per week continuously. The three groups of patients received respectively a mean NTD of 1.8 Gy (low dose group: 6 pts), 4.4 Gy (intermediate dose group: 4 pts) and 9.2 Gy (high dose group: 4 pts). Patients were considered responders if experienced a spleen reduction (≥50%) and a durable pain relief. Response evaluation was carried out 20 days after completing radiation. Treatment toxicity, limited to myelosuppression, was scored according the RTOG scale. *Results.* Patients in the low dose group received 11 RT courses and experienced an excellent outcome both in terms of spleen size reduction (91% of courses) and pain relief (100% of courses); no life-threatening cytopenias (grade 4 RTOG) occurred. Patients in the intermediate dose group received 7 RT courses and had comparable outcomes (72% and 86% of courses with spleen size reduction and pain relief) but a higher incidence (28%) of severe cytopenias (2 on 7 courses). Patients in the high dose group experienced a spleen size reduction in 75% of courses and a pain relief in all. The incidence of RTOG 4 cytopenias was 50%. No patient in this group had retreatment. *Conclusions*. NTD10 1.8 is a NTD value 2-3 fold lower than in other published series. Patients in this group experienced an excellent splenomegaly palliation without any relevant side effect. In this group patients were able to repeat safely splenic irradiation several times so prolonging the symptoms relief largely over than expected

0150

POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA: JAK2V617F MUTATION IS MORE COMMON IN OLDER PATIENTS

M.L. Randi, E. Ruzzon, M. Scapin, F. Tezza, I. Bertozzi, F. Fabris

University of Padua, PADOVA, Italy

Background. Most patients affected by polycythemia vera (PV) and essential thrombocythemia (ET) carry a somatic JAK2 point mutation (V617F). At present, we know that *JAK2V617F* occurs in 90% of PV patients and it is more common in older than in younger ET patients. The aim of the present study is to evaluate the frequency of the mutation in PV and ET patients stratified on the basis of their age. *Patients and methods*. We evaluated 249 consecutive patients affected by myeloproliferative disorders (MPD)(92 males and 157 females, mean age 60±17 years at the time of the study) and regularly followed in our outpatients surgery. 112 were affected by PV and 126 by ET, diagnosed in agreement with the PVSG criteria. The patients were stratified in 4 groups on the basis of their age at the time of the study: younger than 40 years, 40-60 years, 60-75 years and >75 years. The *JAK2V617F* mutation was searched in peripheral blood granulocytes with DNA and allele- specific PCR and Bsa XI digestion. *Results*. Considering all the patients together, but also dividing PV and ET cases, the percentage of mutated *JAK2* patients increased progressively as shown in the Table 1. While around 50% of patients younger that 40 carried the mutation, all but few cases was wild type in the group > 75. Considering all the MPD patients, the increase of mutated cases was statistically significant comparing >75 with all other age groups (p=0.001 *vs* <40, p=0.009 *vs* 40-60 and p=0.03 *vs* 60-75). Within PV patients the comparison between <40 and >75 patients was statistically different (p=0.007) as well as comparing 40-60 patients and >75 (p=0.04). No statistical difference was found in the different groups of ET regarding the percentage of *JAK2V617F* mutated cases. *Conclusions*. The PVSG criteria seems to overestimate younger PV patients possibly because idiopathic erythrocytosis or not-recognized familial cases are more frequent in this age group. In contrast, *JAK2V617F* mutation is common in older patients with PV in agreement with the occurrence of PV mainly in middle-advanced age. We confirm that *JAK2V617F* is more common in older than in younger ET patients.

Table 1. Significant statistical difference respect >75.

Age (years)		PV	ET	TOT
<40	tot	15 *	23	38 *
	V617F	7 (46.6%)	13 (56%)	20 (52.6%)
40-60	tot	23 *	44	67 *
	V617F	14 (60.8%)	28 (63.6%)	42 (62.6%)
60-75	tot	44	39	83
	V617F	30 (68.2%)	26 (66.6%)	56 (67.4%)
>75	tot	33	17	50
	V617F	29 (87.8%)	15 (82.3%)	43 (86%)

0151

UNUSUAL VEIN THROMBOSIS IN PATIENTS WITH POLYCYTHEMIA VERA (PV) AND ESSENTIAL THROMBOCYTHEMIA (ET)

M.L. Randi, M. Scapin, F. Tezza, N. Candeo, I. Bertozzi, E. Duner

University of Padua, PADOVA, Italy

Background. Budd-Chiari (BD) syndrome and portal system thrombosis (PST) are complications of both PV and ET. Moreover, sometimes these patients can develop thrombosis of cerebral veins (CVT) otherwise uncommon. In most cases, the thrombotic event is the presenting feature of the myeloproliferative disorder (MPD) and the causal disease of thrombosis is often difficult to be recognized. The erythroid spontaneous colony (EEC) formation are well known to be associated with both BD and PST also before the clinical evidence of PV or ET. Recently, *JAK2V617F* mutation has been reported to be commonly associated with venous thrombosis in ET. *Aims.* The aim of our study is to clarify if the research of *JAK2V617F* and of EEC formation can help in identifying the presence of PV or ET in patients with uncommon vein thrombosis. *Patients and Methods*. We report 22 consecutive patients (15 females and 7 male, mean age 37.5±14.4 years) with 5 PVT, 3 CVT and 14 BC diagnosed with ultrasonography in the case of splancnic thrombosis and with cerebral magnetic resonance for SVT. In all patients JAK2V617F mutation was searched in peripheral blood granulocytes DNA and allele-specific PCR and Bsa XI digestion were used. Spontaneous erythroid colonies formation (EEC) from peripheral blood mononuclear cells was obtained with a standard method. Concurrently, the diagnosis of PV or ET was made in agreement with the WHO 2001 criteria. *Results*. Our 22 patients resulted affect by MPD (16 ET and 6 PV). JAK2V617F mutation was present in 18 cases (81.1%, 4 PV and 14 ET) while the remaining 4 had JAK2 wild type (WT); 1 PV patient was homozygous for the mutation, while the remaining 17 were heterozygous. EEC development was found in 14 (63.3%,11 ET and 3 PV). The concordance of the texts is resumed in the Table 1.

Table 1.

	$JAK2^{V617F}$ EEC+	$JAK2^{V617F}$ EEC-	$JAK2^{WT}$ EEC-
BC	10 (71.4%)	2 (28.5%)	2 (50%)
PST	3 (60%)	1 (20%)	1 (20%)
CVT	1 (33%)	1 (33%)	1 (33%)
total	14 (60%)	4 (20%)	4 (20%)

Conclusions. We confirm that PV or ET and an unusual vein thrombosis, included cerebral vein thrombosis, have a high prevalence of JAK2V617F mutation. In BC and in PST we found a strong association between the presence of JAK2V617F and development of spontaneous EEC that suggests to use these texts as first line diagnostic tools in patients with splancnic vein thrombosis of unknown origin.

0152

A TIME AND DOSE TO RESPONSE STUDY OF IMATINIB THERAPY FOR CHRONIC HYPEREOSINOPHILIC SYNDROMES

T. Intermesoli,[1] F. Delaini,[1] S. Salmoiraghi,[1] S. Acerboni,[1] V. Guerini,[1] A. Vannucchi,[2] S. Mappa,[2] G. Rossi,[3] V. Rossi,[3] E. Di Bona,[4] A. Carobbio,[1] T. Barbui,[1] A. Rambaldi,[1] R. Bassan[1]

[1]Ospedali Riuniti di Bergamo, BERGAMO; [2]AOU Careggi, FIRENZE; [3]Spedali Civili, BRESCIA; [4]Ospedale S. Bortolo, VICENZA, Italy

Background/Aims. Hypereosinophilic syndrome (HES), chronic eosinophilic leukaemia (CEL) and chronic idiopathic hypereosinophilia (CIH) are rare disorders characterized by unexplained chronic hypereosinophilia with/without organ damage. Imatinib mesylate (IM) may be a potent therapeutic agent for these diseases, mainly targeting an overexpressed PDGFR tyrosine kinase activity. *Methods*. This study aimed to assess 1) the response rate to escalating doses of IM administered for 12 total weeks, 2) timing to response, 3) diagnostic profile of IM-responsive cases, and 4) duration of response following drug withdrawal. Eligible patients had HES, CEL, or severe CIH with eosinophil (EO) count >5x10^9/L. Diagnostic work-up included marrow morphology, standard cytogenetics, molecular genetics to identify *FIP1L1-PDGFRα, ETV6-PDGFRβ* and *JAK2* rearrangements, and immunophenotypic detection (Vβ) of T-cell clones (TCC). IM was provided by Novartis Oncology, Italy, to be administered at 100 mg/d; in case of unsatisfactory response, the drug could be increased by 100 mg/d on a weekly basis and up to a maximum of 400 mg/d. After 12 weeks, IM was stopped and response re-evaluated. A complete response (CR) was defined by an eosinophil count <0.35x10^9/L, associated with disappearance of any previous clinical and hematological abnormality. *Results*. Between X/04-II/07, 25 patients were enrolled: 18 males, median age 53 yy (range 25-80 yy), with a disease history of 0.08-16 yy (median 3), and a previous treatment in 10 (40%). Blood eosinophilia was 0.71-17.5x10^9/L (median 2.3). The diagnosis was HES in 13, CEL in 6 and CIH in 6. Cytogenetics was abnormal in 6 (30%), a TCC was detected in 4 (21%), *FIP1L1-PDGFR4* in 6 (24%), ETV6-PDGFRβ in none and *JAK2* mutation in 1. Twenty-three cases were evaluable for response, while 2 quit the study . An early CR was documented in 11 cases (48%): 3 with CEL and t(4;8) (n=1) or chromosome 5 abnormalities (n=2), 6 with FIP1L1-PDGFRalpha rearrangements, and 2 with HES without rearrangements. No patient with TCC or JAK2 mutation responded. 2/6 (33%) patients with FIP1L1-PDGFRα fusion gene achieved a complete molecular response. As regards timing and dose relationships, a marked drop of blood EO as well as 10/11 CRs occurred after only 1 (92% EO reduction) and 2 (97% EO reduction) weeks of IM 100 mg/d (Figure 1), while a further CR patient received 400 mg/d on week 2 only due to abdominal illness. After 12 weeks, 10 evaluable patients were confirmed in CR (IM dosing: 100 mg/d in 7; 200 mg/d in 3, of whom one had 400 mg/d for one week only). After withdrawal, all cases lost rapidly their response, IM being restarted

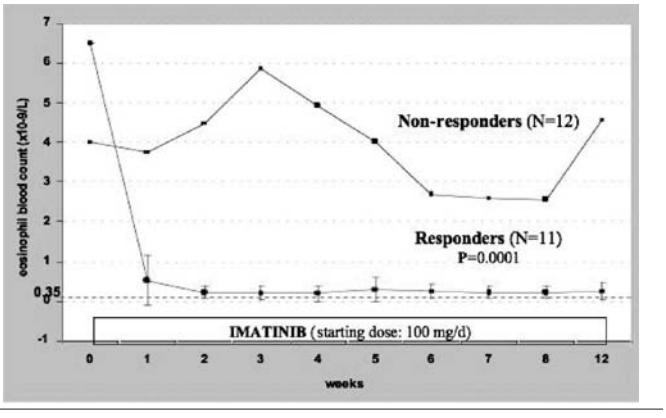

Figure 1.

Conclusions. Low-dose IM (100 mg/d) is confirmed to be rapidly effective in selected HES/CEL/CIH patients (*PDGFR* rearrangements, no abnormal T cell clone). A short therapeutic trial of low-dose IM may be indicated in idiopathic hypereosinophilia patients, particularly with concurrent organ damage, to identify IM-sensitive cases outside the predefined group of PDGFR-rearrangements, before the availability of complex diagnostic tests. Drug withdrawal is on the contrary associated with rapid relapse, so that long-term maintenance is required.

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EUROPEAN STANDARDIZATION OF BCR-ABL MRNA QUANTIFICATION

M.C. Müller,[1] P. Erben,[1] G. Saglio,[2] S. Branford,[3] B. Hanfstein,[1] D. Gosenca,[1] T. Ernst,[1] R. Hehlmann,[1] N.C.P. Cross,[4] A. Hochhaus[1]

[1]Universitätsklinikum Mannheim, MANNHEIM, Germany; [2]Division of Hematology and Internal Medicine, University of Turin, TURIN, Italy; [3]IVMS, ADELAIDE, Australia; [4]Wessex Regional Genetics Laboratory, University of Southampton, SALISBURY, UK

Background. Comparability of molecular results between different laboratories is the prerequisite for their use within large studies and for optimal patient management. International recommendations have defined a standardized way to calculate and express individual results on an International Scale (IS). Utilization of the IS in different laboratories currently relies on comparison of a series of test samples to derive laboratory-specific conversion factors (CFs) which are then validated by exchange of a second set of samples. *Aims.* We sought to develop the IS in 40 European laboratories undertaking BCR-ABL mRNA quantification with highly diverse techniques, i.e. cDNA synthesis, PCR platforms, control genes, external standards, evaluation methods. *Methods*. Triplicates of WBC lysates (10%, 1%, 0.1%, 0.01% dilutions of a CML patients leukocytes in healthy donors leukocytes, n=480) were shipped on dry ice to 40 participating laboratories. A common RNA extraction protocol (TRIzol®) was applied followed by individual protocols for cDNA synthesis, RQ-PCR and evaluation. Results were compared with results from the central reference laboratory (Mannheim). After assessment of linearity, individual CFs were calculated (individual BCR-ABL result x CF = BCR-ABL result according to the International Scale). For validation of the CF 25-30 aliquots of patient WBC lysates were exchanged. *Results.* In 36 of 40 laboratories (90%) CFs were successfully calculated whereas 4 laboratories (10%) failed due to non-linear results. The coefficients of variation (CV) of individual results (n=36) improved from 0.87-1.85 before conversion to 0.28-0.43 after conversion. In order to validate the CFs, 35 of 40 laboratories (88%) provided patient samples which were analyzed in both test and reference laboratories. After conversion, 50% of results (median proportion of samples per laboratory) were within 2-fold of each other (range, 0.5-2.0), 77% were within 3-fold and 92% were within 5-fold. Recalculation of the CFs using Bland-Altman difference plots led to significantly higher concordance rates (<2-fold difference, median 72%, p=0.0002; <3-fold difference, median 89%, p=0.0002; <5-fold difference, median 96%, p=0.0011). Three labs appeared to achieve unsatisfying concordance rates (2-fold, <40%; 3-fold, <65%; 5-fold, 65-90%). *Conclusions*. We conclude that control rounds based on distribution of dilution samples represent powerful tools to check linearity of molecular quantification systems for BCR-ABL mRNA. Furthermore, they facilitate the calculation of CFs for participating laboratories, enabling the expression of results according to the International Scale. An expansion of these efforts to all European countries is ongoing within the European Treatment and Outcome Study (EUTOS) for CML.

0154

RESCUE OF GENOMIC INFORMATION IN 72% OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) WITH NORMAL/FAILED CYTOGENETICS AND IDENTIFICATION OF NF1 DELETION AS THE HALLMARK OF A SUBSET OF NOTCH1-MUTATED T-ALL. A GIMEMA STUDY

C. Matteucci,[1] G. Barba,[1] E. Varasano,[1] A. Vitale,[2] L. Elia,[2] M. Mancini,[2] V. Pierini,[1] R. La Starza,[1] B. Crescenzi,[1] S. Romoli,[1] V. Nofrini,[1] M.F. Martelli,[1] R. Foà,[2] C. Mecucci[1]

[1]Hematology, IBiT Foundation, University of Perugia, PERUGIA, Italy; [2]Hematology, University La Sapienza, ROMA, Italy

Background and Methods. Molecular cytogenetics and advanced wide-genome technologies have provided information on >80% of T-cell acute lymphoblastic leukaemia (T-ALL). We used Metaphase-, Array-CGH (M-, A-CGH), Fluorescence *in situ* hybridization (FISH) and mutational analysis to characterize 32 adult B-cell acute lymphoblastic leukaemia (B-ALL) and 40 T-ALL at diagnosis, from GIMEMA protocol 0496. All cases were non-classified genetically, because of failed conventional cytogenetic analysis, normal karyotype, and negative molecular studies for the most common genomic rearrangements. *Results*. M-CGH detected DNA copy number variations (CNV) in 25/40 T-ALL cases (62.5%) and

13/32 (40.6%) B-ALL. Gains frequently involved entire chromosomes 21 (six cases), 14 (four cases), and X (four cases, one with an Xp amplification) and the following chromosomal regions: 1q (six cases), 17/17q (five cases), 5/5p (four cases, one with a high level amplification at 5p13.2-p15.2). Losses frequently involved the regions of: 9p (eleven cases), 5q and 6q (eight cases each), 11q and 13 (four cases), 9q, 16/16q, 17p (three cases each). CNV were associated with specific lineages: 1q gain was found in 12.5% of B-ALL and 5% of T-ALL; X/Xp gain in 12.5% of B-ALL but not in T-ALL. 9p loss was detected in 6.2% of B-ALL and 22.5% of T-ALL; 5q loss was found in 3.1% of B-ALL and 20% of T-ALL. A-CGH, performed with commercial 3200-3600 BAC arrays with resolution of about 0.85-0.8 Mb, detected changes in 4/6 cases with normal M-CGH and identified additional imbalances in 6 abnormal cases. It revealed a deletion in 17q11.2/NF1 gene in 2 cases (the sole genomic imbalance in one; associated with chromosome 13 deletion in the other). In the latter, BAC array detected a cryptic four-clone deletion (RP11-229K15, RP11 353O18, RP11-142O6, RP11-41C23) on 17q11.2 of 0.5-2 Mb in size. FISH screening with probes for the NF1 gene on 42 cases of T- and B-ALL with fixed cells detected another case of T-ALL case with a cryptic del(17q11.2). None of the B-ALL cases was positive. A FISH screening in additional 20 cases of T-ALL belonging to different GIMEMA protocols showed 2 cases with a microdeletion at 17q11.2 region. Somatic mutations of the NOTCH1, FBW7 and NF1 genes were investigated using Denaturing High Performance Liquid Chromatography in the 5 cases with NF1 deletion. No mutation was detected at FBW7 gene while NOTCH1 was mutated in all cases. Moreover, one case showed a stop mutation at NF1 exon 22 of the nondeleted allele, confirming the tumorigenic mechanism of suppressor gene haploinsufficiency. Thus, NF1 deletion is a genomic event identifying a subgroup of NOTCH1-positive T-ALL. Conclusions. M- and A-CGH integrated failed/normal cytogenetics in T-ALL, rescuing 72% of genetically undefined T-ALL cases (M-CGH alone rescued 62.5 %). A-CGH detected a new microdeletion of the 17q11.2 region, which identified a subset of patients with NOTCH1-positive T-ALL. M-CGH, together with the added value of the higher resolution A-CGH, emerged as valid approaches for genomic characterization of T-ALL.

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CONSTITUTIVE ACTIVATION OF PDGFRA OR PDGFRB BY DIVERSE FUSION GENES CAN RAPIDLY BE DETECTED BY QUANTITATIVE RT-PCR

P. Erben,[1] M.C. Mueller,[2] D. Gosenca,[2] G. Metzgeroth,[2] H. Popp,[2] C. Walz,[2] E. Thomas,[2] R. Hehlmann,[2] N. Cross,[3] A. Hochhaus,[2] A. Reiter[2]

[1]University Hospital Mannheim, MANNHEIM, Germany; [2]III. Med. Klinik, Med. Fak. Mannheim, Univ. Heidelberg, MANNHEIM, Germany; [3]Salisbury District Hospital, SALISBURY, UK

Rapid identification of fusion genes involving PDGFRA or PDGFRB in chronic myeloproliferative disorders or myelodysplasia is compromised by the multitude and heterogeneity of partner genes. We therefore sought to establish quantitative RT-PCR assays (RQ-PCR) by amplification of 3´-mRNA sequences which are fully retained in PDGFRA fusion-gene positive patients (4 fusion genes; 38 patients, 35m, 3f, median age 54 yrs, range 33-75) or PDGFRB fusion gene-positive patients (4 fusion genes; 7 patients, median age 58 yrs, range 19-75). Except for FIP1L1-PDGFRA positive cases, all patients had chromosomal aberrations of bands 4q12 (*PDGFRA*) or 5q31-33 (*PDGFRB*) as assessed by conventional cytogenetics. As external standards for quantification, serial dilutions of plasmids containing non-rearranged *PDGFRA* and *PDGFRB* sequences were employed. ABL transcripts were quantified as an internal control and results were expressed as the ratios PDGFRA/ABL or PDGFRB/ABL. A cut-off point for overexpression of *PDGFRA* and *PDGFRB* (mean+2SD) was determined by analysis of a series of 30 healthy volunteers. At diagnosis, all patients in the study group showed significantly increased normalized transcript levels compared to patients with fusion-gene negative hypereosinophilic syndrome (HES) patients or healthy controls (*PDGFRA*, 0.8 *vs* 0.0062 *vs* 0.0064, $p<0.0001$; PDGFRB, 182 *vs* 3.41 *vs* 5.85, $p<0.0001$). The sensitivity of the assays measured by serial cell dilutions (EOL cells in HL60 cells) and mRNA dilutions from patients with known fusion transcripts differed for PDGFRA (1:1,000) and *PDGFRB* (1:10) quantification. The greater sensitivity for *PDGFRA* was due to the very low expression of this gene in normal leukocytes. Follow-up analyses of *FIP1L1-PDGFRA* positive patients during treatment with imatinib (n=23) revealed a decrease of *PDGFRA* expression to levels comparable to healthy controls in 21 of 23 (91%) patients after a median time of 13 weeks (range 8-67). These results were concordant to fusion gene specific nested RT-PCR which became negative in 19 of 21 cases after a median of 21 weeks (range, 28-67). The RQ-PCR assays may serve as useful adjuncts to diagnostic detection of imatinib-responsive fusions as well as powerful tools to help identify as yet unknown molecular aberrations involving these genes.

0156

MUTATIONS OF THE WT1 TRANSCRIPTION FACTOR ARE ASSOCIATED WITH INFERIOR OVERALL SURVIVAL-AN ANALYSIS IN 368 AML PATIENTS WITH NORML KARYOTYPE <60 YEARS TREATED IN THE AML96 PROTOCOL OF THE DSIL

C. Thiede, T. Illmer, S. Soucek, M. Schaich, G. Ehninger

University Hospital Carl Gustav Carus, DRESDEN, Germany

Background. In patients with AML and normal karyotype, several specific molecular abnormalities have been reported during the last years, including *FLT3-ITD*, *NPM1* and *CEBPA* mutations. The presence of these alterations has an impact on prognosis, and new strategies try to implement them for treatment stratification. More recently, mutations of the WT1 gene have been described in AML patients. Thus far, studies on this mutation are limited in sample size and the precise prevalence and the prognostic impact WT1 mutations is unknown. In an attempt to further characterize patients with AML and normal karyotype (NK-AML), we have studied WT1 mutations in NK-AML patients below the age of 60. We characterized mutations in the mutational hotspot regions of WT1 by high-resolution fragment analysis (Exons 1 and 7) or direct sequencing (Exon 9) in a total of 368 patients treated in the AML96 trial of the DSIL. Results were correlated with other mutations (e.g. FLT3; NPM1) as well as clinical parameters and outcome. *Results*. Mutations of the WT1 gene were found in 38/368 patients (10.3%). The mutations were predominantly located in exon 7 (n=25) and exon 9 (n=12), whereas only 3 mutations were found in exon 1; two patients had mutations in two exons. Among the clinical characteristics, *WT1* mutations were associated with younger age at diagnosis (median *WT1* wt: 49 yrs. *vs* WT1 mut: 42 yrs.; $p=.036$), higher LDH (median *WT1* wt. 434 U/l *vs* WT1 mut709.8 U/l; $p=.001$), but not with patient gender, BM-blasts or leukocyte counts. No significant association was further seen with the presence of FLT3-ITD, TKD or *NPM1* mutations. Patients with WT1 mutations had a comparable CR-rate after double induction chemotherapy (WT1-neg: 70% *vs WT1* pos: 65.8%). However, the probability of overall survival in patients with mutated WT1 was significantly lower (median WT1 neg: 22.6 mo. range: 16.1-29.2 *vs* WT1-mut: 11.5 mo. range 8.4-14.6; $p=0.027$). *WT1* had no significant impact on the probability of disease free survival and the rate of relapse. In a multivariate Cox-proportional hazard model including several clinical (age; WBC counts; LDH) or molecular parameters (NPM1/FLT3-ITD mutational groups), mutant *WT1* remained an independent predictor of inferior OS with a hazard ratio of 1.6 (range: 1.1-2.5). *Conclusions*. *WT1* mutations can be found in a significant proportion of patients with AML and normal karyotype. They are more common in younger patients and appear to be associated with a lower probability of OS. Thus, the detection of WT1 mutations might add further impact to the prognostic classification based on molecular alterations.

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MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) IS A POWERFUL TECHNIQUE TO IDENTIFY PROGNOSTIC RELEVANT CHROMOSOMAL DELETIONS AND DUPLICATIONS IN CLL PATIENTS

M.-J.P.L. Stevens-Kroef,[1] A. Simons,[1] H. Gorissen,[1] T. Feuth,[1] D. Olde Weghuis,[1] A. Buijs,[2] R. Raymakers,[1] A. Geurts van Kessel[1]

[1]Radboud University Nijmegen Medical Centre, NIJMEGEN; [2]University Medical Centre Utrecht, UTRECHT, Netherlands

Background. B-cell chronic lymphocytic leukemia (B-CLL) is characterized by a highly variable clinical course. Characteristic genomic abnormalities such as deletions of the ATM and/or TP53 genes, deletions of the 13q14.3 region and trisomy 12 have shown to provide prognostic information (*Dohner et al. NEJM 343:1910-6*). *Aims*. The still widely and commonly applied karyotyping and FISH techniques for the detection of these aberrations are laborious and expensive. As an alternative, we set out to perform multiplex ligation-dependent probe amplification (MLPA) analysis using commercially available kits. This allowed us to analyze 56 genomic CLL-specific and risk-identifying targets, including the loci mentioned above, in a single experiment. *Methods*. MLPA data of 88 CLL patients were compared with interphase FISH data using the

ATM, centromere 12, RB1, D13S319, and TP53 specific probes in a double blinded fashion. *Results*. Overall, there was a perfect correlation between the MLPA and FISH data if the genetically abnormal clone was present in at least 10% of the cells. Only one case with 8% abnormal cells, as determined by FISH, was not detected by MLPA. Since multiple probes per tested locus and multiple loci were included in the MLPA assay, additional abnormalities not covered by the standard FISH probes used were detected by MLPA, i.e., trisomy of the short arm of chromosome 12, trisomy of chromosome 19 and gain of the MYCN gene. Furthermore in 3 patients small 13q14.3 deletions undetectable by FISH were identified and in another 10 patients deletions only partly covering the 13q14.3 locus were observed. In all these patients the deletions included the non-coding RNA locus DLEU, which represents an alternatively spliced transcript of BCMS gene. This gene has been proposed to be the most likely candidate tumor suppressor in 13q14 involved in the leukemogenesis of B-CLL. *Conclusions*. We have demonstrated that MLPA is a comprehensive and reliable diagnostic tool for the simultaneous identification of different clinically relevant and region-specific genetic aberrations in CLL. Furthermore MLPA has the potention to detect small critical regions of genomic loss or gain.

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PATTERN OF MENINGIOMA 1 GENE (MN1) EXPRESSION IN DIFFERENT GENETIC SUBSETS OF ACUTE AND CHRONIC MYELOID LEUKAEMIAS AND ITS POTENTIAL USE AS A MARKER FOR MINIMAL RESIDUAL DISEASE DETECTION

F. Arruga,[1] F. Messa,[1] E. Gottardi,[1] M. Fava,[1] S. Carturan,[1] C. Panuzzo,[1] E. Bracco,[1] E. Messa,[1] P. Nicoli,[1] A. Rotolo,[1] I. Iacobucci,[2] F. Lo Coco,[3] G. Martinelli,[2] G. Saglio,[1] D. Cilloni[1]

[1]University of Turin, TURIN; [2]University of Bologna, BOLOGNA; [3]University tor Vergata, ROMA, Italy

Background. Meningioma 1 (MN1) gene overexpression has been reported in acute myeloid leukaemia (AML) patients and identified as a negative prognostic factor. The Aims of the study are to characterize the patients presenting with MN1 overexpression and to establish if MN1 transcript could be a useful marker for MRD detection. *Methods.* MN1 has been quantified by RQ-PCR in 158 AML patients of different cytogenetic groups, in 30 CML patients at different phases of disease and in 50 normal controls. In 20 patients bearing a fusion gene transcript (FG) suitable for MRD assessment, we performed a simultaneous analysis of the MN1 and of the FG transcript during follow-up. Sequential MN1 and WT1 analysis was also performed in 10 AML patients lacking other molecular markers and RQ-PCR for NPM mutations in additional 7 cases. *Results.* The MN1 levels were extremely low in normal samples: median value of 136 MN1/104ABL copies in PB (range 9-300) and 254 in BM (range 80-500) and 12,9 (range 11-19) in $CD34^+$ cells. By contrast, about 50% of the AML samples with normal karyotype (NK) showed high MN1expression with a median of 5136 copies/104 ABL copies (range 852-90230) and 6780, (range 1367-15900) in PB. All samples carrying the CBFβ-MYH11 FG expressed a significantly higher amount of MN1 transcript as compared to controls (p<0,0001 in both BM and PB): median 46950, (range 2149-98000) in BM and 34500, (range 1400-67999) in PB. About 50% of the samples with AML1-ETO FG abnormally expressed MN1: median 16950, (range 3500-34000) in BM and 3475, (range 1260-56000) in PB. Finally, the APL samples expressed MN1 values comparable to those of healthy subjects in both BM (p=0,05) and PB (p=0,08). Paired analysis established a remarkable correlation between MN1 expression in PB and BM with a r value of 0,91. No association between FLT3 mutations and MN1 was demonstrated. In contrast, MN1 is typically overexpressed in patients with NPM mutations. 36 out of 47 $NPMc^+$ patients were characterized by abnormal MN1expression. We were unable to confirm the correlation between EVI-1 and MN1 expression (r= 0,06) reported in literature. Finally, MN1 is not expressed in CML in chronic phase (10 cases) but it is highly overexpressed during accelerated (n=10) and blast crisis (n=10) (median 49100 and 62741 respectively). Finally MRD has been detected by RQ-PCR by measuring MN1, FG, WT1 and in 7 cases without FG by NPM as well. In all cases characterized by FG transcript, the longitudinal pattern of MN1 expression always paralleled that of the FG. Furthermore, MN1 strictly paralleled WT1 and NPM in patients without any FG. In all the cases MN1 rose at least two months before relapse. *Conclusions.* these data show that 47% of patients with NK are characterized by abnormal MN1 expression. The overexpression is typical of AML with $NPMc^+$ and inv(16) and CML in accelerated and blastic crisis. MN1 could represent a marker for MRD. Increased MN1 expression in the BM during follow up was always found to be predictive of an impending hematological relapse.

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INFANT ALL PATIENTS WITH T(4;11)/MLL-AF4 FUSION HAVE A DIFFERENT GENOTYPIC PROFILE THAN OLDER ALL CHILDREN

M. Bardini,[1] R. Spinelli,[2] L. Corral,[1] E. Mangano,[3] G. Fazio,[1] I. Cifola,[2] A. Biondi,[1] C. Battaglia,[4] G. Cazzaniga[1]

[1]M. Tettamanti Research Center, MONZA; [2]ITB-Segrate, CNR and Center of excellence CISI, Università degli Studi di Milano, MILANO; [3]CNR and Center of excellence CISI, Università degli Studi di Milano, MILANO; [4]Center of excellence CISI and Dip Science and Biomedical Techologies, MILANO, Italy

Background. Mice models and prenatal studies indicate that in childhood ALL the individual genetic lesions alone are insufficient to generate a full leukemic phenotype, and cooperating oncogenic lesions are required. Recently, genome-wide studies on childhood ALL (1-18 years) identified deletions at several loci, mainly affecting genes that play a critical role in regulating B-cell development and differentiation. By contrast, prenatal and postnatal steps in the pathogenesis of Infant ALL (<1 year at diagnosis) are not defined. Infant ALL is a very aggressive disease, with t(4;11)/MLL-AF4 fusion representing the major subgroup. Although the very short latency suggests that leukemogenic events occur prenatally, mice models indicate that MLL-AF4 alone is not sufficient to induce leukemia, and additional mutations may occur. Also unclear is whether the molecular pathways needed for lymphoid cell differentiation are altered in cases with an MLL rearrangement and, if so, whether these alterations differ between the leukemia of infants and older children. *Aims*. Aim of this study was to detect MLL-cooperating aberrations, undetectable by conventional techniques. More specifically, we searched for Loss of Heterozygosity (LOH) associated or not to copy number alterations. The identification of these lesions could help identifying leukemia pathogenesis, providing the basis for targeted therapy. *Methods*. We have analyzed 28 cases of Infant ALL with t(4;11) at diagnosis and their corresponding samples at remission and relapses, when available (n=18 and n=8 respectively) by using genome-wide single nucleotide polymorphism (SNP) analysis (100K SNP human mapping, Affymetrix). Data were analyzed by using dChip software, and confirmed by CNAG2.0. *Results*. Compared to older childhood ALL patients, a far limited number of deletions/amplifications has been found; only 4/28 patients showed deletions, namely 1p36.13-p36.31 in one patient, 3p11.1-p12.2 plus 7q22.1-q22.2 in another patient, 4q22.3 in a third patient and 8q24.11 plus 14q21.3 in another; while 24/28 Infant ALL did not present any visible structural variation at diagnosis. By contrast, several segmental copy-number neutral (CNN) LOH have been detected and in most cases the same homozygous trait found at diagnosis was also present at remissions. The extension and prevalence of the affected regions was variable; among them 14q21.2 (4/28), 7q31.33-q32.1 (3/28), 8q21.12-q21.3 (2/28) and 8q24.11 (2/28) have been further validated by direct sequencing. At relapse, additional alterations have been detected, such as deletion of 7p in 3 cases, associated to 7q duplication (iso7q) in 2/3 cases. *Summary and Conclusions*. Overall, these results confirm that Infant ALL with t(4;11)/MLL-AF4 fusion represents a biologically unique disease, different from other type of leukemia occurring in older children. While in older children a multistep mechanism (with the involvement of several genes) is required for the full leukemic phenotype, MLL rearrangements *per se* might play a major role on the leukemogenesis. By this approach it could not be excluded that different mechanisms could cooperate with MLL in transforming cells, including point mutations. The functional role of CNN-LOH still needs to be understood: they could either reflect the duplication of oncogenic mutations, or be related to epigenetic mechanisms. A role in the predisposition to leukemia could also be hypothesized.

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COMBINED INTERPHASE FLUORESCENCE *IN SITU* HYBRIDIZATION IS A VALID TOOL FOR FULL GENOMIC INVESTIGATION AND DIAGNOSTIC SCREENING IN T-ALL. A GIMEMA STUDY

R. La Starza,[1] P. Gorello,[1] E. Varasano,[1] V. Pierini,[1] A. Guarini,[2] A. Vitale,[2] A. Bardi,[3] A. Cuneo,[3] M.F. Martelli,[1] R. Foà,[2] L. Elia,[2] C. Mecucci[1]

[1]Hematology, IBiT Foundation, University of Perugia, PERUGIA; [2]Hematology, University La Sapienza, ROMA, Italy; [3]Hematology, University of Ferrara, FERRARA, Italy

Background. In T-cell acute lymphoblastic leukemias (T-ALL) molecular lesions target regulators of cell cycle, proliferation, differentiation, survival and apoptosis in *multi-step* pathogenetic pathways. To identify events concurring in T-ALL development full genetic characterization is needed. *Aims*. To set-up and validate a molecular cytogenetic tool to integrate mutational analysis and PCR and provide a full genetic diagnosis of T-ALL. *Methods*. We designed a combined interphase fluorescence *in situ* hybridization (CI-FISH) assay to study 15 genes/loci that are involved in T-ALL leukemogenesis and validated it in 20 patients enrolled in the GIMEMA protocols with immunophenotype, cytogenetics, mutational analyses, and ALL Multiplex-PCR (*BCR-ABL1, PBX1-E2A, SIL-TAL1, MLL-AF4, MLL-ENL*, and *NUP98-RAP1GDS1*). Phenotypes were pre-T in 8 cases, cortical in 11 and mature in 1. Karyotyping succeeded in 13/20 cases and was abnormal in 6/13. *NOTCH1* mutations were detected in 7/18 cases (38%); FBW7 in 4/18 (16%). Multiplex-PCR detected one MLL-ENL and one SIL-TAL1 rearrangement. *Results*. CI-FISH gave abnormal hybridization patterns in 16/20 patients and detected multiple genomic changes in 10. Del(9p)/CDKN2A was found in 8 patients and del(6q) in five. No TCRA/D translocations were found even though they are estimated to be as frequent as TCRB translocations.1 TCRB was involved in translocations: t(7;11)(q35;p13)/TCRB-LMO2 in two cases, and inv(7)(p15q34)/TCRB-HOXA, t(7;9)(q34;q32) /TCRB-TAL2, and t(7;10)(q34;q24)/TCRB-HOX11 in one case each. Although the incidence of CDKN2A and of TCRB translocations was similar to other reports,[1,2] we found a higher incidence of del(6q) (25% *vs* 4-13%) which was probably due to the genomic clones selected for the minimal common deleted region. MLL-translocations were found in two cases, one of which had been found by Multiplex-PCR. In another patient CI-FISH hybridization signals indicated 6q23/C-MYB had undergone duplication and translocation suggesting a new cytogenetic mechanism may lead to C-MYB over-expression. CI-FISH detected the rare CALM-AF10 in one case and SIL-TAL1 in another. Previously unknown recurrent aberrations included trisomy of chromosome 9 in 1 case and of 11p in 2 cases and deletions involving 7q34 in 3 cases, 5q35/TLX3, 9q34/ABL1, and 12p13 in 2 cases each, and 14q11/TCRA/D in 1. In 2 cases with del(12p) of different extension, a common deleted region included several putative tumour suppressor genes such as *LRP6, DUSP16, DDX47, EMP-1, CREBL2,* and *CDKN1B*. Interestingly, *CDKN1B* haploinsufficiency was recently reported in T-cell prolymphocytic leukemia.[3] In 2/3 patients with del(7)(q34) which lost only the 3' *TCRB* flanking region we hypothesize a new cytogenetic mechanism whereby an unbalanced translocation activated unknown oncogenes. *Conclusions*. CI-FISH identified genetic abnormalities in 80% of cases and multiple changes in 50%. It reliably detected cryptic, rare, and new chromosome changes, TCR rearrangements, molecular lesions involving *promiscuous* genes and oncogene activation by diverse cytogenetic mechanisms. We propose using CI-FISH in the diagnostic screening of T-ALL to supplement mutational analysis and PCR studies and to validate the genetic classification of T-ALL in clinical studies.

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0161

THE COINCIDENCE OF THE JAK2V617F AND 5Q-DELETIONS IS ASSOCIATED TO VARIABLE MYELOPROLIFERATIVE AND MYELODYSPLASTIC PHENOTYPES: REPORT OF 13 CASES

U. Bacher,[1] T. Haferlach,[2] C. Haferlach,[2] T. Weiss,[2] W. Kern,[2] S. Schnittger[2]

[1]University of Hamburg, HAMBURG; [2]MLL Munich Leukemia Laboratory, MUNICH, Germany

Background. There are few studies on the coincidence of deletions of 5q and of the JAK2V617F in the literature. Ingram *et al.* (Leukemia, 2006) reported on 6 patients with initial stages of Myelodysplastic Syndrome (MDS) with this coincidence. *Aim of the Study*. To increase insights in this phenomenon, we evaluated retrospectively the frequency of the JAK2V617F mutation in 69 patients with different entities of MDS and chronic myeloproliferative diseases (CMPD) and a del(5q) and performed further characterization of the 13 patients who had both aberrations in coincidence. *Patients and Methods*. Between August 2005 and February 2008, mutational screening for the *JAK2V617F* was performed in 69 patients with a deletion of 5q as sole aberration or within non-complex aberrant karyotypes. Cases were analyzed by chromosomal banding combined by interphase fluorescence *in situ* hybridization (FISH) and by melting point based PCR analyses for the *JAK2V617F*. Cytomorphology and histopathology were additionally performed, and a diagnosis of chronic myeloid leukemia was excluded by interphase FISH for BCR-ABL. Finally, 13 patients with diverse CMPD and MDS subtypes were observed with a coincidence of del(5q) and *JAK2V617F*, and were further evaluated. *Results*. Frequency of coincidence: 13/69 patients (19%) with a deletion of 5q were positive for the *JAK2V617F* (11 at diagnosis, 2 during follow-up). Characteristics of the 13 patients with a coincidence of *JAK2V617F*/del(5q): There was a strong female preponderance (10 females: 3 males). Range of age was 45.9-86.1 years (median 71.9). Ranges of peripheral blood parameters were as follows: Leukocytes: 4 - 22×10^9/L (median 12.8×10^9/L), thrombocytes: 80 - 1195x10^9/L (median 592×10^9/L), hemoglobin: 90-160 g/L (median 12.8 g/L). Thrombocytosis was present in 6/10 cases, where data were available, 4/10 had anemia, and 5/10 presented with peripheral leukocytosis. 11/13 cases had an isolated del(5q), whereas in one case there was an additional del(20q)(q11), and in another case an additional del(12p)(p11p13). The proximal breakpoints of the deletion were localized in 9 cases at 5(q13-q15), in 4 cases at 5(q22-q23). The distal breakpoints were localized in 11 cases at 5(q33-q35) and in 2 cases at 5q31. Diagnoses of the 13 patients as assessed by cytomorphology, histopathology, and clinical and laboratory parameters were as follows: The most frequent disorder was MDS/CMPD overlap in 5 cases. Two cases each showed "classical"MDS/5q- syndrome, not classifiable CMPD (CMPD-U), or essential thrombocytosis (ET). One case had a CMPD in acceleration with 14% bone marrow blasts. (In one case classification was not possible, as diagnosis was performed during follow-up.) *Conclusions*. A considerable proportion of patients with a deletion of 5q (19% in this analysis) show a coincident *JAK2V617F*. In this study most patients with this coincidence had an overlap between myelodysplastic and myeloproliferative disorders, but there are also cases with the features of *classical* MDS/5q- or with *classical* ET. These variable phenotypes might be explained by additional mutational factors and deserve further investigation. Further studies should focus on the cooperation of the JAK2 and the del(5q) to increase insights in cooperative mechanisms in leukemogenesis in myeloproliferative and myelodysplastic diseases as in their overlap.

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0162

GAIN/AMPLIFICATION OF 1Q21 IS THE MOST POWERFUL GENETIC PROGNOSTIC FACTOR FOR PATIENTS TREATED BY AUTOLOGOUS STEM CELL TRANSPLANTATION

P. Nemec,[1] Z. Zemanova,[2] K. Michalova,[2] J. Tajtlova,[2] H. Greslikova,[1] H. Filkova,[3] R. Zaoralova,[4] I. Spicka,[5] E. Gregora,[6] D. Kralova,[4] R. Kupska,[4] M. Krejci,[7] L. Pour,[7] L. Zahradova,[7] V. Sandecka,[7] Z. Adam,[7] P. Kuglik,[8] R. Hajek[7]

[1]University Research Centre - Czech Myeloma Group, BRNO; [2]Institute of Clinical Biochemistry and Lab. Diagnostics, Charles University, PRAGUE; [3]Department of Experimental Biology, Faculty of Science, Masaryk University, BRNO; [4]University Research Centre - Czech Myeloma Group, Masaryk University, BRNO; [5]1st Medical Department, General University Hospital and 1st Faculty of Medicine, PRAGUE; [6]Department of Clinical Hematology, Faculty Hospital Kralovske Vinohrady, PRAGUE; [7]Internal Haemato-oncology Clinic, Fac. Hospital, Fac. of Medicine, Masaryk Univ., BRNO; [8]Institute of Experimental Biology, Faculty of Science, Masaryk University, BRNO, Czech Republic

Background. The presence of chromosomal aberrations detected by fluorescence *in situ* hybridisation in plasma cells is considered to be important prognostic factor for patients with multiple myeloma (MM). One of the newest mentioned factors is gain/amplification of chromosome band at 1q21. *Aims*. This study is aimed at comparison of incidence and assesment of prognostic impact of amp1q21, del13q14, del17p13, and translocations t(4;14) and t(11;14) in newly diagnosed MM patients. *Methods*. A total of 91 newly diagnosed patients with median age 58 years, median follow-up 33.9 months received 4 cycles of VAD followed by high dose of melphalan (200 mg/m^2) supported by peripheral blood stem cells. Cytoplasmic interphase FISH (cIg-FISH) analyses were assesed utilizing commercial DNA probes (Abbott-Vysis, Il, USA and Kreatech Biotechnology, NL, USA) in plasma cells labeled by the AMCA anti-IgL antibodies (Vector Laboratories, Inc.). *Results*. Chromosomal abnormalities were assessed in 91% MM patients. Amp1q21 was detected in 47% (43/91) patients, del13q14 and del17p13 were detected in 54% (48/89) and 29% (25/87) patients, respectively; t(4;14) and t(11;14) were detected in 31% (18/59) and 44% (35/79) patients, respectively. A significant association between amp1q21 and del17p13 incidences was observed (p=0.032). Similarly, a trend towards association between amp1q21 and del13q14 incidences was observed (p=0.088). Among patients with amp1q21 ORR (CR+PR) was 82.4% (14/17); among patients lacking amp1q21 ORR was 90.6% (20/22) (p=0.636). Similarly, no difference in ORR for any other studied aberration was observed. Comparison of PFS median in positive *vs* negative patients for each aberration was as follows: For amp1q21 reached 15.6 *vs* 37.5 months; p=0.013; for del13q14 reached 21.7 *vs* 30.1 months; p=0.614; for del17p13 reached 17.1 *vs* 31.7 months; p=0.059; for t(4;14) reached 18.1 *vs* 31.9 months; p=0.582 and for t(11;14) the PFS median was not yet reached *vs* 22.5 months; p=0.435, respectively. Comparison of OS median in positive *vs* negative patients for each aberration was as follows: For amp1q21 reached 28.0 *vs* 51.0 months; p<0.001; for del13q14 was not yet reached *vs* 40.8 months; p=0.388; for del17p13 reached 28.6 *vs* 47.7 months; p=0.095; for t(4;14) was not yet reached *vs* 38.2 months; p=0.701 and for t(11;14) reached 35.9 months *vs* not yet reached; p=0.663, respectively. *Summary and Conclusions*. Our data suggest that amp1q21 is associated with reduced PFS and OS intervals in patients treated with autologous transplantation. Similarly a trend towards shorter survival for del17p13 positive patients was observed. Moreover, negative prognostic impact of amp1q21 can be boosted by presence of any other studied chromosomal aberration, which implicates much poorer outcome for patients carrying those aberrations together (data not shown). Considering observation of negative impact of amp1q21 either with or without correlation of any other studied aberrations, amp1q21 seems to be the most powerful independent prognostic factor for MM patients.

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GENE COPY NUMBER CHANGES IN MYELODYSPLASTIC SYNDROMES

I. Borze,[1] E. Juvonen,[2] E. Elonen,[2] S. Knuutila[1]

[1]Haartman Institute, HELSINKI, Finland; [2]Department of Medicine, Division of Hematology, Helsinki University Central Hosp, HELSINKI, Finland

Background. Myelodysplastic syndromes (MDS) are a group of bone marrow malignances characterized by dysplasia and ineffective hematopoiesis involving one or more myeloid lineages. MDS evolve to acute myeloid leukemia (AML) in many cases. These disorders occur predominantly in elderly people (median age is 50-70 years old). MDS can appear as *de novo* (without history of some toxic exposure) or as therapy-related (related to chemotherapy and radiotherapy) disorder. Cytogenetic abnormalities vary with MDS type, but are present in about 30-50% of all MDS cases. Specific chromosome abnormalities known in MDS are loss of whole 5 or 7, 5q-, 7q-, trisomy of 8, and Y-. Additionally can be observed: 20q-, 17p-. In special losses of chromosomal material are frequent. *Aims.* We screed the entire genome of 25 *de novo* MDS for gene copy number changes, in order to detect microdeletions and microduplications, using high-density 244k oligonucleotide-based array CGH (Agilent Technologies). *Methods.* DNA was extracted from archive bone marrow samples using standard methods. The digestion, labeling and hybridisation were done according to manufacture's protocol. The array slides were scanned with Agilent's cofocal scanner. The data was processed with Feature Extraction software v9.1 and analyzed with CGH Analytics software v3.4 (Agilent Technologies). *Results.* The array CGH results showed small chromosomal aberration in addition to that detected by karyotyping tehnique. In 56% of the cases we were able to point out new chromosomal microaberrations. The most common aberrations were deletions of 5q and 7q with common region located at 5q21.3q32 and 7q22.1q33. In three cases we found a complex molecularkaryotype, and in addition four cases exhibit only one or two new microalterations. *Summary.* Based on our array result and also of some previous reports, the aCGH is a powerful technique to reveal small chromosomal aberrations in MDS. In our data these new microaberrations were located at chromosome: 3q, 7q, 11q, 18q, 17q.

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MOLECULAR CYTOGENETIC STUDY OF IMMUNOFLUORESCENTLY LABELED PLASMA CELLS AND PROGNOSTIC SIGNIFICANCE OF CLONAL CHROMOSOMAL ABERRATIONS IN 208 PATIENS WITH MULTIPLE MYELOMA

Z. Zemanova,[1] K. Michalova,[1] J. Tajtlova,[1] L. Pavlistova,[1] A. Oltova,[2] H. Filkova,[2] P. Kuglik,[2] P. Nemec,[3] D. Kralova,[3] M. Holzerova,[4] J. Balcarkova,[4] M. Jarosova,[4] J. Rabasova,[2] M. Hruba,[5] H. Fischlova,[5] I. Spicka,[1] E. Gregora,[6] Z. Adam,[7] V. Scudla,[8] V. Maisnar,[9] M. Schutzova,[10] R. Hajek[7]

[1]General University Hospital and 1st Faculty of Medicine, Charles University, PRAGUE 2; [2]Department of Medical Genetics, Faculty Hospital, BRNO, Czech Republic; [3]Myeloma Basic Research Centre, BRNO; [4]Department of Hemato-Oncology, Faculty Hospital, OLOMOUC; [5]Institute of Medical Genetics, Faculty Hospital, PLZEN; [6]Department of Clinical Hematology, Faculty Hospital Kralovske Vinohrady, PRAGUE; [7]Department of Internal Medicine, Hematooncology, University Hospital, BRNO; [8]Department of Internal Medicine, Medical Faculty, Palacky University, OLOMOUC; [9]Department of Clinical Hematology, Faculty Hospital, HRADEC KRALOVE; [10]Department of Hematooncology, Faculty Hospital, PLZEN, Czech Republic

Finding of clonal chromosomal aberrations in plasma cells is considered as one of the most important and independent prognostic factor in patients with multiple myeloma (MM). The aim of the study was to assess the frequency and prognostic value of the most common chromosomal aberrations in a homogenous series of patients with newly diagnosed multiple myeloma enrolled to the phase III trial CMG 2002. In this study 208 patients enrolled until December 31, 2005 were included. All patients underwent single autologous bone marrow transplantation according to protocol of the trial after 4xVAD induction chemotherapy and were randomized after transplantation to two maintanance/consolidation treatment arms. All patients gave written informed consent to the proposed therapeutic procedure and to the provision of samples for research purposes. Cytoplasmic Ig-FISH (cIg-FISH) analyses were done on plasma cells labeled by the Amca Anti-Human kappa-chain, Amca Anti-Human lambda-chain and Amca Anti-goat IgG monoclonal antibodies (Vector Laboratories). cIg-FISH was done by locus-specific DNA probes (Abbott-Vysis, Des Plaines, Illinois, USA and Kreatech Biotechnology B.V, Amsterdam, The Netherlands). To rule out the presence of a false positive result, cut-off values were established at 20% for detection of deletions and at 10% for detection of translocations and gains, respectively. All patients were systematically examined by conventional G-banding technique and by cIg-FISH for the deletion/monosomy of chromosome 13 and IgH gene rearrangements. Consequently, in patients with previously proven rearrangement of IgH, cIg-FISH with LSI IGH/CCND1 and/or LSI IGH/FGFR3 probes for detection of translocations t(11;14)(q13;q32) and

t(4;14)(p16;q32) was performed. In addition, 137 patients were also screened for the deletion of 17p13 and 106 cases for amplification of 1q21 chromosomal region were examined. Chromosomal changes were observed in 83.6% of the patients. Aberrations of chromosome 13 and IgH rearrangement were present in 52.9% and 58.6% of cases, respectively. t(11;14)(q13;q32) was detected in 18.5% of cases (only in two of them as sole abnormality), and t(4;14)(p16;q32) in 15.8% of cases. Statistical analyses confirmed association of IgH aberrations with significantly shorter progression free survival (PFS, p=0.040). We did not confirmed better prognosis of t(11;14)(q13;q32). Prognostic impact of monosomy/deletion of 13q was not proved. Deletion of 17p13 was detected in 21.6% of patients, its association with shorter survival in this study was not found. Amplification of 1q21 was detected in 37.7% of cases, we confirmed its association with significantly shorter survival (OS, p=0.020 and PFS, p=0.001). Finding of cumulative two and more aberrations was in our cohort of patients with MM accompanied by significantly lower progression free and overall survival (p=0.007 and p=0.001, respectively). Comprehensive molecular cytogenetic analysis of genetic changes in plasma cells of patiens with MM should further extend our understanding of the disease, hopefully enabling improvements in the targeted treatment of the patients.

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METHYLATION STATUS OF THE PROMOTER OF 25 TUMOUR SUPPRESSOR GENES IN CHRONIC MYELOMONOCYTIC LEUKAEMIA (CMML)

E. Such,[1] A. Valencia,[1] J. Cervera,[1] M.L. Senent,[1] I. Luna,[1] E. Marco,[1] M. Mallo,[2] F. Solé,[2] S. Oltra,[1] R. Collado,[3] A. Vicente,[4] V. Amigo,[5] J.C. Hernández-Boluda,[6] E. Luño,[7] O. Fuster,[1] E. Barragan,[1] P. Bolufer,[1] M.A. Sanz,[1] G. Sanz[1]

[1]Hospital Universitario La Fe, VALENCIA; [2]Hospital del Mar, BARCELONA; [3]Hospital General Universitario de Valencia, VALENCIA; [4]Hospital de La Ribera, VALENCIA; [5]Hospital Arnau de Vilanova, VALENCIA; [6]Hospital Clinico de Valencia, VALENCIA; [7]Hospital Central de Asturias, OVIEDO, Spain

Background. Epigenetic inactivation of tumour suppressor genes by promoter methylation is a common event in human cancers. In myelodysplastic syndromes (MDS) aberrant methylation of some tumour suppressor genes, such as p15INK4B, is a frequent event. The most common method for identifying this alteration is the methylation specific PCR (MS-PCR). As the number of genes hypermethylated in cancer is increasing a methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) technique has been recently developed. This technique allows the detection of the methylation status of 25 sequences that correspond to a set of tumor suppressor genes that are frequently silenced by methylation in different tumours in a single reaction. *Aims*. To study the methylation status of 25 tumour suppressor genes in DNA samples of 31 patients with CMML by means of MS-MLPA. *Patients and methods*. We studied 31 CMML bone marrow samples at the time of diagnosis [23M/8F, median age: 62 (range:52-85); median Hb: 11.1 g/dL (range: 7.2-14.1); WBC: 21.8×10^9/L (range: 3-42.8) and platelets: 196.5×10/L (range: 4.0-478)]. Genomic DNA was extracted using standard protocols (Qiamp DNA Mini kit). MLPA reagents were obtained from MRC-Holland (*www.mrc-holland.com*). Bone marrow DNA from healthy donors was used as negative control. CpGenome™ Universal Methylated DNA (Chemicon, Millipore) was used as a positive control. Quantification of the methylation status was done by dividing the peak area with the combined areas of the control probes lacking the target sequence of the HhaI. Finally, the relative peak area of each target probe from the digested sample was compared with those obtained from the undigested sample. Aberrant methylation was scored when the calculated methylation percentage was >10%. *Results*. The analysis of diagnosed CMML patients by MS-MLPA, showed aberrant methylation in p15INK4B (6/31, 19%), CDH13 (5/31, 16%), IGSF4 (3/31, 10%), FHIT (3/31, 10%) and RASSF1 (1/31, 3%) promoter regions. The vast majority of patients showed no methylation (18/31; 58%) or just one methylated sequence (8/31; 26%). By contrast, 13% (4/31) of patients showed two or three methylated genes. To validate these findings, MS-PCR was carried out to amplify CpG regions in p15INK4B. Methylation status of p15INK4B was confirmed by both methods in all the patients. The aberrant methylated genes found in the experiment belong to different molecular pathways involved in cell progression and differentiation: cell cycle (p15INK4B, RASSF1 and FHIT) and cell adherence (CDH13 and IGSF4). All of these genes are found frequently inactivated by aberrant methylation in haematological malignancies. No significant correlation was found between methylation status of the different genes and the clinical or biological characteristics. *Conclusions*. MS-MLPA appears to be a new valid method for multiplex detection of aberrant methylation patterns of CpG islands of several genes in just one assay. We found that methylation of these tumour suppressor genes is uncommon in patients with CMML. However, 13% of the patients show abnormal methylation in two or three genes. Clinical relevance of these findings should be explored.

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FISH IMPROVES THE DETECTION OF 5Q31 DELETION IN MYELODYSPLASTIC SYNDROMES (MDS) WITHOUT CYTOGENETIC EVIDENCE OF 5Q-

M. Mallo,[1] L. Arenillas,[1] B. Espinet,[1] M. Salido,[1] J.M. Hernández,[2] E. Lumbreras,[2] M. Del Rey,[2] E. Arranz,[3] S. Ramiro,[4] P. Font,[4] O. González,[4] M. Renedo,[4] J. Cervera,[5] E. Such,[5] G.F. Sanz,[5] E. Luño,[6] C. Sanzo,[6] M.J. Calasanz,[7] J. Mayans,[8] C. García-Ballesteros,[8] V. Amigo,[8] R. Collado,[9] I. Oliver,[9] F. Carbonell,[9] E. Bureo,[10] A. Insunza,[10] L. Yañez,[10] M.J. Muruzabal,[11] E. Gómez-Beltrán,[12] R. Andreu,[12] P. León[13]

[1]Hospital del Mar, BARCELONA; [2]Centro de Investigación del Cáncer, Universidad de Salamanca., SALAMANCA; [3]Hospital Universitario La Princesa, MADRID; [4]Laboratorio Gemolab, MADRID; [5]Hospital Universitario La Fe, VALENCIA; [6]Hospital Universitario Central de Asturias, OVIEDO; [7]Universidad de Navarra, PAMPLONA; [8]Hospital Arnau de Vilanova, VALENCIA; [9]Consorcio Hospital General Universitario, VALENCIA; [10]Hospital Universitario Marqués de Valdecilla, SANTANDER; [11]Hospital Sierrallana, TORRELAVEGA; [12]Hospital Universitario Dr Peset, VALENCIA; [13]Hospital Universitario 12 de Octubre, MADRID, Spain

Background. Myelodysplastic syndromes (MDS) are a group of clonal haematopoietic stem cell diseases characterised by dysplasia and ineffective haematopoiesis in one or more myeloid cell lines. More than 50% present cytogenetic aberrations at diagnosis time. Partial or complete deletion of the long arm of chromosome 5 is the most frequent abnormality in MDS; it supposes 10-15% of all *de novo* MDS. To note, there is a distinction between patients with 5q- as a sole alteration and patients diagnosed as *5q- syndrome*, a new MDS entity accepted by the WHO. Recently, a new drug (lenalidomide) has been approved in the US for the treatment of patients diagnosed of MDS with the 5q31 deletion. This drug can reduce transfusion requirements and reverse cytologic and cytogenetic abnormalities. *Aims*. The aim of the present study is to apply the FISH technique in patients diagnosed of MDS in which the conventional banding cytogenetics (CBC) study had shown a normal karyotype, absence of metaphases or an abnormal karyotype without evidence of del(5q). As a result, FISH would allow the detection of the 5q deletion in cases in which has not been detected by CBC. In consequence, these patients might be candidates to receive treatment with lenalidomide. *Patients and Methods*. We present a retrospective study based on 717 patients referred by institutions affiliated to the Spanish Haematological Cytogenetics Working Group (GCECGH). Patients were divided into two groups: Group A, includes 638 patients who did not present the 5q deletion at diagnosis by CBC and Group B (79 patients), in which CBC analysis revealed the 5q deletion (positive control group). In all of them, LSI EGR1 (5q31) SpectrumOrange/D5S23, D5S721 SpectrumGreen Probe (Abbot Molecular Inc.™) was applied to test the 5q deletion. We also performed whole chromosome paintings (Metasystems™) in order to define some karyotype aberrations. *Results*. In group A, the 5q deletion was detected by FISH in 38 cases (6%). Most of the positive cases were diagnosed as *5q- syndrome*. The deletion was observed in 13 cases with a normal karyotype, 11 without metaphases in the CBC study, nine with an aberrant karyotype with implication of chromosome 5, and five with altered cytogenetics and others chromosomes involved. In group B, the 5q deletion was observed by CBC and confirmed by FISH in all cases. We compared the proportion of cells with 5q- detected by CBC *vs* FISH; after applying a statistical analysis we did not find significant concordances in the detection of cells carrying 5q deletion by CBC or FISH. *Conclusions*. The application of FISH 5q31 improves the detection of 5q deletion in 6% compared with CBC. FISH 5q31 will be mandatory to apply when there is a suspected *5q- syndrome* by clinical and/or morphology (64.28%), CBC without metaphases (20.37%) or an aberrant karyotype with chromosome 5 involved (no 5q- chromosome) (81.81%). It would be recommendable to apply FISH 5q31 in any *de novo* MDS. FISH 5q31 might help in the selection of possible candidate patients to receive treatment with lenalidomide.

Infectious diseases, supportive care

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WHICH IS THE OPTIMAL DOSAGE OF VALGANCICLOVIR AS PRE-EMPTIVE THERAPY FOR CYTOMEGALOVIRUS INFECTION IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT?

A. Candoni, E. Simeone, M. Tiribelli, R. Fanin

Division of Hematology, UDINE, Italy

Introduction. CMV infection is a common complication after allogeneic hematopoietic stem cell transplant (HSCT). Valganciclovir (VGC) is a pro-drug of ganciclovir (L-Valyl ester), orally available, that was first approved for prevention of CMV disease in high-risk (donor positive, recipient negative) solid organ transplants (1800 mg/day as a standard dose). However the optimal dosage of the drug in HSCT has not been yet established. *Aim and Methods*. We report our experience using two different dosages of VGC as pre-emptive CMV therapy in HSCT. During a 12 months period VGC was administered on an outpatient basis to 30 consecutive HSCT recipients with a CMV positive antigenemia, detected after a median time of 86 days (range: 59-480) from transplant; 18/30 patients underwent HSCT from unrelated and 12/30 from related donors. Seventeen patients (57%) received a RIC regimen. At the detection of CMV infection, 21 patients (70%) had an acute or chronic GVHD for which they were receiving immunosuppressive therapy. The pp65 antigenemia assay were positive in all cases with a mean number of positive nuclei of 22,5±43. The first 15 cases received VGC 1800 mg/day (GROUP A) while the following 15 patients were treated with VGC 900 mg/day (GROUP B). The treatment with VGC was continued in all cases until the CMV antigenemia became negative in two consecutive samples, or until the treatment failure (increase of antigenemia in two consecutive samples). The two groups resulted well balanced, without significant differences in age, sex, underling diseases, transplant type, mean number of positive nuclei or CMV serology status at HSCT. *Results*. Overall, 27/30 (90%) cases obtained a complete clearance of antigenemia (14/15 in the GROUP A and 13/15 in the GROUP B). The median duration of VGC therapy was 18 (range 8-64) and 22 (range 8-61) days, respectively (p=ns). No cases of CMV disease were reported. A total of fifteen (50%) patients experienced one or more recurrence of CMV infection after discontinuation of VGC therapy, without significant differences in the two groups (8/15 *vs* 7/15). Seven cases required i.v. therapy with foscavir, but no patient developed CMV disease. Only three patient (2 in the GROUP A and 1 in the GROUP B) developed a mild deterioration of renal function that required dose halving (VGC 900 mg/day in the two from GROUP A, 450 mg/day in the GROUP B case). Overall, 14/30 patients (47%) experienced hematologic toxicity. Mild and transient neutropenia was reported in 12 cases (40%), transient anemia in 9 (30%) and transient thrombocytopenia in 12 patients (40%) respectively, without significant differences between two groups even if there was a trend toward higher incidence of hematological side effects in the 1800 mg/day dose group. *Conclusions*. Our experience confirms that pre-emptive therapy with VGC after allogeneic HSCT is safe and effective, with a rapid clearance of CMV antigenemia. However, in this outpatient setting, the relapse rate was not negligible (50% of cases) even if we did not observe any case of CMV disease. Our data suggest that a lower dose of VGC (900 mg/day) has comparable efficacy than the standard dose (1800 mg/day) in clearing CMV antigenemia. Therefore, in the setting of allogeneic HSCT, the suitable VGC dose for pre-emptive CMV therapy could be lower than the 1800 mg/day previously suggested. However, additional prospective and randomised studies with close monitoring of both pharmacokinetics parameters and CMV antigenemia and viremia are warranted to confirm these data and to allow the definition of the optimal dose and duration of VGC pre-emptive therapy in allogeneic HSCT recipients.

0168

VORICONAZOLE PROPHYLAXIS AMONG AUTOLOGOUS STEM CELL TRANSPLANT PATIENTS

T. Gastinne, S. Le Gouill, F. Gay-Andrieu, A. Clavert, C. Touzeau, V. Safar, J.-L. Harousseau, P. Moreau

University Hospital of Nantes, NANTES, France

Invasive fungal infections remain one of the leading causes of death among recipients of hematopoietic stem-cell transplants (SCT), especially in allogeneic SCT but also in autologous SCT. Because of a long period of building works in our hospital, we initiated, in July 2005, a systematic administration of oral voriconazole in patients receiving high dose chemotherapy and autologous SCT. We performed a retrospective observational study to describe the incidence of IA and other invasive fungal infection in autologous SCT. We report no cases of IA among 99 SCT recipients who received voriconazole prophylaxis compared with a 5% (5/95) incidence among those receiving no systemic antifungals for prophylaxis (p<0.05). Thus, voriconazole was effective in preventing IA during neutropenia of patients, who underwent autologous HSCT. Between July 2005 and June 2006, eighty-seven patients underwent ninety-nine autologous SCT at the Hematology department of the Nantes University Hospital. Patient characteristics are included in Table 1.

Table 1. Characteristics of patients receiving voriconazole prophylaxis versus no prophylaxis.

	No prophylaxis group (n=87)	Voriconazole group (n=87)
Single autologous SCT	79	75
Double autlogous SCT	8	12
Number of HSCT	95	99
Mean age	54	54
Sex M (%)	47 (54)	48 (55)
Reason for transplant, n (%)		
Non hodgkin Lymphoma	41 (47)	42 (48)
Hodgkin'sdisease	12 (14)	13 (15)
Multiple myeloma	32 (37)	32 (37)
Other	2 (2)	-
Line of treatment, n (%)		
First	75 (79)	83 (84)
Second	19 (20)	15 (15)
Third	1 (1)	1 (1)
Conditionning regimen, n (%)		
BEAM	55 (58)	51 (52)
Melphalan 100	6 (6)	1 (1)**
Melphalan 200	31 (33)	39 (39)
Melphalan 220	0	3 (3)
Mitoxantrone-cytarabine	2 (2)	4 (4)
Other	1 (1)	1 (1)
IA, n (%)	5 (5)	0**
Invasive Zygomycosis	0	0
Mortality due to fungal	1	0

** P < 0,05

Forty-two patients were treated for non-Hodgkin lymphoma (48%), and thirty-two (37%) for multiple myeloma. The majority of the autologous SCT were performed during first line treatments. BEAM (BCNU, Etoposide, Aracytine and Melphalan) and Melphalan (200 mg/m^2) were the most common conditioning regimen. Median duration of neutropenia was 8 days. Out of these 99 autologous SCT, no patient met the criteria for definite or probable IA. The cohort of patients receiving voriconazole prophylaxis was retrospectively compared with autologous SCT recipients receiving no antifungal prophylaxis. Between July 2004 and June 2005, 95 autologous HSCT were performed on 87 patients and no patient received antifungal prophylaxis. These two cohorts did not significantly differ in terms of demographic characteristics, disease, type of conditioning or of duration of the neutropenia. In this non-prophylaxis group, 5 patients (5%) met the criteria for probable IA. The difference in incidence of IA among these 87 patients compared with those receiving voriconazole prophylaxis (0 on 99 autologous SCT) was statistically significant (p<0.05). In both groups, we encountered neither zygomycoses, nor candidosis. One patient receiving no antifungal prophylaxis presented a fatal hemoptysis. However, mortality related to fungi did not show a statistical difference, with no death in the voriconazole group compared to one death in the non-prophylaxis group. Thus, voriconazole was safe and effective in preventing IA during neutropenia of patients, who underwent autologous HSCT. Voriconazole offers an alternative for selected patients and for a short period of time.

0169

IS DOMESTIC TAP WATER A RISK FACTOR FOR INFECTIONS IN NEUTROPENIC PATIENTS?

H. von Baum, M. Bommer, A. Forke, J. Holz, P. Frenz, N. Wellinghausen

Ulm University Hospitals, ULM, Germany

Background. It has been questioned whether tap water from the domestic environment of neutropenic patients poses a risk for infections with waterborne pathogens like *Legionella spp., P.aeruginosa*, nontuberculous mycobacteria (NTM) or moulds, respectively. *Aims.* We therefore conducted a prospective study to assess i) the prevalence of these pathogens in the tap water of hemato-oncologic patients and ii) the risk for acquiring an infection with the aforementioned pathogens at home. *Material and Methods*. Cold and warm tap water samples were collected in the bathrooms of neutropenic patients, decontaminated (for NTM culture) and

cultured quantitatively on several specific media. Species identification was performed using standard microbiological methods including 16S rDNA sequencing or line hybridization assays for NTM. If the patients were re-admitted to the hospital all infectious episodes were analyzed with regard to the pathogens of interest. *Results*. 65 patients were included into the study. *P. aeruginosa* or *Legionella spp.* were cultivated from the tap water of 7 (11%) and 6 (9%) homes, respectively. Cultures for NTM were completed in 45 cases yet with mycobacteria present in 42 samples (93 %). Species included *M. chelonae, M. diernhoferi, M. fortuitum, M. fluoranthenivorans, M. frederiksbergense, M. gilvum, M. gordonae, M. hodleri, M. mucogenicum, M. scrofulaceum, and M. tusciae*. Moulds could be cultivated from the water of 36 households (55%), *Aspergillus spp.* (2) and *Mucor spp.*(3) were isolated only rarely. During the observation period infections due to *P. aeruginosa* were noted in 7 patients and one patient had a bloodstream infection with *M. chelonae*. Typing of the clinical and water mycobacterial isolate is underway. No legionellosis was diagnosed. Moulds were recovered from only two clinical samples. In the case of one patient, however, *Cladosporium spp.* was identified in his bronchial lavage as well as in his domestic water sample. *Conclusions*. Community drinking water contains several facultative pathogens which can pose a risk to immunocompromised patients. Our preliminary data suggest that a minority of patients develops infectious complications due to bacterial pathogens acquired from water sources at home.

0170

LOW MORTALITY OF INVASIVE FUNGAL INFECTIONS IN PATIENTS UNDERGOING INTENSIVE CHEMOTHERAPY FOR ACUTE LEUKAEMIA: EXPERIENCE FROM A SINGLE INSTITUTION NOT USING ROUTINE ANTIFUNGAL PROPHYLAXIS

F.H. Hitz, K. Boggian

Kantonsspital St.Gallen, ST.GALLEN, Switzerland

Background. Patients undergoing intensive chemotherapy for acute leukaemia (AL) are at high risk for invasive fungal infections. Proven and probable fungal infections in this patient population range between 8% - 15% and are lethal in approx. 30% of cases. Recent reports therefore emphasise the importance of prophylactic antifungal therapy for the prevention of lethal invasive fungal infections. However, antifungal prophylaxis may favour the emergence of resistant fungal strains. As an alternative close clinical monitoring of AL patients with the prompt introduction of antifungal therapy in case of clinically suspected fungal infection may be equally effective to control the mortality due to fungal infections in neutropenic AL patients. *Aims*. Retrospectively analyse the incidence and mortality of fungal infections in neutropenic AL patients undergoing intensive chemotherapy in the absence of prophylactic antifungal treatment, and to compare this to the published incidence and mortality observed in the presence of antifungal prophylaxis. *Methods.* We retrospectively analysed 326 neutropenic episodes from 126 consecutive patients treated for *de novo* or relapsed acute leukaemia (AL) between 2001 and 2007. Median age at diagnosis was 52 years (range: 18-75 years). All patients received intensive cytotoxic therapy. Autologous stem cell transplantation was performed in 5 patients; no patients receiving allogeneic transplantations were included. The mean duration of neutropenia observed was 17.6 days. Antifungal prophylactic treatment or routine laboratory screening tests (e.g. galactomannan test) were not used. Patients were examined daily for clinical signs of infection, and infectious diseases specialists were routinely involved into clinical decision making. Antifungal therapy was initiated in case of persisting neutropenic fever in the presence of broad spectrum antibiotic treatment, or when radiologic imaging results from febrile, neutropenic patients were suggestive of invasive fungal infection. Proven, probable and possible fungal infections were defined according to EORTC guidelines. *Results.* Possible fungal infection was diagnosed in 20/326 (6.1%) neutropenic episodes (6 possible candida infections of liver/spleen and 14 cases of possible pulmonary aspergillosis). Proven invasive fungal infections were diagnosed in 16/326 (4.9%) neutropenic episodes. Invasive candidiasis was diagnosed in 7 patients, invasive aspergillosis in 8 patients while a generalised mucor infection was identified in one patient. Importantly, none of the suspected or proven invasive fungal infections in neutropenic AL patients was lethal. *Summary and Conclusions.* We performed a single-institution, retrospective analysis of the incidence and mortality of invasive fungal infections in AL patients undergoing intensive chemotherapy without receiving prophylactic antifungal treatment. Employing this strategy we observed 11% proven or suspected invasive fungal infections, which is similar to the incidence reported for this patient population when antifungal prophylaxis is performed. However, we did not observe mortality in any of the 36 proven or suspected invasive fungal infections. Our results suggest that close interdisciplinary clinical monitoring of AL patients during chemotherapy-induced neutropenia may be an effective strategy for controlling the rate of fatal fungal infections.

0171

IS SERUM INTERLEUKIN-8 (IL-8) A USEFUL MARKER TO DISCRIMINATE LOW OR HIGH PROBABILITY OF INFECTION IN HOSPITALIZED PATIENTS WITH NEUTROPENIC FEVER?

Y.H. Tromp

University Medical Center, GRONINGEN, Netherlands

Background. Chemotherapy-induced neutropenic fever is frequently noticed in patients treated with intensive chemotherapy. However, only half of the patients demonstrate a clinically or microbiologically proven infection. Recently, it was shown that patients in the out patient clinic with neutropenic fever can be categorized in subgroups with a low or high probability of infection. The low-risk group was characterized by the absence of clinical and physical abnormalities and an IL-8 level <60 ng/l (*JCO 2005;20:7437*). *Aims*. To demonstrate whether IL-8 in conjunction with CRP is a relevant parameter to select hospitalized patients with chemotherapy-induced neutropenic fever with a low or high probability of bacterial infection. *Methods*. Hospitalized patients treated with high-dose chemotherapy and developing chemotherapy-induced neutropenic fever (neutrophiles $\leq 0.5\times10^9$/L, or leucocytes 1.0×10^9/L in combination with temperature >38.5°C) were enrolled after informed consent. Before starting with intravenous antibiotic treatment, all patients underwent physical and radiological examination, blood samples for IL-8 and CRP, and bacteriological cultures were taken. Treatment with antibiotics was continued until the 5th afebrile day and was adapted according to culture results. In case of persistent fever without a pathogen, antifungal medication was added. IL-8 and CRP monitoring was repeated at day 1, 3, 5 and 7. Primary end point was the predictive value of serum IL-8 level in conjunction with CRP for the presence of a clinically (CDI) or microbiologically (MDI) documented infection or fever of unknown origin (FUO). Secondary end point was the IL-8 level in relation to response to treatment. *Results*. 93 febrile episodes in 74 patients were evaluated. Based on clinical and microbiological examinations, 43% of the febrile episodes were categorized as MDI, 10% as CDI and 47% as FUO. Median IL-8 was lower in the FUO group compared to CDI/MDI (70 respectively 214 ng/L, $p<0.0005$), whereas median CRP levels were equal (105 mg/L *vs* 135 mg/L, $p=0.1$). In 25% of the febrile episodes the IL-8 level was <60 ng/L, predominantly in the FUO group (43%) compared to CDI/MDI (8%). In 3 of 4 patients with CDI/MDI and serum IL-8 <60 ng/l at the start, the IL-8 level increased ≥60 ng/l after 2-3 days of follow-up. FUO and CDI/MDI patients with a serum IL-8 ≥60 ng/l and responsive on i.v. antibiotic treatment showed after 3 days a rapid decline of IL-8 levels, i.e. 92% and 72% respectively. In non-responding patients however, median IL-8 level remained ≥60 ng/l at day 3, i.e. 95 ng/L in FUO (n=15), and 113 ng/L in CDI/MDI (n=36). *Conclusions*. These results demonstrate that the serum IL-8 level is a useful marker to identify hospitalized FUO patients with neutropenic fever with low probability of infection especially when follow-up samples are obtained. These findings might in future be translated to limit the period of antibiotic treatment in this subgroup of patients.

0172

MATERNAL HIV INFECTION AND ITS IMPACT ON CORD BLOOD CYTOKINES AND B CELL ONTOGENY

I.G.H. Lorand-Metze, E. Borges-Almeida, M.M.S. Vilela, H. Milanez, B.M. Abramczuk, K. Metze

State University of Campinas, CAMPINAS, Brazil

Background. The introduction of antiretroviral therapy (HAART) in HIV+ pregnant women drastically decreased vertical transmission of HIV to their offspring. However, immunological deficiencies have been found in newborns of HIV+ mothers, affecting their response to vaccines given in the neonatal period. *Aims*. We studied the B lymphocyte maturation in cord blood from newborns of HIV+ mothers using HAART and compared it with that of normal newborns. We also examined the lymphocyte subsets and cytokine profiles in the cord blood and compared it to the values found in their mothers at the 35. week of gestation. *Methods*. cord blood from newborns of 36 HIV+ mothers (9 were drug users and were analyzed separately) and 15 newborns of healthy mothers was examined. All mothers were invited to participate and gave informed consent. Lymphocytes were analyzed by flow cytometry using the com-

binations: CD45/CD34/CD19/CD22, cCD79a/cIgM/ CD19/CD10, sIgM/CD34/CD19/CD10, CD5/CD19/CD45 and CD8/ CD4/CD3. The BCG-and PHA-stimulated production of IL2, IL4, IL7, IL10, IL12, IFN-gamma and TNF-α from mothers and infants were quantified using ELISA. *Results*. delivery took place in week 39 (36-41) in normal mothers and in week 38 (36-40) in HIV+ mothers. Mothers' age was similar in all three groups. Only 13 mothers had a detectable viral load: mean 2.7 logs (1.73-5.6). Concerning cord blood lymphocyte subsets, the 3 groups differed in the percentage of $CD3/CD8^+$ cells (higher in the newborns of drug users) and that of $CD19/CD5^+$ cells (higher in the newborns of all HIV^+ mothers). Maturation of B-lymphocytes was similar in newborns of normal mothers and HIV+ ones not using drugs, but in the offspring of drug users, increased numbers of immature cells were seen. In a multiple regression, the number of CD19/CD5+ cells could be calculated by the formula: % B CD5+ = 21.8 x "group"- 0.18 "IL-7 (cord)"+ 0.28 "INF (cord)"+ 6.3 "TNF (cord)"- 10.6 "(smoking mother)". Concerning the cytokine profile, there was a good correlation between cord and mothers' serum level, except for TNF-alpha. In mothers with drug abuse, very low levels of IL-7 and high levels of TNF were observed. *Conclusions*. exposed newborns present alterations in T and B subsets as well as in cytokine profile in cord blood. This may be due to exposure in utero to HIV proteins, maternal cytokine transplacental transfer, as well as the use of HAART by the mother during pregnancy. In newborns of drug users this effect is enhanced despite effective HAART treatment. These changes may affect effectiveness of neonatal vaccination.

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0173

INCIDENCE OF HUMAN HERPES VIRUS TYPE 7 (HHV-7) INFECTIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES WHOSE DISEASE COURSE IS ACCOMPANIED BY NEUTROPENIA

G. Lejniece,[1] D. Auzina,[1] A. Lejnieks,[1] S. Kozireva,[2] S. Chapenko,[2] M. Chistjakov,[2] Z. Nora,[2] M. Murovska[2]

[1]*The National center of Hematology, Riga East Hospital, Clinic Linezers, RIGA;* [2]*A. Kirchenstein Institute of Microbiology and Virology Riga Stradins University, RIGA, Latvia*

Background. Neutropenia is form of host-defence dysfunction that is closely related to an increased risk of infections including herpes virus infections. HHV-7 is ubiquitous lymphotropic herpes virus which infects cells of immune system including $CD4^+$ and $CD34^+$ cells and modulates their functions. Like other herpes viruses HHV-7 undergo latency and in immunocompromised individuals may become reactivated leading to severe complications including neutropenia and contribute further toward immunosuppression. The aim of this work was to evaluate the frequency of HHV-7 infection in patients with hematological malignancies whose disease course is accompanied by neutropenia and to determine the possible involvement of HHV-7 in development of this dysfunction. Materials and methods. 82 patients with neutropenia including 25 MDS, 19 ALL, 9 NHL, 14 MM patients and 15 patients with other hematological diseases were enrolled in this study. 45 apparently healthy donors were examined as a control group. The presence of latent/persistent and active HHV-7 infection was determined by detection of HHV-7 DNA in whole blood and cell-free plasma, respectively, using nested PCR. The plasma levels of TNF-alpha and IL-6 were determined in 12 patients with active infection, 22 - with latent/persistent infection, 30 without HHV-7 infection and 10 blood donors using quantitative ELISA. Results. Latent/persistent HHV-7 infection was observed in 46 (56.1%) of 82 patients as well as in 26 (57.7%) of 45 blood donors. Active HHV-7 infection was detected in 20/46 (43.5%) patients and in 1/26 (3.8%) blood donors. The frequency of active HHV-7 infection was significantly higher in patients with neutropenia compare to the blood donors (20/46 *vs* 1/26 p=0.00030). Five patients with active HHV-7 infection had febrile neutropenia and splenomegalia. The mean level of TNF-α in patients with active HHV-7 infection was higher (40.2 pg/mL) than those in patients with latent/persistent (25.8 pg/mL) and patients without HHV-7 infection (25.5 pg/mL) as well as in blood donors (7.9 pg/mL). The mean level of IL-6 in patients with active (16.5 pg/mL) and latent/persistent (10.7 pg/mL) HHV-7 infection was similar to this in patients without HHV-7 infection (19.2 pg/mL). *Conclusion*. The results showed that active HHV-7 infection is frequent event in patients with hematological malignancies whose disease course is accompanied by neutropenia and leads to increase of TNF-alpha level. In some cases active HHV-7 infection could be the cause of febrile symptom and splenomegalia. Taking into account that HHV-7 is able promote maturation of granulocyte lineage in infected CD34+ cells we suggest that it could be implicated in the pathogenesis of neutropenia in such patients.

0174

IN HIGH RISK ACUTE LEUKEMIA PATIENTS EARLY ANTIFUNGAL THERAPY AND DIAGNOSTIC PROCEDURES IMPROVE THE OUTCOME OF INVASIVE MOULD INFECTIONS

A. Nosari, M. Riva, A. Volonterio, M. Montillo, L. Marbello, A. Brizio, A. Molteni, C. Vanelli, C. Gabutti, G. Nador, E. Morra

Niguarda Cà Granda Hospital, MILAN, Italy

Background. Most guidelines recommend empirical antifungal therapy (EAT) for persistent febrile neutropenia, but this recommendation is now controversial. Risk for invasive mould infections (IMI) is particularly elevated in patients during induction or salvage phase of chemotherapy in acute leukaemia (AL) setting. In selected patients at high risk for IMI, EAT could continue to be the most effective and safer choice. *Aims.* To evaluate efficacy and safety of EAT in acute leukaemia patients. *Methods*. We reviewed the records of 137 consecutive acute leukaemia patients (108 acute myeloid leukemia, 20 acute lymphoblastic leukemia, 6 myelodysplastic syndromes, 3 blastic crisis of chronic myeloid leukemia) treated from January 2003 to January 2008 with empirical antifungal therapy during induction or salvage phase of chemotherapy and therefore considered at very high risk of IMI. Prophylaxis with itraconazole oral solution 5 mg/kg/day was performed to all patients, except those with acute lymphoblastic leukemia. At the beginning of EAT early diagnostic procedures with chest CT scan (within 48 hours) and galattomannan antigen test for the first three alternate days were performed. *Results*. Amphotericin B (AMB)-based frontline EAT was administered at fourth day of fever with negative cultures in 126 pts, voriconazole in 4 pts and caspofungin in the other 7 cases due to previous nephrotoxicity. We found 56 FUO (40.9%), and 81 fungal infections, 52 possible , 22 probable and 7 proven (4 aspergillosis, 3 zygomycoses, 1 fusariosis, with a comorbility of Aspergillus Niger and Mucor infection in one patient). Seventynine patients (62.7%) changed AMB-based frontline therapy due to: nephrotoxicity (41 pts, 32.5%), infusional reaction (26 pts, 20.6%), cutaneous reaction (2 pts), no response to antifungal therapy (5 pts, 4%). Improvement or cure were seen in the majority of patients. Only 2 of 81 patients died during antifungal therapy. Fungal attributable mortality of our population was 2.5%. At discharge the patients were treated, if necessary, with oral voriconazole 400 mg/day (50 pts) or posaconazole (3 pts: 2 aspergillosis, 1 zygomycosis) until the disappearance of fungal lesions. During aplasia of the following chemotherapy cycles secondary prophylaxis with oral voriconazole or AMB-based treatment was administered. Fifteen patients, 2 with residual pulmonary lesions, underwent bone marrow transplantation (4 autologous, 6 related allogeneic, 4 unrelated allogeneic, 1 aploidentical stem cell transplants) with liposomal amphotericin 3 mg/kg/day as secondary prophylaxis; one of the 2 pts with residual pulmonary lesions died due to Aspergillus terreus reactivation during neutropenic phase of allogeneic transplant. *Conclusions*. Compared with our previous study (*Nosari et al, Am J Hematol 2001;68:231*) performed between January 1989 and July 1999, an earlier antifungal therapy and diagnostic procedures reduced mortality (27% *vs* 2,5%) and improved the outcome of high risk acute leukemia patients. Our observations probably reflect a better management of these patients mainly due to the use of new antimycotics and more effective diagnostic and therapeutic approaches.

0175

CHANGING EPIDEMIOLOGY OF INVASIVE FUNGAL INFECTIONS AMONG HAEMATOLOGICAL PATIENTS: RESULTS OF 46 MONTH SURVEILLANCE STUDY

C. Cattaneo, E. Borlenghi, F. Bracchi, M.A. Capucci, M. Micheletti, A.M. Pelizzari, A. Re, G. Rossi

Spedali Civili, BRESCIA, Italy

Background. Invasive fungal infections (IFI) can cause severe morbidity and mortality among haematological cancer patients. Acute leukaemia (AL), particularly myeloid, during first induction, is the major predisposing factor outside allogeneic transplant. Aims. To evaluate the recent evolution of IFI epidemiology among haematological non-allotransplant patients. Methods. From April '04 to January '08, 2360 admissions to our Institution were monitored and all febrile/infectious episodes were prospectively recorded. Haematological diagnoses were mainly AL/myelodysplastic syndrome (MDS), lymphoma/chronic lymphocytic leukemia (CLL) and myeloma (35.4%, 35.2% and 18.1% respectively). Patients with >7-days expected neutropenia received antifungal prophylaxis with itraconazole 400 mg/day p.o. and nebulized amphotericin-B. Infections were considered "microbiologically proven" (MPI) when

microrganisms were isolated from infection sites. IFI were defined as probable/proven according to EORTC/MSG criteria. Infections were correlated with haematological diagnosis, status of disease, neutropenia and central venous catheter (CVC). Results. Among 952 fever/infections recorded, 385 were MPIs. They were more frequent in AL/MDS (50.1% and 53% of cases, respectively), than in lymphoma/CLL (31.9% and 31.4%) or myeloma (14.7% and 14.5%)). There were 34 probable/proven IFI, i.e. 8.8% of MPI. Thirty-five fungal pathogens were isolated (1 mixed). Aspergillus spp was responsible for 22 (64,7%) cases (12 A. fumigatus, 2 A. niger, 1 A. terreus, 1 A. flavus, 6 A. not specified), Candida spp for 6 (17,6%) (2 C. parapsilosis, 1 C. giullermondii, 1 C. famata, 1 C. kefyr, 1 C. tropicalis), Mucor spp for 2, and Cryptococcus spp, P. boydii, P. jirovecii and Fusarium spp for 1 case each. IFI occurred more frequently in patients with lymphoma/CLL (2% of admissions) and myeloma (1.6%) than in AL/MDS, where only 10 cases were diagnosed (1.2%), only 4 of which during induction. IFI occurred more in refractory/relapsed patients (61.8% of cases), than at diagnosis (17.6%), during postchemotherapy aplasia in AL (11.8%), or in patients without evidence of disease (8.8%). In 68% of cases patients developed IFI while on corticosteroids, whereas neutropenia was present in 53% of cases, and was not a statistically significant risk factor for IFI in febrile patients (3.2% *vs* 4.1%). By univariate analysis a diagnosis of lymphoma/CLL and active underlying disease were significant risk factors among patients with MPIs, both for the presence of IFI (14% *vs* 6.4%, $p<0.05$; 12.6% *vs* 2.2%, $p<0.01$, respectively) and for aspergillosis (9.9% *vs* 3.8%, $p<0.05$; 8.1% *vs* 1.4%, $p<0.01$ respectively), whereas neutropenia turned out to be protective for IFI (11.3% *vs* 7.4%, $p<0.01$), as well as diagnosis of AL/MDS both for IFI and aspergillosis (4.9% *vs* 13.3%, $p<0.01$; 2.7% *vs* 8.5%, $p<0.05$, respectively). Candidemia was not associated with CVC. Conclusions. This surveillance study completely redefined the epidemiology of IFI and aspergillosis among haematological non-allotransplanted patients admitted at our Institution, showing that IFI and aspergillosis are currently developing predominantly in those with active haematological disease, particularly lymphoma/CLL in advanced stage, often without neutropenia and on corticosteroids, whereas they do not significantly impact on patients with AL, even during induction treatment. The role of the prophylactic measures adopted as well as of other unrecognized local factors needs to be further explored.

0176

SINGLE INSTITUTION RETROSPECTIVE ANALYSIS OF BRONCHOALVEOLAR LAVAGE AS ETIOLOGICAL DIAGNOSTIC TOOL FOR PULMONARY INFILTRATES IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

F. Onida, P. Usardi, C. Annaloro, M.L. Ranzi, G. Saporiti, C. Olivares, C. Boschetti, L. Rosso, A. Maraschini, M. Colombi, A. Grancini, A. Della Volpe, N.S. Fracchiolla, G. Lambertenghi Deliliers

Ospedale Maggiore Policlinico Mangiagalli e Regina Elena, University of Milan, MILANO, Italy

Background. Due to both disease- and therapy-related immunodeficiency, patients with hematological malignancies are at high risk to develop severe respiratory infections, which bear to high mortality rates. Identification of the etiological causes is of extreme usefulness for selection of the most effective treatment. *Aims*. With this study we aimed to retrospectively evaluate the etiological diagnostic value of fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) in febrile patients with hematological malignancies and radiological evidence of pulmonary infiltrates. *Methods*. From January 2004 to December 2007, 56 bronchoscopies with BAL were performed in 52 patients in whom pulmonary infiltrates were unveiled by CT scan. Six patients were untreated, whereas all the others had received a previous chemotherapy-based treatment, including hematopoietic stem cell transplantation (HSCT) in 25 patients (12 allogeneic, 13 autologous). Underlying diseases were acute leukaemias in 27 patients (9 ALL, 18 AML), blastic phase of CML in 3 patients (2 Philadelphia-negative), lymphomas in 13 patients, and other malignancies in 9 patients. *Results*. Overall, a microbiological positivity was documented in 34 BAL samples (60%) obtained from 30 patients. Findings were classified as due to possible contamination in 7 cases (21%), even though only in 3 of these the same microorganisms were detected by the weekly nasal and oropharyngeal surveillance swabs. Eight samples (23%) were positive for Aspergillus fumigatus, whereas gram-negative bacterial pathogens were found in 5 samples (15%). Pneumocystis carinii was isolated in 9 samples (26%), while in 1 patient there was identification of Mycobacterium tuberculosis. CMV-DNA was detected in two cases, one positive also for *Aspergillus*. Among the 5 patients who underwent more than one BAL, dissimilar microbiological findings were detected in 4. Early termination of bronchoscopy was required in one patient who experienced acute respiratory distress requiring intubation. Antibiotic treatment was modified as a result of microbiological findings in 55% of patients, whereas among patients whose BAL was negative only 8 did not require empirical modification of the antibiotic scheme. As far as the outcome of pneumonia is concerned, death as a consequence of infection-related respiratory failure occurred in 3 out of the 30 patients who had positive BAL findings (10%), and in 4 out of the 22 patients (18%) with sterile BAL. *Conclusions*. In neutropenic patients fiberoptic bronchoscopy represent the most feasible etiological diagnostic tool, while other methods are dangerous and of difficult execution. Regarding BAL findings, we did not observe significant differences between patients who were previously subjected to HSCT and patients who were not. Also, no differences were noted between patients having a single localized pulmonary infiltrate and patients with multiple lesions or diffuse infiltrates. The identification of pathogens (Candida, Gram-positive bacteria) whose causative association with infectious pulmonary lesions is doubtful raise the question as to how to define criteria to establish their actual etiological role. In this setting, periodical surveillance by mucosal swabs may be viewed as an useful tool.

0177

ITRACONAZOLE *vs* AMPHOTERICIN B AS EMPIRICAL ANTIFUNGAL THERAPY FOR PERSISTENT FEVER IN NEUTROPENIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES : EVIDENCE FROM A META-ANALYSIS OF 618 PATIENTS

M. Yoshida

Teikyo University School of Medicine, KAWASAKI, Japan

Background. There is still no consensus on the efficacy of antifungal therapy for persistent neutropic fever with hematological malignancies. *Aims*. To compare the efficacy and overall tolerability of itraconazole with those of amphotericin B as empirical antifungal therapy for persistent neutropenic fever in patients with hematological malignancy. *Methods*. This study is a meta-analysis of patients with persistent neutropenic fever with hematological malignancy from three comparative clinical trials of itraconazole and amphotericin B. *Results*. In this study, 618 patients with hematological malignancy were included. Publication bias and statistically significant heterogeneity was not observed among the analyzed studies. Itraconazole was statistically significant in response rate compared with amphotericin B [odds ratio (OR) = 0.63, 95% confidence interval (CI): 0.46-0.87](Figure 1).

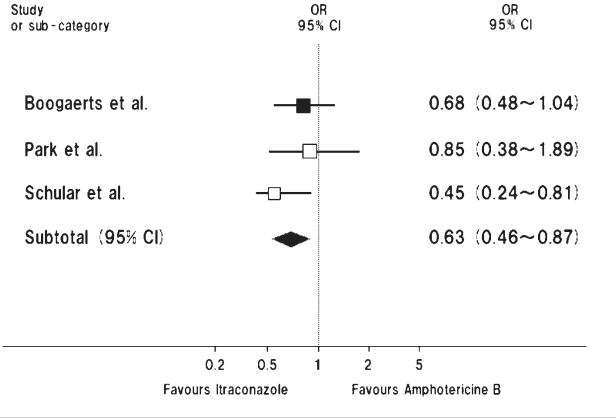

Figure 1. Result of the Meta-analysis: Response Rate.

Fewer patients were withdrawn due to the development of adverse effects associated with itraconazole when compared with amphotericin B [odds ratio (OR) = 0.30, 95% confidence interval (CI): 0.19-0.46]. Fewer patients were inferior in result of composite outcome score with itraconazole when compared with amphotericin B[odds ratio (OR) = 0.59, 95% confidence interval (CI): 0.42-0.84]. *Summary and Conclusions*. These data suggest that itraconazole is more effective than amphotericin B and also showed advantages regarding tolerability. This study confirm the role of itraconazole as useful and safe agent in empirical antifungal therapy of febrile neutropenic patients with hematological malignancy.

0178

NEUTROPHIL VOLUME DISTRIBUTION WIDTH: A NEW AUTOMATED HEMATOLOGIC PARAMETER FOR ACUTE INFECTION

A.E. Papakonstantinou,[1] A. Chondrothanasi,[2] E. Christodoulaki,[2] I. Grafakos,[2] A. Skourbouti,[2] A. Lavda,[2] P. Safioleas,[2] C. Manti[2]

[1]*Thriasion Hospital, ATHENS, Greece;* [2]*Thriassion Hospital, ELEUSINA, Greece*

Introduction. Review of peripheral blood smears can yield important diagnostic information through the identification of the morphologic changes characteristically seen in reactive neutrophils during infection. Such changes include the presence of toxic granulation, toxic vacuolization, and Döhle bodies in the cytoplasm. Younger forms (left shift), such as bands and metamyelocytes, also can be identified. This approach, however, is labor-intensive and time-consuming because it requires manual examination. Furthermore, the results are subjective because they depend on human interpretation, and only a few hundred cells can be analyzed for any given sample. The Coulter LH 750 hematology analyzer has a ability to measure specific parameters of neutrophil populations like mean and standard deviation (SD) of cell volume (MVI,SDVI), conductivity (MCI,SDCI), and light scatter (MSI, SDSI). These so-called positional parameters (PP) can detect morphologic changes in neutrophil population and can be an additional indicator for diagnosing acute infection. *Aims*. To investigate the value of the neutrophil SDVI, generated by VCS technology of the Coulter LH 750 hematology analyzer, as an additional predictor of acute infection. *Materials and Methods*. Total white blood cell count, percentage of neutrophils, and SDVI data from 64 patients with positive blood cultures for bacteria and from 54 age-matched healthy control subjects were prospectively analyzed. We then studied whether changes in SDVI correlated with patients' WBC counts (less or greater than 11000/μL)and neutrophil percentage (less or greater than 85%). The PP was obtained by the Coulter LH 750 hematology analyzer (Beckman Coulter, Fullerton, CA, USA). Comparisons between means were performed by analysis of variance. *Results*. A significant increase in the SDVI was observed in the bacteremic patients compared with the controls (26,8 *vs* 20, 63, $p<0,001$). Such increase was observed even in patients with white blood cell counts less than 11000/ μL) (25,4 *vs* 20,63, $p<0,001$) or with percentage of neutrophils less than 85% (25,8 *vs* 20,63, $p<0,001$). The more dramatic increases were seen in patients with leukocytosis (27,94 *vs* 20,63, $p<0,001$) or with neutrophilia (27,6 *vs* (27,6 *vs* 20,63, $p<0,001$).Tables 1 and 2.

Table 1.

	Control	Patients	P
Number	54	64	
SDVI mean	20.63	26,8	<0,001

Table 2.

	Control	Patients <11000	>11000	Neut perc <85%	>85%
Num	54	29	35	29	35
SDVI mean	20.63	25,4	27,94	25,8	27,6
P		<0.001	<0,001	<0.001	<0,001

Conclusions. The SDVI increases in acute infection 2) Using an SDVI cutoff of 23, as in bibliography, seems that SDVI is a good predictor of acute infection 3) The SDVI increase correlated significantly with leukocytosis and neutrophilia but was observed even in patients with white blood cell counts less than 11000/mL or with percentage of neutrophils less than 85%.4) As a quantitive parameter, the SDVI has potential for use as an additional indicator for diagnosing acute infection.

0179

VORICONAZOLE AS SALVAGE THERAPY IN IMMUNOCOMPROMISED PATIENTS WITH PULMONARY ASPERGILLOSIS WHO RECEIVED FIRST LINEE ANTIFUNGAL THERAPY WITH CASPOFUNGIN

E. Simeone, A. Candoni, M. Chiozzotto, R. Fanin

Division of Hematology, UDINE, Italy

Introduction. The efficacy of voriconazole in the treatment of IFI (including CNS mycosis and rare mycosis) has been clearly demonstrated in large multicentric randomized trials. However, to date, limited preclinical and clinical studies are avaible about voriconazole salvage therapy after caspofungin.[1–3] *Patients and Methods*. Here we report the results of voriconazole salvage therapy in a small but omogeneous group of 14 hematologic patients (12 AML, 1 CML, 1 NHL) who had a proven (5) or probable (9) pulmonary Aspergillosis that received caspofungin as a first line therapy, without CR (6 PR, 1 SR, 7 NR). The loading dose of voriconazole was 6 mg/Kg intravenously or 400 mg b.i.d. orally, followed by maintenance therapy of 4 mg/kg intravenously or 200 mg b.i.d orally. *Results*. The median duration of voriconazole therapy was 37 days (range 14-68). Eight of 14 patients (57%) obtained a complete resolution (CR) of IFI and one, who was a non responder to CAS, obtained a SR. Table 1 reported the results of Voriconazole salvage therapy. No significant drug-related clinical adverse events were recorded. IFI-related death occurred in 3/14 cases (21%); IFI-unrelated death occurred in 2/14 (14%) patients, who died for complications related. *Conclusions*. voriconazole is an effective option as a salvage therapy for Aspergillosis with lung involvment (Complete Response rate 57%) after first line therapy with caspofungin. The tolerance was good without discontinuation of therapy due to advers events. However we have to underline that in our limited experience, the success of voriconazole salvage therapy was associated with neutrophils recovery in the majority (7/8) of responsive patients.

Table 1.

Pts	Disease (status)	First line therapy (duration-days)	IFI Response	ANC at STOP	Salvage therapy (duration-days)	IFI Response	ANC at STOP	Status
1.CM	AML-(CR)	CASPO (16)	PR	800	VORICO (35)	CR	> 1000	Alive
2.FF	AML-(CR)	CASPO (17)	PR	200	VORICO (38)	CR	> 1000	Alive
3.RS	AML-(CR)	CASPO (16)	PR	300	VORICO (58)	CR	> 1000	Alive
4.MD	AML-(RES)	CASPO (16)	PR	0	VORICO (32)	CR	> 1000	Alive
5.MT	AML-(RES)	CASPO (21)	PR	300	VORICO (68)	CR	600	Death-Leukemia
6.TL	AML-(CR)	CASPO (16)	SR	0	VORICO (65)	CR	> 1000	Alive
7.MM	AML-(REL)	CASPO (8)	NR	0	VORICO (52)	CR	> 1000	Death-Leukemia
8.PS	AML-(RES)	CASPO (15)	NR	200	VORICO (64)	CR	> 1000	Alive
9.CM	AML-(RES)	CASPO (14)	NR	0	VORICO (15)	NR	0	Death-Infection
10.RA	CML-(SCT)	CASPO (14)	NR	> 1000	VORICO (36)	NR	> 1000	Alive
11.RA	AML-(CR)	CASPO (9)	NR	200	VORICO (22)	NR	> 1000	Death-Infection
12.BB	NHL-(REL)	CASPO (26)	NR	200	VORICO (36)	NR	800	Alive
13.BN	AML-(RES)	CASPO (21)	NR	200	VORICO (38)	SR	> 1000	Alive
14.OL	AML-(RC)	CASPO (18)	PR	100	VORICO (14)	NR	500	Death-Infection

CAS, Caspofungin; VORICO, Voriconazole; AML, Acute Myeloid Leukemia; CML,Chronic Myeloid Leukemia; CR, Complete Response; RES, Resistance; REL, Relapse; PR, Partial Response; SR, Stable Response; NR, Not Response; ANC, Absolute Neutrophils Count/μL; SCT, Stem cell Transplantation.

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0180

HOW COMPLEMENT AMPLIFICATION CAN BE STIMULATED AT THE ONSET OF A SYSTEMIC INFLAMMATORY RESPONSE IN SEPSIS

H.U. Lutz,[1] S. Fumiax,[1] J.S. Goede,[2] M. Fischler,[3] A. Luginbühl,[1] S. Frick,[4] P. Fodor[4]

[1]ETH Zurich, ZURICH; [2]University Hospital Zurich, Hematology Department, ZURICH; [3]University Hospital Zurich, Intensive Care Unit, ZURICH; [4]Stadtspital Zurich, Department of Internal Medicine, ZURICH, Switzerland

Background. The systemic inflammatory response syndrome (SIRS) in sepsis is triggered by excess C5a generation via the amplifying C3/C5 convertase, but it has remained unclear how complement amplification is stimulated. It is, however, known that neutrophils release elastase that becomes effective, despite the many plasma inhibitors, because small molecular weight elastase inhibitors ameliorate SIRS (Okayama *et al.* J Anesth. 2006;20:6-10). Elastase can cleave IgG to F(ab')2 (*Baici et al. Scand. J. Immunol. 1980;12:41-50*) and F(ab')2-containing immune complexes (F(ab')2-IC) of any specificity are known to stimulate complement amplification, but require an unknown plasma factor (*Reid. Immunology. 1971;20:649-658*). *Aims.* The aims were 1) to identify this plasma factor by *in vitro* experiments and 2) to investigate whether F(ab')2 fragments are generated in septic patients at the onset of SIRS and whether F(ab')2-IC play a role in complement amplification at the onset of SIRS. *Methods.* 1) We generated F(ab')2-IC from a naturally occurring antibody (NAb) to spectrin and spectrin dimer and studied their potency a) to form potent C3 convertase precursors (Jelezarova *et al.* J Biol. Chem. 2003;278:51806-1812) and b) to stimulate complement amplification by using radiolabelled C3 (Lutz *et al.* Blood 1996;88:184-193). 2) We gelfiltered plasma from controls and septic patients at the onset of SIRS and determined the total concentrations of F(ab')2 and of anti-hinge NAbs in three pools (enriched in immune complexes, IgG, or F(ab')2). On plasma of these patients we studied the concentration of released elastase and formation of factor Bb by using ELISA methods. *Results.* 1) We show that absorption of human plasma on F(ab')2 from human IgG prevented F(ab')2-IC from stimulating complement amplification and thus depleted the required plasma factor. The factor was purified from IVIG as those NAbs that bind to F(ab')2, but not to intact IgG. These *anti-hinge NAbs* restored complement amplification by F(ab')2-IC in absorbed plasma. Anti-hinge NAbs must have formed secondary immune complexes with F(ab')2-IC, in which the conformationally stabilized, shortened heavy chain captured dimeric C3b as shown by 2-D SDS PAGE with ester bond cleavage between the dimensions, implying generation of C3b2-F(ab')2-IC. Such complexes are as potent as C3b2-IgG complexes, which have a long half life and are 7-10 more effective C3 convertase precursors than immobilized C3b (*Lutz and Jelezarova. Mol Immunol. 2006;43:2-12*). This process may stimulate complement amplification at the onset of SIRS in humans. Indeed, plasma from nine septic patients at the onset of SIRS contained up to 3 µg/mL F(ab')2 fragments. The concentrations of F(ab')2 and of factor Bb correlated linearly with that of released elastase. Moreover, the F(ab')2 fragments migrated on Sephacryl S300 together with anti-hinge NAbs as ICs with MW of up to ~750kDa in all of the 9 patients studied. Conclusion: These findings provide for the first time a plausible mechanism of how complement amplification may be stimulated at the onset of a SIRS.

0181

HBV MARKERS IN ACUTE LEUKAEMIA PATIENTS TREATED WITH MYELOSUPPRESSIVE CHEMOTHERAPY: A MONOCENTRIC EXPERIENCE

A. Ferrari,[1] M. Pacilli,[2] E. Conte,[3] E. Montefusco,[3] M.A. Aloe Spiriti,[2] C. Tatarelli,[3] R. Porrini,[3] M. Di Fonzo,[4] S. Angeletti,[4] M. Marignani,[4] B. Monarca[2]

[1]Un. La Sapienza II facoltà Az.Osp. Sant'Andrea, ROMA; [2]University La Sapienza II facoltà Az. Ospedaliera Sant'Andrea Hematology, ROMA; [3]University La Sapienza II facoltà Az. Ospedaliera Sant'Andrea Hematology, ROMA; [4]University La Sapienza II Facoltà Az. Osp. Sant'Andrea Gastroentherology Dep., ROMA, Italy

Background. Reactivation of hepatitis B virus (HBV) infection has been mainly described in patients with antibodies against HBV (anti-HBs and/or anti-HBc) undergoing chemotherapy or bone marrow transplantation for lymphoproliferative disorders. In these patients antibodies against HBV may be lost, and reappearance of markers of active viral replication (HBsAg, anti-HBcIgM, HBeAg and HBV-DNA) may occur. We describe our experience about HBV in patients treated for Acute Leukaemia (AL). *Aims and Methods*. From November 2003 to November 2007 were referred in our Hematology Unit 28 consecutive patients with AL, elegible for aggressive treatment (17 M, 11 F, median age 48 yrs, range 29-67, 18 AML, 10 ALL). At diagnosis, all patients were tested for HBV markers: of these, 13, diagnosed before 2005, were screened only for HbsAg, while the remaining 15, seen from 2005, were screened for the whole panel. All 28 patients underwent intensive chemotherapy +/- transplant procedures; follow-up for HBV serology were performed at least 6 - 12 months from diagnosis. *Results*. At diagnosis, among all patients,25 resulted negative for HBV serological pattern: of these, 9 died for progression disease within 6 months, while 16 mantained at follow up the negativity of HBV serological panel. Three out of 28 patients (10%) resulted positive for HBV screening at diagnosis with the following pattern: one was positive for HBcAb, HBeAb and HBsAb, another was positive for HBcAb and HBsAb, the last was positive only for HBcAb. During follow up of these 3 patients, the first one (ALL patient) started lamivudine prophylaxis before underwent cord blood transplant: he died because of transplant related event without showing HBV seroreversion. The other 2 patients showed an HBV seroreversion; both patients showed an HBsAg positivity and increase of serum HBV-DNA level, so that antiviral treatment with lamivudine was started. One patient (AML, in 1st CR from 9 months) experienced an acute flare hepatitis: in spite of lamivudine treatment he showed a progressive increase in bilirubine and liver enzymes; therapy with adefovir and steroids was added resulting an improvement of hepatic parameters. Three months later HBV-DNA level was < 200 copies/mL, with normal hepatic values. The other patient (AML in a dismielopoietic phase after autologous SCT) after three months of lamivudine had normal hepatic tests but a serum HBV-DNA level already > 200 copies/mL. *Conclusions*. Evaluation of complete HBV panel is important in patients treated with highly myelosuppressive chemotherapy because of the high mortality risk in HBV reactivation in acute leukaemia patients HBcAb positive and/or HBsAb positive. The question of antiviral prophylactic treatment remains still unresolved.

0182

HEPATITIS B VIRUS (HBV) REACTIVATION CAN OCCUR IN SPITE OF LAMIVUDINE PROPHYLAXIS IN HBV CARRIERS TREATED WITH CHEMOTHERAPY. AN ANALYSIS OF 17 CASES

M. Drera, A.M. Pelizzari, M. De Vecchi, A. Tucci, L. Bettini, M. Puoti, G. Rossi

Spedali Civili di Brescia, BRESCIA, Italy

Background. Prophylactic lamivudine has proven effective for preventing hepatitis B virus (HBV) reactivation during chemotherapy (CT). However HBV breakthrough viremia or hepatitis can still occur during prophylaxis or after its withdrawal, but its characteristics and outcome of such prophylaxis failures are largely unknown except for a few case reports. *Aims*. To analyse the characteristics and prognosis of a series of cases of HBV reactivation occurring during or after lamivudine prophylaxis in HBV onco-hematologic carriers. *Methods*. All cases of HBV reactivation observed from 8/1999 to 2/2008 in hematologic HBV-positive patients undergoing lamivudine prophylaxis at our Institution (100 mg/d from the start to 1-6 months after the end of CT) were retrieved. Viral breakthrough was defined as an at least one-log serum HBV-DNA copies increase, acute hepatitis as a sudden rise in serum ALT to >5x the UNL or >3x the baseline level. *Results*. Of 17 patients (pts) identified, 5 had HBV reactivation while on lamivudine prophylaxis for a median of 7 mos (range 3-10) (*breakthrough reactivation*). In 12 reactivation occurred 2,8 mos (range 1,5-3) after the end of lamivudine prophylaxis whose median duration had been 9,3 mos (range 3,5-17,5) (*late reactivation*). Median age was 60 ys (43-72); F/M was 0.54. Diagnoses included indolent NHL (6), CLL (5), aggressive NHL (3), AML (2) and myeloma (1). Antineoplastic treatment included high-dose CT with/without SCT (7), purine analogue-contaning CT (5), anthracyclin-based CT (2), chlorambucil (2). Monoclonal antibody (antiCD20/antiCD52) were used in 10 cases. At baseline all cases were anti-HBc-positive, 14/17 were HBsAg positive, 5/12 had detectable serum HBV-DNA and 3 had chronic hepatitis. HBV reactivation occurred as increase in HBV-DNA copies in the 5 pts with CLL, and as acute hepatitis in 12 cases, never as acute liver failure. YMMD mutation was found in 2/7 cases tested. *Breakthrough reactivation* during prophylaxis did not differ from *late reactivation*, except for a predominance of male sex (4/5) and of a diagnosis of indolent NHL/CLL (5/5). Reactivation was managed by continuing or resuming lamivudine (100 mg/d), with/without adefovir or tenofovir. Hepatitis resolved in 11/12 cases, one returned to chronic hepatitis. HBV-DNA decreased in all and normalized in 10/12 evaluable cases. After a median follow-up

of 21 mts (range 4-89) 15/17 pts are alive. Lamivudine has been stopped in 4 cases in remission after 8,5-18 mos and in 1 for inefficacy. It has been maintained in 10 cases, in 6 in combination with other antivirals. No liver-related death has occurred. Hematologic treatment could be continued with few modifications. Two pts have died of lymphoma but overall results do not differ from HBV-negative patients. *Conclusions*. HBV reactivation in spite of lamivudine prophylaxis is not rare. It appears mainly in pts with indolent NHL or CLL, a disease in which it seems to characteristically occur in the absence of significant hepatitis. Both *breakthrough* and *late reactivation* are generally mild and can be successfully managed in most cases by continuing or resuming lamivudine, alone or in combination. They do not seem to affect the overall prognosis of patients.

0183

EFFECTIVE SECONDARY ANTI-FUNGAL PROPHYLAXIS WITH VORICONAZOLE

L. Dignan, V. Grigoriadou, F. Maxwell, S. Evans, A. Riddell, B. Shaw, M. Ethell, J. Treleaven, G. Morgan, M. Potter

The Royal Marsden Hospital, SURREY, UK

Background. Systemic fungal infections (SFIs) are common in patients undergoing allogeneic transplant or intensive chemotherapy. There is also a high incidence of reactivation in those requiring further treatment. Voriconazole has proven efficacy against candida and aspergillus species, excellent oral bioavailability and compliance is generally good. A randomised controlled trial (RCT) has supported its use in treatment of SFI (Herbrecht R *et al.* NEJM 2002; 347: 408-15) but data in the prophylactic setting is limited. Small studies suggest that it may prevent fungal reactivation during further treatment in patients with leukaemia. (*Cordonnier C et al. BMT 2004;33: 943-8; Trifilo S et al. BMT 2007; 40(5)*:451-6). *Aims*. To assess whether voriconazole is an effective secondary antifungal agent in a larger group of patients with haematological malignancy. *Methods*. A retrospective chart review was undertaken to identify patients who had received voriconazole from Jan 2004 to June 2007. Patients were included if they had a possible, probable or proven SFI and went on to receive further treatment for haematological malignancy. All patients had their original CT scans independently assessed by a radiologist to confirm likelihood of fungal infection. CT changes were categorised as major e.g halos, air crest, cavitation or minor e.g. nodules. Patients who had normal CT scans or those with consolidation, effusions or ground glass changes only were excluded. Informed consent was undertaken as part of the consent to treatment of haematological malignancy. *Results*. Twenty-two patients received oral voriconazole 200mg bd during the study period (14 acute leukaemia, 4 lymphoma, 4 chronic leukaemia) . 20 patients had a previous diagnosis of possible fungal infection and 2 had proven infection according to European Organisation for Research and Treatment of Cancer criteria. 5 patients had major changes and 16 had minor changes on chest CT scan. 4 patients had complete remission of fungal infection at time of further treatment and 17 were in partial remission. 1 patient had candida in blood cultures but a normal CT. 5 patients underwent allogeneic BMT, 7 received steroid treatment (average 75mg of prednisolone) for graft versus host disease, 8 received chemotherapy for acute leukaemia or lymphoma and 2 received alemtuzumab and prednisolone for CLL. The average duration of treatment was 121 days (range 21-278). Patients generally tolerated voriconazole well. 3 patients developed deranged liver function tests, one had visual disturbance and one developed cerebellar signs. Voriconazole was stopped in 3 patients and an alternative anti-fungal agent used in 2 of these cases. Five patients received further IV anti-fungal treatment. 3 had worsening CT scan appearances and 2 were treated empirically. At 6 months following diagnosis of SFI, 2 patients had died. Causes of death were *E. Coli* septicaemia and myocardial infarction. *Summary/conclusion.* We report a retrospective review of patients receiving secondary antifungal prophylaxis with voriconazole which is, to the best of our knowledge, larger than that previously published. This review suggests voriconazole may be an effective secondary prophylactic agent in this high risk group. A RCT would be useful to further investigate this issue.

0184

THE ROLE OF BRONCHOALVEOLAR LAVAGE (BAL) IN EVALUATING NEW PULMONARY INFILTRATES ON COMPUTED TOMOGRAPHY IN HAEMATOLOGY PATIENTS WITH NEUTROPENIC FEVER UNRESPONSIVE TO BROAD-SPECTRUM ANTIBIOTICS

T. Todd, D. Enoch

Cambridge University Hospitals NHS Foundation Trust, CAMBRIDGE, UK

Background. Pulmonary complications are a significant cause of morbidity and mortality in immunocompromised haemato-oncology patients, with invasive fungal infection being one of the commonest. Chest computed tomography (CT) with bronchoalveolar lavage (BAL) for further investigation of new pulmonary changes identified on CT has emerged as a widely used approach in investigating patients with neutropenic fever unresponsive to broad-spectrum antibiotics. However, studies of BAL are conflicting with diagnostic yields of between 9 and 80%. Most studies suggest its role in diagnosing non-infectious aetiologies is limited. Complication rates are high in haematology patients (up to 15%) and recent studies have failed to demonstrate a beneficial impact on patient outcome of therapy changes made in response to BAL findings. The optimum role of BAL in the diagnostic pathway of this high risk population remains unclear. *Aims*. To establish the diagnostic yield, complication rate, therapeutic and outcome impact of BAL in neutropenic haematology patients with fever unresponsive to broad spectrum antibiotics and new pulmonary changes on CT. *Methods*. All haematology in-patients undergoing CT chest for antibiotic unresponsive neutropenic fever between September 2003 and December 2006 at our centre were retrospectively identified from a radiology database. The CT findings, microbiology, histopathology and admission records were reviewed. Specimen processing was audited against British Society for Medical Mycology (BSMM) guidelines. Death and admission length data were collected on all patients with new pulmonary changes on CT. Antigen tests were not available during the study period. *Results*. 191 patients (198 episodes) received chest CT scans, of which 68 had significant new radiological changes. All patients had received ≥3 daysof broad spectrum antibiotics (usually piperacillin-tazobactam, vancomycin and gentamicin in combination) and had commenced liposomal amphotericin B (n=66) or voriconazole (n=2). Median age was 33 years with equal sex distribution. The commonest underlying conditions were acute myeloid leukaemia (31), acute lymphoblastic leukaemia (12) and allogeneic stem cell transplant (10). 24 underwent BAL, three with transbronchial biopsy. Median time from CT to BAL was 7 days. In 4 cases no sample reached the laboratory, twenty had samples sent for microbiology, 12 for cytology and 3 for histology. Despite largely meeting BSMM standards (microbiology 95%, cytology 92% and histopathology 100%) no fungus was identified. Klebsiella, pseudomonas aeruginosa and herpes simplex virus were identified in 1 case each. The klebsiella and pseudomonas had also been grown on blood cultures and treatment was only altered for the herpesvirus. No non-infective diagnoses were established. Three patients had respiratory distress post BAL (none had had biopsy), one required ventilatory support. There was no significant difference in rate of death during admission or duration of admission between those who did or did not have BAL. *Summary/ Conclusion*. The diagnostic yield of BAL was low, there were life-threatening complications, the results infrequently changed treatment and patient outcome was not improved. A diagnostic strategy either excluding BAL or delaying it until antifungal therapy proves ineffective or newer fungal antigen detection techniques have proven negative may, for most of this patient group, be more appropriate.

Multiple myeloma - Biology

0185

BISPHOSPHONATE RELATED OSTEONECROSIS OF THE JAW IS ASSOCIATED WITH POLYMORPHISMS OF THE CYTOCHROME P450 CYP2C8 IN MULTIPLE MYELOMA: A GENOME WIDE SINGLE NUCLEOTIDE POLYMORPHISM ANALYSIS

E. Sarasquete,[1] R. Garcia-Sanz,[1] L. Marin,[1] M. Alcoceba,[1] M.C. Chillon,[1] A. Balanzategui,[1] C. Santamaria,[1] J. Blade,[2] J. De la Rubia,[2] J. Petit,[2] M.T. Hernandez,[2] J. Lahuerta,[2] M. Gonzalez,[1] F. San Miguel[2]

[1]Hospital Universitario de Salamanca, SALAMANCA; [2]GRUPO ESPAÑOL DE MIELOMA GEM/PETHEMA. RED ESPAÑOLA DE MIELOMA (G03/136), SPAIN, Spain

Background. Bone destruction is probably the most representative hallmark of Multiple myeloma (MM). Bisphosphonates (BP) are the most widely used and effective antiresorptive agents for the treatment of disease associated with excessive osteoclast-mediated bone resorption. Recently, an increasing body of the literature has suggested the association between the use of bisphosphonates and the development of osteonecrosis of the jaws (ONJ). However, the reason why some patients develop ONJ (incidence between 2.4% to 12.8%) and why the majority do not remains unresolved. Several environmental factors have been related with a higher risk of ONJ developing, but no genetic variables have been describe to be associated with ONJ up to now. *Aims*. To asses the potential role of genetics in ONJ development. *Methods*. We performed a genome wide association study using 500.568 SNPs in two series of MM patients included in the same therapeutic protocol and which also received the same BP therapy: 22 cases (MM with ONJ) and 65 matched controls (MM without ONJ). Witten informed consent was obtained from all participants. *Results*. All patients included in this study were selected from the global population enrolled in the Spanish GEM-2000 protocol. Clinical and biological characteristics, response to treatment and survival rates were similar in both subsets of patients. For each SNP, we tested the allelic association with the ONJ presentation or not. The first analysis found four SNPs mapped within the cytochrome P450, subfamily 2C polypeptide 8 gene (CYP2C8) that could be associated with this complication (rs1934951, rs1934980, rs1341162 and rs17110453). The SNP rs1934951 (Intron 8) was significantly associated with ONJ (Overall P-value=1.07E-06? Pc, Bonferroni corrected P value =0.02). This SNP displayed an overrepresentation of the T allele in the group of cases (0.475 *vs* 0.125). Furthermore, the heterozygous genotype CT was present in 66% of the cases vs. 25% of the controls, whereas the homozygous genotype CC was more frequent in the control population, 75% vs, 19%. Even more, homozygote individuals for the T allele were exclusive of individuals with ONJ (14.3% vs. absence). On the other hand, rs1934980 and rs1341162 are allocated in the intron 5 whereas rs17110453 lies in the 5' near gene region. These SNPs showed a significant correlation with ONJ presentation ($p=4.23\times10^{-6}$, p=6.2210-6 and p=2.1510-6 respectively), although the association did not remain statistically significant after the Bonferroni correction (pc=0.09, pc=0.13 and pc=0.46 respectively). The calculated odds ratio of the risk conferred by the SNP rs1934951 assuming a dominant model of inheritance was 12.75 (95% confidence interval (CI) =3.7-43.5) associated with the presence of one or two T alleles. The odds ratios for rs1934980, rs1341162 and rs17110453 assuming the dominant model too, were as follows: 13.88 (95% CI =4.0-46.7), 13.27 (95% CI =3.5-49.9) and 10.2 (95% CI =3.2-32.1) respectively. *Conclusions*. Our data suggest that the rs1934951 polymorphism, as well as other SNPs in linkage disequilibrium with it, may play a role as a risk factor for developing ONJ in MM patients receiving BPs therapy. This data support genetic analyses in MM patients before starting the BP therapy.

0186

ARRAY CGH HIGHLIGHTS GENETIC DIFFERENCES BETWEEN MGUS AND MYELOMA

L. Chiecchio,[1] G. Dagrada,[1] R.K.M. Protheroe,[1] D.M. Stockley,[1] G.J. Morgan,[2] N.C.P. Cross,[1] C.J. Harrison,[1] F.M. Ross[1]

[1]University of Southampton, SALISBURY, UK; [2]Institute of Cancer Research, SUTTON, UK

Multiple myeloma (MM) usually shows complex chromosome abnormalities, many of which are also seen in SMM and MGUS. In order to find potential changes mediating transformation from pre-malignant stages to MM, we analysed an initial set of 57 plasma cell dyscrasias on the Agilent 244k array comparative genomic hybridization (aCGH) platform. Interphase FISH (iFISH) was used to define different groups for aCGH analysis. The cohort comprised cases with t(4;14) (8 MM, 5 SMM, 2 MGUS); t(11;14) (1 PCL, 1SMM, 1 MGUS); t(14;16) (1 PCL, 7 MM, 5 SMM, 1 MGUS); t(14;20) (4 MGUS); hyperdiploid cases lacking immunoglobulin heavy chain rearrangements (IgHr) (1 PCL, 9 MM, 2 SMM, 7 MGUS); hypodiploid cases lacking IgHr (1 PCL, 1 MM). The iFISH results of patients at different stages of the disease were almost indistinguishable, while aCGH revealed marked differences in complexity. Surprisingly, the simplest profiles belonged to MGUS cases with t(14;20); all four of whom have been stable for at least four years. Among gained regions, 1q was more frequent in MM and PCL, 17/28 (61%) *vs* 10/28 (36%) in pre-malignant cases; three of the latter progressed to MM at 7, 33 and 44 months, while the other seven are still stable (median FU 41 mo). iFISH on 484 cases (376 MM, 41 SMM, 67 MGUS) confirmed the lower frequency in MGUS ($p=0.001$ *vs* MM) and significant associations with t(4;14) ($p<0.001$), deletion 13q ($p<0.001$) and t(14;20) ($p<0.007$). Two recurrent homozygous deletions (HD) involved members of the NF-kB pathway: TRAF3 (14q32.32; 3 cases) and BIRC2/BIRC3 (11q22.1; 2 cases). Other HD were 1p32.3 (CDKN2C & FAF1; 3 cases) and 9p21.3 (CDKN2A; 2 cases). Heterozygous deletions for the same loci were also detected. No HD were found in stable MGUS or SMM, although one SMM with TRAF3 HD progressed to active MM within a year. HD were also rare in hyperdiploid MM lacking IgHr. Multiple other NF-kB pathway genes are dysregulated in MM by loss or gain of function, leading to constitutive activation of the non-canonical pathway; among these only LTBR, on 12p13.31, was abnormal in our cohort (within one interstitial gain of 0.9Mb). iFISH in a further 237 patients (40 MGUS, 12 SMM, 185 MM) showed BIRC2/3 deletion in 9 (4.9%) MM cases with 6/9 (67%) HD, and TRAF3 deletion in 43 (20%) patients, including 6 MGUS and 3 SMM; all 6 HD were in MM cases. Both deletions were associated with deletion 13q (TRAF3 $p=0.0039$; BIRC2/3 $p=0.01$) and non-hyperdiploidy ($p=0.01$ for both). Occasional MM cases showed multiple gains and losses of discrete adjacent regions on a few chromosomes. Amplification was rare: one hyperdiploid case with no IgHr had amplified different regions of 8q including MYC and of 11q including CCND1. MYC amplification was also found in one PCL with t(11;14). It is clear that standard iFISH abnormalities are insufficient to predict progression of MGUS/SMM. This study suggests that different secondary genetic events contribute to this process and may preferentially occur in association with specific primary abnormalities.

0187

BASED ON DIFFERENTIAL EXPRESSION OF ANGIOGENIC GENES ALONE, BEING POLYCLONAL PLASMABLAST (PPC), BONE MARROW PLASMA CELL (BMPC) AND MULTIPLE MYELOMA CELL (MMC) CAN BE PREDICTED, BUT BEEING EARLY STAGE/ VS. THERAPY-REQUIRING MULTIPLE MYELOMA CAN NOT

D. Hose,[1] J. Moreaux,[2] T. Meiner,[3] J.-F. Rossi,[2] A. Benner,[4] K. Mahtouk,[2] A. Seckinger,[3] C. Hei,[4] M. Hundemer,[3] T. Rème,[2] J. Hillengass,[3] K. Herde,[5] U. Bertsch,[3] J. DeVos,[2] S. Wenisch,[5] V. Pantesco,[2] A. Jauch,[6] E. Jourdan,[2] H. Goldschmidt,[3] B. Klein,[2] T. Möhler[3]

[1]Universitätsklinikum Heidelberg, HEIDELBERG, Germany; [2]INSERM U847 and CHU Montpellier, MONTPELLIER, France; [3]Universitätsklinikum Heidelberg, Medizinische Klinik V, HEIDELBERG, Germany; [4]Abteilung für Biostatistik, DKFZ, HEIDELBERG, Germany; [5]Lab. für Exp. Unfallchrurgie, Universitätsklinikum Giessen, GIESSEN, Germany; [6]Institut für Humangenetik, Universitätsklinikum Heidelberg, HEIDELBERG, Germany

Background. Angiogenesis is a hallmark of active multiple myeloma and a therapeutic target, e.g. by thalidomide. Others and we have shown this to be due to a differential and novo expression of

pro/antiangiogenic genes in MM as well as an effect of increased plasma cell number. *Aim* of this study was to investigate based on the expression of (anti)angiogenic genes alone (192 gene consensus list determined by medline search), i) whether for respective samples being PPC, BMPC or MMC can be predicted, ii) the expression differences between these entities, iii) if being early stage *vs* therapy requiring MMC can be predicted. *Patients and Methods*. 187 newly diagnosed MM/MGUS-patients (65 training (TG) / 122 independent validation group (VG)), 14 normal donors (ND) and 12 *in vitro* generates PPC samples were included. Bone marrow aspirates were CD138-purified by activated magnetic cell sorting. RNA was *in vitro* transcribed and hybridised to Affymetrix HG U133 A+B GeneChip (TG) and HG U133 2.0 plus arrays (VG). Expression data were gcrma-normalised and the empirical Bayes algorithm used. P-Values were adjusted using the Benjamini-Hochberg method (Bioconductor). The PANP-algorithm was used to identify expressed genes. iFISH was performed on purified MM-cells using probesets for chromosomes 1q21, 9q34, 11q23, 11q13, 13q14, 15q22, 17p13, 19q13, 22q11 and the translocations t(4;14) and t(11;14). Selected expression data were verified by real time RT-PCR and western blotting. *Results*. (i) On TG and VG, being BMPC or PPC is predicted by a 17 gene predictor (31 probe sets) without error. Being MMC is predicted with an error rate of 6.9/4.7 % with an overall-error rate of 5.8/4.3 % in TG/VG, respectively. (ii) 5 proangiogenic genes/7 probe sets (*HGF, ADM, CXCL2, IGF1, Met*) and 1 antiangiogenic/1 probe set (SerpinF1) are differentially expressed between these entities concordantly in TG and VG. Most patients with MGUS and MM display 1 or more over expressed angiogenic gene. (iii) Attribution of MMC to early *vs* therapy requiring MM can not be predicted. *Conclusion*. PPC, BMPC, and MMC differ in expression of (anti)angiogenic in a way that being either of the three can be predicted on the expression of these genes alone. To the contrary, the (rather small) expression differences between MMC of early-stage/therapy requiring MM-patients do not allow a prediction, giving evidence that angiogenesis related genes are activated early during disease progression. This can be taken as rationale for early intervention using antiangiogenic compounds.

0188

BONE MICROENVIRONMENT CELLS REVEALS A DIFFERENT GENE EXPRESSION PROFYLING IN MULTIPLE MYELOMA PATIENTS AS COMPARED TO HEALTHY SUBJECTS: POTENTIAL RELATIONSHIP WITH THE BONE STATUS

K. Todoerti,[1] G. Lisignoli,[2] F. Morandi,[3] L. Agnelli,[1] S. Colla,[3] M. Crugnola,[3] C. Manferdini,[2] D. Verdelli,[1] K. Codeluppi,[2] G. Lambertenghi-Deliliers,[1] V. Rizzoli,[3] A. Neri,[1] N. Giuliani[3]

[1]*Università di Milano, Fondazione IRCCS Policlinico, MILANO;* [2]*Laboratorio di Immunologia e Genetica, Istituti Ortopedici Rizzoli, BOLOGNA;* [3]*Ematologia e Centro trapianti midollo osseo, Università di Parma, PARMA, Italy*

Background. Multiple myeloma (MM) is characterized by the high capacity to induce osteolytic bone lesions. Osteoblasts (OB) formation is impaired in MM patients mainly due to the block of the Runx2/Cbfa1 activity and consequently the suppression of the osteogenic differentiation of human mesenchymal cells (MSC)/osteoprogenitor cells. *Aims.* To find the gene expression alterations in the bone microenvironment cells and their potential relationships with the occurrence of bone lesions in multiple myeloma (MM) patients. *Methods*. MSC and OB cells were isolated, without *in vitro* differentiation, from bone biopsies obtained by iliac crest of 24 MM patients, 10 MGUS subjects and 8 healthy donors (N) who underwent orthopedics surgery. Bone status was determined by total X rays scan and MRI for the spine. Cell proliferation was evaluated in relationship with growth substrate (bone and glass) and cell phenotype by flow cytometry and immunohistochemistry. Gene expression profiling analysis of isolated MSC and OB cells was performed using GeneChip® Affymetrix HG-U133A arrays. Hierarchical agglomerative clustering and dendrogram generation were used to search for natural groupings in the profiles of the most variable genes across the whole panel using the dChip software. Supervised analysis was performed using the Significant Analysis of Microarrays software. *Results*. Both MSC and OB cells showed a higher cell doubling rate in MM patients as compared to N. Higher expression of alkaline phosphatase and *Runx2* was observed in OB as compared to MSC cells in both N and MM patients without osteolytic lesions, but not in osteolytic ones. An unsupervised analysis of the most variable genes across the dataset generated a hierarchical clustering with the two major branches distinguishing MSC and OB cellular types. A multi-class analysis of N, MGUS and MM patients identified 43 differentially expressed genes in MSC cells, mainly characterizing N *vs* MM and MGUS samples. A supervised analysis between N and MM samples identified 78 genes (57 up-regulated and 21 down-regulated) differentially expressed in MSC and 29 genes (16 up-regulated and 13 down-regulated) in OB. Notably, genes encoding the Homeobox class proteins, such as HOXB2-6-7, were up-regulated in both MSC and OB of MM patients as compared to N. As regards the bone status, a total of 36 up-regulated and 9 down-regulated genes were found differentially expressed in MSC from osteolytic *vs* non-osteolytic MM patients, whereas MGUS-MSC exhibited an intermediate transcriptional profile between osteolytic and non-osteolytic MM patients. A distinct pattern of gene expression profiling was also observed in MSC *vs* OB when osteolytic and non-osteolytic MM patients were compared (51 *vs* 20 differentially expressed genes, respectively). On the other hand, few genes were found differentially expressed in OB cells in relationship with the presence of bone lesions. CONCLUSIONS. A distinctive transcriptional fingerprint was identified in isolated MSC and OB cells of MM patients as compared to N subjects, which mainly correlated with cell proliferation. Moreover, a different gene expression profile was observed in MSC cells of MM patients according to the presence/absence of bone lesions, highlighting the critical role of the block of the osteogenic differentiation.

0189

THE TYROPHOSTIN ADAPHOSTIN (NSC680410) INHIBITS MULTIPLE MYELOMA BONE MARROW ANGIOGENESIS IN *IN VITRO* AND *IN VIVO*

K. Podar, J. Zhang, G. Tonon, M. Sattler, S. Vallet, M.S. Raab, D. Chauhan, K.C. Anderson

Dana-Farber Cancer Institute, BOSTON, USA

Background. Marked anti- proliferative activity of the tyrophostin adaphostin (NSC680410) was previously demonstrated in a variety of hematologic malignancies including chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), acute myelocytic leukemia (AML), and Multiple Myeloma (MM). Specifically, in MM we identified a new mechanism by which drug- induced upregulation of c-Jun inhibits proliferation and induces apoptosis via caspase- triggered c-Abl cleavage. *Aims*. To demonstrate an antiangiogenic role for adaphostin in MM and to delineate molecular mechanisms mediating these effects. *Methods and Results*. Here we show that adaphostin (NSC680410), similar to bortezomib, additionally inhibits tumor angiogenesis within the MM bone marrow (BM) microenvironment. Specifically, adaphostin induces marked downregulation of total and nuclear c-Myc protein expression in MM cells followed by a significant Hif-1 decrease. Consequently, VEGF secretion is downregulated in a dose- and time-dependent manner. In contrast, neither specific knockdown of c-Abl expression nor exogenous overexpression of caspase- cleavage- induced c-Abl fragment abrogate adaphostin- induced c-Myc and Hif-1α downregulation. Taken together, these results indicate the existence of a c-Myc-/ Hif-1α- dependent, but c-Abl- independent, pathway modulating MM cell production and secretion of VEGF. Our data further demonstrate activity of adaphostin within the BM microenvironment. Specifically, adaphostin inhibits maturation of osteoclasts and, similar to bortezomib, VEGF secretion triggered by adhesion of MM cells to BMSCs and mature osteoclasts. Consequently, conditioned media derived from adaphostin-treated co-cultures markedly inhibit endothelial cell growth and tubule formation in a dose- dependent manner. In addition, adaphostin inhibits MM cell adhesion to extracellular matrix proteins vitronectin, fibronectin, and osteopontin. Functionally, these effects are predominantly mediated via adaphostin- induced downregulation of β3- integrin protein and activity and disturbance of lipid rafts within the plasma cell membrane as evidenced both by use of β3- integrin neutralizing antibodies and deletion of caveolin-1 by antisense methodology. Furthermore, we also demonstrate a direct anti- angiogenic effect of adaphostin induced by inhibition of endothelial cell proliferation, and the induction of cell apoptosis. Finally, these *in vitro* data were confirmed using an *in vivo* xenograft mouse model of human MM. Specifically, marked tumor growth inhibition was accompanied by both downregulation of Hif-1α and CD31 expression in tumors isolated from adaphostin- treated animals *vs* control animals as evidenced by western blot analysis, as well as immunohistochemistry. *Summary/conclusions*. In summary, these data identify new molecular mechanisms leading to VEGF production and secretion in the MM bone marrow microenvironment and provide the rationale for the clinical evaluation of adaphostin to target both MM cells and the BM milieu to improve patient outcome in Multiple Myeloma.

0190

EARLY CONSOLIDATION WITH BORTEZOMIB, THALIDOMIDE AND DEXAMETHASONE IN MM PATIENTS IN CR OR VGPR FOLLOWING AUTOLOGOUS TRANSPLANTATION INDUCES MOLECULAR REMISSIONS

A. Palumbo,[1] F. Cavallo,[1] G. Pagliano,[1] S. Ferrero,[1] L. Santo,[1] V. Magarotto,[1] P. Pregno,[2] M. Grasso,[2] A.M. Liberati,[2] T. Caravita,[2] F. Pisani,[2] T. Guglielmelli,[2] C. Crippa,[2] L. De Rosa,[2] D. Drandi,[1] M. Boccadoro,[1] M. Ladetto[1]

[1]A.O.U. San Giovanni Battista, TORINO, Italy; [2]Italian Multiple Myeloma Network, GIMEMA, ITALY, Italy

Background. Autologous transplantation (ASCT) is unable to induce molecular remission (MR) in MM as opposed to allogeneic transplantation. The achievement of MR is an important requisite for long-term disease control. It is unknown whether the use of new non-chemotherapeutic agents following ASCT might ensure further cytoreduction allowing patients to enter this status which seems associated with a reduced risk of relapse. *Aims*. In this study an early consolidation regimen including Bortezomib, Thalidomide and Dexamethsone (VTD) was employed in patients achieving CR or VGPR in the post-ASCT setting and strict molecular monitoring was performed by qualitative and quantitative PCR to evaluate if VTD may induce further cytoreduction and to verify if a proportion of those patients could enter MR. *Methods.* Patients were eligible if they had the following: 1) presence of a molecular marker based on the IgH rearrangment; 2) a documented complete or very good partial remission (CR or VGPR) following ASCT. The VTD had to be started within 6 months from ASCT for a total of 4 cycles. Each cycle consisted of: a) Bortezomib at the dose of 1.6 mg/m^2 as an IV injection once weekly (on days 1, 8, 15, 22) followed by a 13-day rest period (days 23-35); b) Thalidomide at the initial dose of 50 mg/day PO once daily, with increments of 50 mg every 7 days to acceptable tolerance (maximum 200 mg); c) Dexamethasone 20 mg/day PO once daily, on days 1 to 4, 8 to 11 and 15 to 18 followed by a 17-day rest period (days 19-35). Minimal residual disease (MRD) was evaluated on bone marrow samples at study entry, after 2 courses of treatment, at the end of treatment and then at six months intervals. MRD was evaluated using clone-specific primers by nested PCR and real time PCR. *Results.* 40 patients were enrolled and were valuable at study entry: 94% of them were PCR-positive following ASCT. 18% of patients did not receive the whole planned treatment, including one toxic death. 12 patients improved their responses from ASCT: 9 patients (36%) from VGPR to CR, 3 patients (12%) from nearCR to CR. 17% of patients converted to PCR negativity after 2 VTD courses and 27 patients are valuable at the end of the program with a PCR-negativity rate of 22%. Subsequent follow-up samples are available in 22 patients. MR persisted in all PCR-negative patients assessed at 6 months (four cases) and at 6 and 12 months (two cases). Seven relapses/progressions occurred and were all among PCR-positive patients. Real-time PCR has been performed in ten persistently PCR-positive patients with a >0.5 log tumor reduction in eight of them (median 1.2 log; range 0.2-2.1). *Conclusions*. VTD consolidation can convert a proportion of CR/VGPR MM patients from PCR-positivity to PCR-negativity and has a measurable anti-tumor effect also in persistently PCR-positive patients. In this study we demonstrate that new non chemotherapeutic agents have activity on MRD persisting following ASCT and indicate that MR is an achievable goal also outside the allogeneic transplantation setting.

0191

DISTINCT PATHOGENIC SUBGROUPS IN MULTIPLE MYELOMA DEFINED BY QUANTITATIVE GENE EXPRESSION, GENOMIC ABERRATIONS, AND VDJ GENE STRUCTURE

D. Kienle,[1] P. Liebisch,[1] C. Vatter,[1] E. Wiedenmann,[1] A. Brodbeck,[1] P. Lichter,[2] H. Döhner,[1] S. Stilgenbauer[1]

[1]University of Ulm, ULM; [2]German Cancer Research Center, HEIDELBERG, Germany

Background. Clinical course of multiple myeloma (MM) is highly variable. Recurrent chromosomal aberrations such as translocations, deletions or chromosomal extra copies may lead to a dysregulation of critical genes (e.g. affecting *CCND1, CCND3, FGFR3, MAF, TP53*) resulting in plasma cell immortalization. Some of these chromosomal abnormalities were associated with poor prognosis such as translocation t(4;14) and deletion 17p13, however, the mechanisms mediating the aggressive clinical course are poorly understood. Additionally, a restricted usage of specific VH genes has been reported implicating a role for specific B-cell receptor rearrangements in MM biology. *Aims*. To identify potential pathomechanisms and disease subgroups in MM patients based on the analysis of interrelations between genomic abnormalities, quantitative expression of genes involved in critical genomic regions and MM pathogenesis, and VDJ configuration. *Methods*. Purification of plasma cells of 117 patients to a median percentage of 85% was reached using immunomagnetic separation. FISH screening for the detection of genomic abnormalities at 1q21.2, 9q34, 11q25, 13q14, 17p13, and 14q32, as well as t(4;14), and t(11;14) was performed in all cases. Transcripts of the following candidate genes were quantified using real-time RT-PCR: *CCND1* (coding region and 3`UTR), *CCND2, CCND3, FGFR3, MAF, MMSET, MUM1, TACC, RB1, p16, E2F1, p27, p21, CDK4, TP53, ATM, MDM2, MCL-1, BCL-XL, BCL-2, c-MYC, BTBD3, ITGB7, CX3CR1, TYMS, FNTA, EIF3S12, and CXCL12*. VDJ configuration was analyzed in 100 cases by direct sequencing. *Results*. Major genomic subgroups could be defined by 1) t(4;14) (20% of all cases), or 2) t(11;14) (25%), or 3) no abnormality at 14q32 (14q32normal; 39%). In 16% of the cases the translocation partner remained unknown. Compared to 14q32normal cases, the t(4;14) subgroup showed a characteristic overexpression of *FGFR3* and *MMSET* (critical genomic region) but also of *CCND2* and CDK4. In addition to a high-level overexpression of *CCND1*, the t(11;14) subgroup was characterized by a significant overexpression of *CCND3* and BCL2, and a downregulation of *BTBD3*. Upregulation of *CCND1* was observed in approx. 50% of 14q32normal cases when compared with t(4;14) cases (median expression 80 *vs* 0.4) but was lower than in t(11;14) cases (median 915). No gene-dosage deregulation of RB1, TP53 or MCL1 transcript levels could be detected in cases harboring a genomic alteration at the respective locus (13q14, 17p13, 1q21). Most MM exhibited heavily mutated VH genes, (median VH homology 92.7%). The t(4;14) subgroup showed a significantly lower VH homology compared to the t(11;14) and the 14q32normal subgroup (median 89.2%, 93.2%, and 92.9%). VH gene usage was balanced between the genomic subgroups except for V3-21, which was exclusively observed in cases with t(4;14). *Conclusions*. Genomic subgroups defined by t(4;14), t(11;14), and 14q32normal were associated with highly characteristic gene expression patterns pointing to distinct biologic subgroups, which is supported by the non-random distribution of VH mutations and VH gene usage. Aberrant expression of candidate genes from critical genomic regions was associated with a deregulation of cell cycle and apoptosis regulators pointing to underlying pathomechanisms. To elucidate the clinical impact of these findings, clinical data will be supplemented and correlated with the molecular features.

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EFFECT OF LENALIDOMIDE-BASED REGIMENS ON BONE REMODELING IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

E. Terpos,[1] D. Christoulas,[2] E. Kastritis,[2] E. Katodritou,[3] M.C. Kyrtsonis,[4] X. Papanikolaou,[4] D. Maltezas,[4] N. Stavropoulos,[4] K. Tsionos,[4] P. Repousis,[4] G.A. Pangalis,[4] K. Zervas,[3] O. Sezer,[5] M.A. Dimopoulos[2]

[1]251 General Air Force Hospital, ATHENS, Greece; [2]Department of Clinical Therapeutics, University of Athens Medical School, ATHENS, Greece; [3]Department of Hematology, „Theagenion Cancer Center, THESSALONIKI, Greece; [4]Greek Myeloma Study Group, GREEK SOCIETY OF HEMATOLOGY, Greece; [5]Department of Hematology and Oncology, Charité - Universitaetsmedizin Berlin, BERLIN, Germany

Background/Aims. Lenalidomide plus dexamethasone is very effective for the management of refractory/relapsed multiple myeloma (MM). However, there is very little information for its effect on myeloma bone disease. The aim of this study was to evaluate the effect of lenalidomide-based regimens on bone remodeling in relapsed/refractory MM. *Patients/Methods*. We evaluated 71 patients (44M/27F; median age 67 years) with refractory/relapsed MM: 58 received lenalidomide at the standard dose of 25 mg/day x 21d, every 28 days, with either high (n=38) or low (n=20) dose dexamethasone (RD), while 13 patients received the combination of lenalidomide (15 mg/day x 14d), low dose dexamethasone plus bortezomib (1 mg/m^2, on days 1,4,8,11 every 21 days; BDR). All but one patient were under zoledronic acid therapy; this patient had developed ONJ and refused to re-start bisphosphonate. The following serum indices were measured on cycle 1/day 1, and then on the last day of cycles 3, 6, and 9: (i) osteoblast inhibitor dickkopf-1 (Dkk-1); (ii) osteoclast regulators: *sRANKL* and osteoprotegerin (OPG); (iii) bone resorption markers: CTX and TRACP-5b; and (iv) bone formation markers: bone-specific alkaline phosphatase (bALP) and osteocalcin. These markers were also evaluated in 35 healthy controls of similar gender and age.

Results. At baseline, 21 patients had no lytic lesions (group A), while 11 had 1-3 lytic lesions (group B) and 39 had >3 lytic lesions and/or a pathological fracture (group C) in skeletal survey. Patients at baseline had increased levels of Dkk-1, sRANKL, and bone resorption markers (p<0.01) and reduced levels of osteocalcin (p<0.01) compared to controls. Group C patients had increased sRANKL/OPG, CTX, TRACP-5b, Dkk-1 and reduced OC compared with all others (p<0.02 for all comparisons). To-date, 50 patients have completed 3 cycles of therapy (38 RD and 12 BDR), while 26 have completed 6 cycles (19 RD and 7 BDR) and 12 (10 RD and 2 BDR) 9 cycles of therapy. The objective response was 58% in RD (34/58 patients) and 53% (7/13) in BDR. The administration of lenalidomide-based regimens produced a reduction of sRANKL and sRANKL/OPG ratio after 6 cycles of therapy (p=0.02) that continued after 9 cycles. Furthermore, a reduction of Dkk-1, which was started after 3 cycles (p=0.01) and continued after 6 and 9 cycles of therapy was accompanied by an increase in bALP during the same period (p<0.01). The reduction of Dkk-1 and the increase of bALP was higher in BDR than in RD (p=0.03 and 0.006, respectively). Moreover, BDR patients showed also a dramatic increase of osteocalcin after the 3rd cycle (p<0.001). During study period the patient who did not receive bisphosphonate had a pathological fracture during the 5th RD cycle. No other SREs were observed. *Summary and Conclusions*. Lenalidomide-based regimens affect bone metabolism through the reduction of sRANKL/OPG and Dkk-1 levels. BDR may have a stronger beneficial effect on bone formation than RD, reflecting the bone anabolic effect of bortezomib and/or the lower dose of dexamethasone used in these patients. Further studies are needed to exact final conclusions for the effect of lenalidomide on bone metabolism in relapsed/refractory MM.

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GENE EXPRESSION PROFILES FOR MOLECULAR CLASSIFICATION OF MUTLIPLE MYELOMA

A. Broyl,[1] D. Hose,[2] Y. Knegt, de,[3] H. Lokhorst,[4] H. Goldschmidt,[4] M. Duin, van,[1] P. Sonneveld[1]

[1]Erasmusmc, ROTTERDAM, Netherlands; [2]University of Heidelberg, HEIDELBERG, Germany; 3Erasmus University Medical Center, ROTTERDAM, Netherlands:[4]Utrecht University Medical Center, UTRECHT, Netherlands

Background. Multiple Myeloma (MM) is a malignant plasma cell disorder, characterized by presence of a monoclonal protein, immunodeficiency, anemia, renal failure and bone lesions. In newly diagnosed patients the median response rates vary from 50% with Melphalan/Prednisone to 80% with high-dose therapy (HDT). New agents like Bortezomib (Bor), a proteasome inhibitor, and Thalidomide (Thal), an antiangiogenic and immunomodulatory drug, have shown efficacy in 40% of patients with relapsed/ refractory MM and are now studied in first-line treatment. Classical prognostic classifications like serum beta2-microglobulin, albumin and chromosomal aberrations have insufficient predictive power to estimate long term outcome with these targeted agents. We have set out to design a prognostic index based on molecular and disease related markers of MM. *Aims. We* have included 800 patients with MM in a large multicenter, prospective phase III trial to evaluate the effect of targeted therapy with HDT. In order to gain new insights in the pathogenesis of MM to design a prognostic index based on molecular profiling, gene expression profiles (GEP) were assessed in purified myeloma cells obtained at diagnosis. Hierarchical clustering was performed to define subgroups of patients with unique pathogenetic events. *Methods*. GEP of 204 CD138 magnetic cell selected (MACS) myeloma plasma cells with a PC purity >80% was performed using Affymetrix GeneChip U133 plus 2.0 arrays. Analysis of the gene arrays included hierarchical clustering to subdivide groups of myeloma patients and identification of differentially expressed genes in the different groups using BRB-array tools, which we verified using RT-PCR. All patients were treated according to trial protocol with Bor in combination with Adriamycin, Dex (PAD, (arm A) followed by HDT or with Vincristine, Adriamycin and Dex (VAD, arm B) followed by HDT (HOVON65/GMMG-HD4). *Results*. Based on correlated GEPs and 3 known translocations involving the immunoglobulin heavy chain region at 14q32, we subdivided myeloma patients into clusters. We determined differentially expressed genes in the different clusters and correlated the clusters with chromosomal aberrations like 13q loss, 1q loss, gain of 9, 11 and 15 and with relevant clinical data like bone lesions. We describe the clusters showing distinct gene expression signatures in combination with specific chromosomal aberrations and clinical characteristics. *Summary/ Conclusions*. Hierarchical cluster analysis in combination with 3 known translocations involving the immunoglobulin heavy chain region at 14q32 led to the subdivision of myeloma patients into clusters, each with a specific molecular signature, and/or unique chromosomal patterns. The response and survival data of the molecular subgroups have been partly identified and will be further analyzed.

0194

A CELLULAR PROTEOME MAP OF HUMAN MULTIPLE MYELOMA

W. Drach,[1] A. Slany,[2] V. Sagaster,[3] N. Gundacker,[2] V. Haudek,[2] H. Wimmer,[2] C. Zielinski,[3] C. Gerner[2]

[1]Medical University of Vienna, VIENNA; [2]Medical University of Vienna, Institute for Cancer Research, VIENNA; [3]Medical University of Vienna, Dept. of Medicine I, VIENNA, Austria

Background and Aims. Molecular profiling identifies proteins characteristically deregulated in malignant diseases. Characteristic biomarkers may be useful to support diagnosis and patient stratification, while the recognition of aberrant cell activities and cell survival strategies may lead to the development of specifically designed pharmacologic strategies. We therefore performed proteomic profiling of primary human multiple myeloma (MM) cells in order to define the impact of protein expression abnormalities in MM. *Methods*. Plasma cells were isolated from bone marrow of patients with MM or MGUS and forwarded to proteome analysis based on 2D gel electrophoresis in addition to shotgun analysis by nano-LC-MS/MS. Erythrocytes, platelets and plasma as well as quiescent and activated lymphocytes, monocytes, endothelial and dendritic cells from healthy donors were processed in an identical manner for comparative analysis. The resulting data were interpreted with the aid of a home-made SQL database. *Results*. Among about thousand proteins identified in MM cells, we found aberrant expression of proteins involved in fatty acid beta-oxidation (VLCAD), unfolded protein response/ER-stress (ARMET protein, cytosol aminopeptidase), oxidative stress (ste20/oxidant stress responsive kinase 1), interferon response (MX1), iron uptake (transferrin receptor/CD71), DNA modification (transforming protein ERG, methyltransferase-like protein 7A), apoptosis and survival (apoptosis-inducing factor 1, hsp75), protein synthesis (ribosome-binding protein, Unc-13 D), cell adhesion (CD9/tetraspanin-29, LYRIC/metastatic adhesion protein), signaling (sts-1) and cell-cell interaction (Cystatin F, basigin/collagenase stimulatory factor, stem cell growth factor, small inducible cytokine B7, PD-ECGF/Gliostatin). *Conclusion*. The presently applied proteome analysis strategy, based on the systematic investigation of purified primary human cells, allowed us to find characteristic alterations in MM cells. Several proteins directly relate to known aberrant cell activities such as elevated protein synthesis and secretion resulting in ER-stress, which makes the cells sensitive to proteasome inhibitor treatment. Work is in progress to use quantitative assessment of such proteins for patient stratification, identification of response predictors, and biomarkers for the distinction of MM and MGUS.

0195

SERUM ANGIOPOIETIN-1 TO ANGIOPOIETIN-2 RATIO IS AN INDEPENDENT PROGNOSTIC FACTOR FOR SURVIVAL IN NEWLY DIAGNOSED PATIENTS WITH MULTIPLE MYELOMA: RESULTS FROM THE GREEK MYELOMA STUDY GROUP ON 174 PATIENTS

E. Terpos,[1] K. Anargyrou,[1] E. Katodritou,[2] E. Kastritis,[3] A. Pouli,[4] E. Michali,[5] S. Delimpasi,[6] E. Verrou,[2] D. Margaritis,[7] K. Tsionos,[1] K. Stefanoudaki,[8] K. Zervas,[2] M.A. Dimopoulos[3]

[1]251 General Air Force Hospital, ATHENS; [2]Department of Hematology, „Theagenion„ Cancer Center, THESSALONIKI; [3]Department of Clinical Therapeutics, University of Athens Medical School, ATHENS; [4]Department of Hematology, St Savvas Anticancer Hospital, ATHENS; [5]Department of Hematology, G. Gennimatas„ General Hospital, ATHENS; [6]Department of Hematology, Evangelismos General Hospital, ATHENS; [7]Department of Hematology, Democritus University of Thrace School of Medicine, ALEXANDROUPOLIS; [8]Department of Hematology, A. Fleming General Hospital, ATHENS, Greece

Background and Aims. Angiogenesis represents an essential step of disease progression in several hematological malignancies, including multiple myeloma (MM). Angiopoietin-1 (Ang-1) and its natural antagonist angiopoietin-2 (Ang-2), both ligands for the receptor tyrosine kinase Tie-2, are essential cytokines for angiogenesis process. Maturation and stabilization of the vascular wall are critically regulated by Ang-1 binding to Tie-2 receptor, while Ang2- antagonizes Tie-2 binding and induces vessel destabilization, which leads to the angiogenic sprouting. Vascular endothelial growth factor (VEGF), angiogenin and basic fibroblast growth factor (bFGF) are also potent stimulators of both physiological

and pathological angiogenesis. The aim of this study was to evaluate the serum levels of the above angiogenesis cytokines and explore possible correlations with clinical data, including survival, in newly diagnosed, untreated, MM patients. *Patients and Methods*. One hundred and seventy-four newly-diagnosed MM patients were evaluated (92M/82F; median age: 66 years, range: 40-94 years). According to ISS, 55 patients had stage 1, 61 stage 2 and 58 stage 3 myeloma. Serum levels of Ang-1, Ang-2, VEGF, VEGF-A (the major angiogenesis component of VEGF), angiogenin, and bFGF were evaluated using ELISA methodology (R&D Systems, Minneapolis, MN, USA, for all, except VEGF-A: Diaclone, Bensancon, France). *Results*. MM patients had increased serum levels of Ang-2 ($p<0.0001$) and angiogenin ($p<0.0001$), and a borderline increase of VEGF-A ($p=0.08$) compared with 21 controls of similar age and gender. Ang-1 levels were not different between patients and controls; thus the ratio of Ang-1/Ang-2 was reduced in MM. Patients with ISS 3 had increased serum levels of angiogenin and reduced Ang-1/Ang-2 ratio compared with all others ($p=0.003$ and 0.04, respectively). Interestingly, patients with lytic disease (n=97) had increased levels of Ang-2 compared with those who had no lytic lesions in skeletal survey ($p=0.008$). Ang-2 correlated with LDH (r=0.267, $p=0.0001$), CRP (r=0.24, $p=0.007$), VEGF (r=0.317, $p<0.0001$) and bFGF (r=0.29, $p=0.0001$), while angiogenin correlated with β2-microglobulin (r=0.249, $p=0.001$) and Hb (r=-0.265, $p=0.001$). The median survival of all patients was 47 months and the median follow-up was 19.6 months. The univariate analysis revealed that ISS stage ($p<0.001$), serum LDH ($p=0.003$), age ($p=0.02$), bone disease status ($p=0.0001$), serum creatinine ($p=0.017$), and the ratio of Ang-1/Ang-2 predicted for survival. Patients with serum Ang-1/Ang-2 of below or equal to the median value of 6.03 had a median survival of 27.8 months, while patients with Ang-1/Ang-2 values of above this median value had a median survival of 53 months ($p=0.033$). The multivariate analysis based on a Cox regression model revealed that only serum LDH ($p=0.001$), Ang-1/Ang-2 ratio ($p=0.001$) and bone disease status ($p=0.001$) could independently predict for survival in our cohort of patients. *Summary & Conclusions*. These results reveal for the first time in MM patients the correlation of reduced Ang-1/Ang-2 ratio with advanced disease and highlight the role of Ang-1/Ang-2 pathway in the biology of plasma cell growth as reflected by its influence on survival. These observations reveal Ang-1/Ang-2/Tie-2 system as a possible target for the development of novel anti-myeloma agents.

0196

MICRORNA GENES ASSOCIATED WITH GENOMIC ALTERATIONS OR GENE EXPRESSION MODULATION CONTRIBUTE TO ENHANCE GENETIC HETEROGENEITY IN MULTIPLE MYELOMA

M. Lionetti, L. Mosca, L. Agnelli, D. Ronchetti, S. Fabris, A Andronache, L. Nobili, G. Ciceri, G. Lambertenghi-Deliliers, A. Neri

University of Milan, Fondazione IRCCS Ospedale Policlinico, MILAN, Italy

Background. Multiple myeloma (MM) is characterized by high genomic instability affecting both ploidy and structural rearrangements, as well as frequent copy number (CN) variations of specific chromosomal regions. It was reported that miRNA-genes often reside in regions representing hot spots for chromosomal abnormalities, which may contribute to their deregulation, and it was demonstrated that intronic miRNAs expression levels are frequently associated with those of the corresponding host-gene. *Aims*. Identification of molecular pathways potentially involved in MM pathogenesis using integrative analysis of genome-wide and gene expression profiling and micro-RNA expression data, in a panel of human myeloma cell lines (HMCLs). Methods. CN data were generated on Affymetrix GeneChip® Human Mapping 250K Nsp arrays, and the local DNA CN variations were calculated using the DNAcopy Bioconductor package based on circular binary segmentation. The miRNA expression data were generated on Agilent miRNA microarrays (representing 470 human mature miRNAs), and normalized using the AromaLight Bioconductor package. Gene expression data were generated on GeneChip® HG-U133A arrays, and normalized using the RMA Bioconductor package. Results. Our microarray analysis in a panel of 18 HMCLs revealed the presence of gains and losses involving entire chromosomes or specific regions, at a variable frequency up to 70%. We excluded miRNA-genes having multiple genomic locations and focused on miRNAs mapped within regions showing increased CN in at least 2 HMCLs, and we selected miRNAs having expression values of at least 2-fold their mean across the whole panel. Among the identified correlations, we report miR-92b (1q22) and miR-27a (19p13.12) in 4 HMCLs; miR-23a (19p13.12) in 3; and miR-520c (19q13.1), miR-214 (1q24.3), miR-22 (17p13.3) and miR-193b (16p13.12) in 2. Most of the identified miRNAs were already reported as deregulated in different human cancers, such as miR-23a in bladder cancer; miR-27a has oncogenic activity in MDA-MB-231 breast cancer cells, stimulating survival and angiogenesis; miR-214 decreases apoptosis in HeLa cells and is deregulated in ovarian cancer, where it induces cell survival through targeting PTEN; miR-520c is involved in tumor migration and invasion. The same integrative approach was used to detect correlations between miRNAs and the corresponding host-gene expressions, looking for those miRNAs whose variation (2-fold higher or lower) to the mean across the whole panel corresponded to the same variation of the host-gene in the gene expression profile panel. A strict correlation ($p<1\times10^{-5}$) was identified between miR-335 and MEST gene (mapped to 7q32.2), and miR-342 and EVL (14q32.2); the correlation coefficient on the whole panel were 0.86 and 0.88, respectively. A minor but significant correlation was identified between miR-625 and FUT8 (14q23.3) (R=0.53, $p<0.05$). These data suggest a strict influence of the same promoter activity on miRNA and host-gene expression. *Conclusions*. We showed that miRNAs expression in HMCLs could be affected by the presence of genomic lesions or may correlate with host-genes modulation suggesting a possible role in the molecular pathogenesis of MM. Our study represents the basis for further investigations aimed at functionally characterizing specific miRNAs in MM.

0197

IDENTIFICATION OF NEOPLASTIC PLASMA CELLS BY FLOW CYTOMETRY: LOOKING FOR THE BEST MARKER(S)

P. Mancuso, A. Calleri, P. Antoniotti, J. Quarna, A. Agazzi, S. Sammassimo, G. Pruneri, G. Martinelli, F. Bertolini

European Institute of Oncology, MILANO, Italy

Background. Bone marrow (BM) examination by trephine biopsy (BM-TB) is routinely performed during staging and follow-up of patients affected by plasma cells (PCs) disorders such as multiple myeloma (MM) and MGUS. The use of multiparametric flow-cytometry (FC) is currently restricted to clinical research studies. However, the presence of monoclonal antibodies specific for PCs (CD138, CD38), and the identification of markers candidate to discriminate normal *vs* neoplastic PCs makes this procedure suitable also for clinical applications. *Aims of the study*. To compare the phenotype of $CD45^{-/dim}$ PCs *vs* $CD45^{+}$ PCs and of monoclonal *vs* polyclonal PCs. 2) To identify which neoplastic PC marker identified by FC ($CD45^{-}$, $CD19^{-}$, $CD56^{-}$, cytoplasmatic λ/γ) better correlates with BM-TB. 3) To analyze discordance between FC and BM-TB. *Methods*. By four- (2000-2005) and six-color (2005-2008) FC, we analyzed 336 bone marrow aspirates from 130 MM patients before or after treatment. PCs were identified as $CD138^{+}CD38^{++}$. A panel of monoclonal antibodies including CD45, CD19, CD56, CD20, CD117, and CD126 and citoplasmatic λ/γ was used to characterize PC phenotype. At the same time, BM-TB was performed. *Results*. We compared the phenotype of $CD138^{+}CD38^{++}$CD45-/dim *vs* $CD138^{+}CD38^{++}CD45^{+}$ PCs for $CD19^{-}$ (92 *vs* 66%) $CD20^{+}$ (15 *vs* 12%) $CD56^{+}$ (72 *vs* 57%) $CD126^{+}$ (9 *vs* 16%) $CD117^{+}$ (46 *vs* 42%) expression and cytoplasmatic λ or γ chain restriction (94 *vs* 58%). When selected for light chain restriction *vs* polyclonal PCs, the phenotype was $CD19^{-}$ (92 *vs* 30%), $CD20^{+}$ (15 *vs* 7%), $CD56^{+}$ (71 *vs* 40%), $CD126^{+}$ (14 *vs* 8%) $CD117^{+}$ (49 *vs* 16%) $CD45^{neg/dim}$ (54 *vs* 8%). Concordance between FC and BM-TB was 80% according to light chain restriction, 77% according to CD19-, 66% according to $CD56^{+}$, 60% according to CD45-/dim PC phenotype. As light chain restriction showed the highest correlation with BM-TB, we analyzed in detailed the reason of discrepancy observed in discordant samples (20% of al samples). In 14.4 % of cases FC was positive and BM-TB negative. Fifty-two percent of these samples were performed after chemotherapy and the average frequency of monoclonal PC was 1% (range 0.2-3%). Among FC-BM-TB+ samples, the most frequent reason of discordance was the dilution of bone-marrow samples with blood, as confirmed by the absence of eritroblasts and myeloid precursor as reported by morphological assessment. *Conclusions*. Combined assessment of clonality and immunophenotype by FC is an useful tool for the diagnosis and follow up of PC disorders, and seems particularly promising for the investigation of the minimal residual disease. Light-chain PC restriction showed the best rate of correlation with BM-TB. However, FC sensitivity seems to be higher when the an aberrant PC phenotype (rather than clonality) is analyzed. Further studies are ongoing to evaluate the prognostic values of CD117+ PC.

0198

MICRO-RNA15A AND MICRO-RNA16 EXPRESSION IN MULTIPLE MYELOMA

S.L. Corthals,[1] Y. Knegt, de,[2] M. Schoester,[2] H.B. Beverloo,[2] K.H. Lam,[1] P. Sonneveld[1]

[1]Erasmus University Medical Center, ROTTERDAM; [2]Erasmus Medical Center, ROTTERDAM, Netherlands

Background. Multiple Myeloma (MM) is characterized by the accumulation of malignant plasma cells in the bone marrow (BM). MM has a profound genetic instability, leading to chromosomal abnormalities. Deletion of chromosome 13 is observed in more than 50% of MM cases and is an early event in MM pathogenesis. Because of its poor prognostic impact it has become clinical practice to diagnose the presence of chromosome 13 deletions by fluorescence *in situ* hybridization (FISH) in patients with newly diagnosed MM. The adverse prognostic role of these deletions suggests the presence of MM tumor suppressor gene(s) on chromosome 13q. Micro-RNAs (miRs) are a new class of small non-coding single stranded RNAs. MiRs negatively regulate gene expression by binding to partially complementary sites in messenger RNAs (mRNAs) and may function as tumor suppressors and oncogenes. miR-15a and miR-16-1, located on chromosome 13q14, are frequently down-regulated or deleted in B-cell chronic lymphocytic leukaemia (CLL). It has been shown that miR-15a and miR-16-1 are able to bind BCL2, an anti-apoptotic protein, and negatively regulate its function. Downregulation or deletion of miR-15a or miR-16-1 might therefore result in increased expression of BCL2, subsequently inhibiting apoptosis. *Aims.* The aim of this study is to evaluate the expression of miR-15a and miR-16 in Multiple Myeloma and assess their role in the pathogenesis of Multiple Myeloma. *Methods.* Thirty-four Multiple Myeloma samples were obtained from newly diagnosed MM patients. Abnormalities of chromosome 13q14 were determined using whole genome SNP analysis using the Illumina Infinium HumanHap550 Genotyping BeadChip and FISH analysis. Probes used in this study include retinoblastoma-1 (RB1) and D13S319. The mature miR-15a and miR-16 expression levels in CD138 magnetic cell selected (MACS) myeloma plasma cells (purity >80%) were determined using the TaqMan MicroRNA Assay. Cytospin slides with MM plasma cells were used for immunocytochemical analysis of Bcl2 protein expression. *Results*. FISH analysis showed a chromosome 13 deletion in 15 patients (44.1%). However, 17 patients (50%) were determined to have chromosome 13 deletion by whole genome SNPchip analysis, indicating 2 false negative cases by FISH analysis. Both miR-15a and miR-16 are expressed in all 34 MM samples, although the level of expression varies. There was no correlation between chromosome 13 status of the MM patients and miR-15a and miR-16 relative expression levels. The Bcl2 protein expression in MM plasma cells demonstrates variation within and between patient samples, however, didn't show correlation with miR-15a or miR-16 relative expression levels. *Summary and Conclusions.* In this study we determined whether loss of chromosome 13q14 is correlated with miR-15a and miR-16 expression in Multiple Myeloma patients. We did not observe such a correlation. All chromosome 13 deletions in this study are heterozygous, therefore, compensation by the non-deleted allele could explain the expression levels of miR-15a and miR-16 detected. Furthermore, the observed miR-15a and miR-16 expression levels could be due to the fact that a highly correlated cluster of miRs located on chromosome 3 continues to be expressed. These data suggest that miR-15a and miR-16 do not explain the poor prognosis associated with chromosome 13 deletions.

0199

FREQUENCY OF C3435T POLYMORPHISM IN A NORTHERN IRISH POPULATION AND ASSOCIATIONS WITH OVERALL SURVIVAL IN MYELOMA

S. Drain,[1] M.A. Catherwood,[2] M.B. Drake,[2] P.J. Kettle,[2] T.C.M. Morris,[2] H.D. Alexander[2]

[1]University of Ulster, COLERAINE; [2]Belfast City Hospital, BELFAST, UK

Background. Multi-drug resistance has been demonstrated as a significant factor in the successful management of malignancy. One major source of this resistance has been identified as the P-glycoprotein (P-gp) drug efflux pump, which has wide substrate specificity and is one of many characterised ATP-binding cassette (ABC) membrane transporters. As this protein is encoded by the gene *MDR-1*, functional aberrations within this genomic region (7q21) are of great interest. Single nucleotide polymorphisms (SNP) have been investigated within *MDR-1* with many contributing to a change in the amino acid. The *MDR-1* C3435T SNP, previously reported as silent, has been significantly associated with altered P-gp substrate specificity due to the generation of a rare codon, leading to conformational changes in the protein product. Previous publications have demonstrated associations with this SNP and the clinical outcome of haematological malignancy such as acute leukaemia. Recent studies of multiple myeloma (MM) patients have investigated this SNP within Italian and Dutch cohorts, with a lack of concordance with regard to outcome. Thus, the role of the SNP in outcomes in MM remains unclear. *Aims*. The aim of this study was to investigate the prevalence of the C3435T SNP in a Northern Irish (NI) MM cohort compared with a NI age matched normal cohort and to determine its effect on disease outcome. *Methods*. Patients with a diagnosis of MGUS or MM (n =140) and age and gender matched healthy controls (n =140) were recruited. Preliminary SNP analysis was performed by polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) on 87 MM cases and 92 controls. Survival statistics were obtained using univariate cox proportional hazards regression analysis and Kaplan-Meier plots while the prevalence of the SNP within the NI cohort was determined using χ^2. *Results*. No significant difference was observed in the incidence of the C3435T SNP in the MM cohort compared with controls and the incidences in both groups concur with NCI recorded Caucasian prevalence. Longer overall survivals (OS) were observed for the CT and TT genotypes compared with patients with a CC genotype (p=0.014) (Figure1). Relative to CT, the hazard ratio (HR) for TT was 2.67 (95% CI, 0.96-7.40; p=0.059) and for CC was 4.75 (95% CI, 1.65-13.64; p=0.004). *Conclusions*. The prevalence of the C3435T SNP within our cohort is in keeping with NCI Caucasian data and indicates no correlation between the SNP and incidence of MM. However, overall survival of myeloma patients was significantly associated with genotype, with wild-type CC patients demonstrating shorter survival. Interestingly, the heterozygous CT genotype had longest OS, indicating an optimum phenotypic contribution for this genotype. This initial data is in concordance with previous work in an Italian MM patient cohort and supports the use of C3435T genotyping in the initial diagnosis and risk stratification of MM patients. Study of larger patient cohorts is required to confirm these findings and will allow the investigation of treatment strategy in relation to these survival trends.

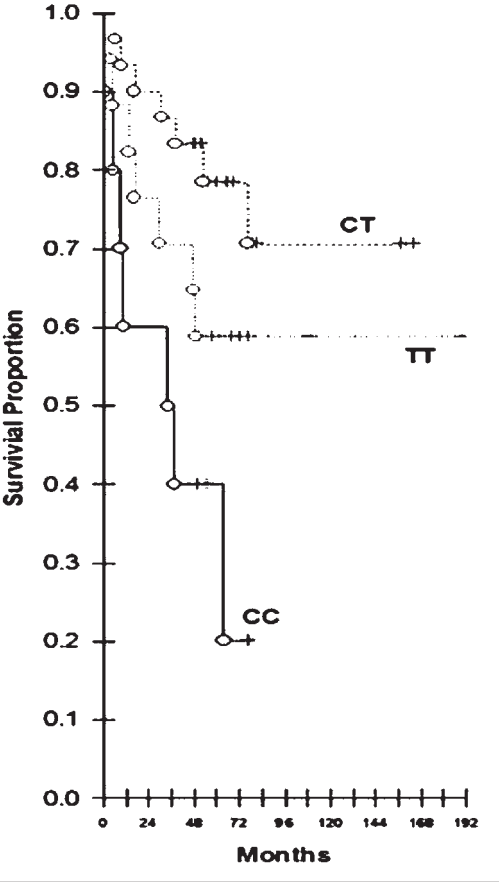

Figure 1

0200

VASCULAR ENDOTHELIAL GROWTH FACTOR, OSTEOPONTIN AND NF-kB/P65 EXPRESSION IN MULTIPLE MYELOMA

S. Stifter,[1] T. Valkovic,[2] A. Nacinovic-Duletic,[2] K. Matus an-Ilijas,[3] K. Lucin,[3] I. Seili-Bekafigo,[2] D. Rendic Petranovic,[2] N. Jonjic[3]

[1]School of Medicine, Medical University Rijeka, RIJEKA, Croatia; [2]Clinical Medical Center Rijeka, Internal Clinic, Department of Hematology, RIJEKA, Croatia; [3]Department of Pathology, School of Medicine, Medical University Rijeka, RIJEKA, Croatia

Background. Previous studies have provided insights into the pathogenesis of multiple myeloma (MM), and defects involving proliferation, apoptosis, and angiogenesis which are believed to be important. Since, chemoresistence is a major problem in the treatment of patients with MM the new strategies of therapy development have been pointed towards expansion of new molecular targets. The importance of the nuclear factor-κ B (NF-κB) in the proliferation and anti-apoptotic effect in normal and tumor cells has been determined. However, the role of NF-ÎB in the pathogenesis of the MM still remains unclear. *Aims.* The aim of this study was to analyze the angiogenic potential of myeloma cells by identification of vascular endothelial growth factor (VEGF) and osteopontin (OPN) expression in connection with NF-κB/p65 activity. *Methods*. The bone marrow trephine sections obtained from 48-patients (22 men and 26 women, median age, 68.3 years; ranged 48- 89) diagnosed with MM, including clinical data regarding clinical stage (13 stage I, 18 in stage II, and 17 in stage III, according to Durie), and therapy response were unrolled in this study. Angiogenesis was immunohistochemically evaluated by measuring the intratumoral microvessel density (MVD) with anti-CD34, while VEGF, OPN, Ki-67 and NÎF-B/p65 were analyzed by using double immunohistochemistry; CD138 being used for detection of myeloma cells. The VEGF and OPN expression was evaluated as VEGF H-score and OPN H-score, multiplying the intensity with the percentage of positive myeloma cells. *Results*. Statistical analyzes show significant correlation between VEGF expression and MVD ($p<0.001$, $rp=0.609$), and between OPN expression and activated NF-κB/p65 ($p<0.033$, $rp=0.325$). In addition, a co-expression in myeloma cell for VEGF and OPN was observed ($p<0.012$). A strong reverse correlation was found between VEGF and Ki-67 ($p<0.008$, $rp=-0.389$) and weak between OPN and Ki-67 ($p=0.098$, rp =-0.255). Cytoplasmic NF-ÍB/65 shows almost significant correlation with OPN ($p=0.055$, $rp=0.294$) and nearly opposite relationship with VEGF expression ($p=0.064$, $rp=-0.278$). *Conclusions*. Increased production of VEGF protein by myeloma cells confirmed the increased intratumoral vascularity or angiogenic potential of tumor cells. However, the autocrine mitogenic potential of VEGF and OPN by evaluating the expression of these proteins could not be established. Co-expression of these two investigated proteins, VEGF and OPN on myeloma cells should be better determined in order to elucidate their probably synchrony role in angiogenesis and other functions. Specially, since the OPN expression is associated with activated NF-κB/p65 what implies a diverse functional activity of these cells.

0201

ELEVATED SERUM TIMP-1 LEVELS CORRELATE WITH ADVANCED STAGE AND PREDICT REDUCED SURVIVAL IN NEWLY-DIAGNOSED MULTIPLE MYELOMA PATIENTS

E. Terpos,[1] M.A. Dimopoulos,[2] V. Shrivastava,[3] K. Leitzel,[3] D. Christoulas,[2] M. Migkou,[2] M. Gavrietopoulou,[2] E. Eleftherakis-Papaiakovou,[2] K. Anargyrou,[1] E. Kastritis,[2] P. Hamer,[4] W. Carney,[4] A. Lipton[3]

[1]251 General Air Force Hospital, ATHENS, Greece; [2]Department of Clinical Therapeutics, University of Athens Medical School, ATHENS, Greece; [3]Penn State University/Hershey Medical Center, HERSHEY, PA, USA; [4]Oncogene Science/Siemens HealthCare Diagnostics, CAMBRIDGE, MA, USA

Background and Aims. Tissue inhibitor of metalloproteinase-1 (TIMP-1) is a natural metalloproteinase (MMP) inhibitor that binds to and inactivates mainly MMP-9. TIMP-1 has multifunctional roles in tumorigenesis: inhibition of the catalytic activity of metalloproteinases, growth promotion, inhibition of apoptosis, and regulation of angiogenesis. Increased TIMP-1 has been associated with an unfavorable prognosis in many cancers including breast, colorectal, gastric, head and neck, lung cancer, and lymphomas. *In vitro* studies have revealed *TIMP-1* overexpression by myeloma cell lines. Furthermore, *TIMP-1* promotes myeloma cell invasion across basement membranes. The aim of this study was to evaluate the serum levels of TIMP-1 in newly-diagnosed, previous untreated myeloma patients and explore possible correlations with clinical and laboratory data, including survival. *Patients and Methods*. Fifty-five patients with newly-diagnosed myeloma (25M/30F, median age: 69 years) were evaluated. Eleven patients had stage 1 disease according to ISS, while 27 had stage 2 and 17 stage 3 myeloma. Serum TIMP-1 was determined before the administration of any therapy, including bisphosphonates, using ELISA (Oncogene Science/ Siemens HealthCare Diagnostics, Cambridge, MA, USA) along with a series of serum markers of bone remodeling (NTX, CTX, TRACP-5b, bALP and osteocalcin) and osteoblast/osteoclast regulators (dickkopf-1, sRANKL and osteoprotegerin). The above bone markers were also evaluated in 25 healthy controls of similar age and gender. *Results*. The mean serum TIMP-1 level of all patients was 431.9 ng/mL (SD 198.1 ng/mL). Twenty-six patients (17M/9F; 47%) had elevated values of TIMP-1 (upper normal limit 324 ng/mL for males and 454 ng/mL for post-menopausal women). Patients had also increased levels of dickkopf-1, sRANKL, sRANKL/OPG ratio and bone resorption markers (NTX, CTX, and TRACP-5b) ($p<0.01$ compared with healthy controls). TIMP-1 serum levels correlated with β2-microglobulin ($r=0.414$, $p<0.01$), albumin ($r=-0.416$, $p<0.01$), osteocalcin ($r=0.325$, $p=0.01$), CTX ($r=0.314$, $p=0.01$), NTX ($r=0.306$, $p=0.02$), and LDH ($r=0.295$, $p=0.03$). More importantly, *TIMP-1* correlated with ISS (ANOVA $p=0.005$). Patients with ISS 3 disease had higher levels of TIMP-1 (mean±SD 557.8±234 ng/mL) compared with those who had ISS 1 (311±90.6 ng/mL; $p=0.001$) or ISS 2 disease (405.5±165.6 ng/mL; $p=0.021$). Furthermore, patients with lytic disease (n=43) had increased levels of TIMP-1 (457.7±205 ng/mL) compared with all others (313.6±107.6 ng/mL; $p=0.05$). The median follow-up was 31 months after blood collection and 16/55 patients have died. The median survival since time of blood collection has not been reached yet. Patients who had TIMP-1 level of above the mean value had a median survival of 37 months, while in all others the median survival has not been reached yet ($p=0.04$). *Summary and Conclusions*. Our study provides evidence for the first time that increased serum level of *TIMP-1* correlates with advanced myeloma and predicts for reduced survival in myeloma patients. These results suggest that *TIMP-1* participates in myeloma pathogenesis and strongly support that serum TIMP-1 deserves further study to determine its predictive and prognostic biomarker potential in a larger cohort of myeloma patients.

0202

INCREASING FREQUENCY OF MCL-1 mRNA EXPRESSION THROUGH THE STAGES OF MULTIPLE MYELOMA

A.K. Mylin,[1] T. Rasmussen,[2] M. Lodahl,[2] I.M. Dahl,[3] L.M. Knudsen[4]

[1]Rigshospitalet, University of Copenhagen, COPENHAGEN, Denmark; [2]Herlev Hospital, University of Copenhagen, HERLEV, Denmark; [3]Tromsø University Hospital, TROMSØ, Norway; [4]Odense University Hospital, ODENSE, Denmark

Background. The protein Mcl-1 (Myeloid cell leukaemia-1), a member of the Bcl-2 (B-cell lymphoma/leukaemia-2) family of antiapoptotic proteins, is demonstrated to be critical for proliferation and survival of myeloma cells *in vitro*, suggesting that Mcl-1 is an attractive target for future therapies in multiple myeloma (MM) (1). Furthermore, a recent study has shown that overexpression of Mcl-1 protein in myeloma cells *in vivo* is associated with relapse and short event-free survival (2). *Aims.* Here, we investigated frequency and prognostic impact of Mcl-1 mRNA expression in myeloma cells from a large group of patients with different stages of MM including the premalignant stage monoclonal gammopathy of undetermined significance (MGUS). *Methods.* The study population consisted of 10 MGUS patients and 113 newly diagnosed MM patients with immunophenotypic aberrant plasma cells in BM aspirates. All MGUS patients had IgG or IgA isotype and were stable for minimum 24 months after BM sampling. Forty MM patients received intensive therapy with high-dose chemotherapy followed by autologous stem cell transplantation (ASCT), 63 MM patients received conventional therapy, and the remaining 10 MM patients were observed without treatment. All samples were obtained after informed consent according to the Helsinki II declaration. Five human myeloma cell lines (HMCL) were also investigated. After fluorescence activated cell sorting (FACS) of 100 immunophenotypic aberrant plasma cells directly into polymerase chain reaction (PCR) tubes, a cDNA archive was generated using global reverse transcription (RT)-PCR. Expression levels of Mcl-1, Bcl-2 and Bcl-xL normalized to β-actin were determined in each cDNA archive by real-time PCR. Normalized mRNA expression above 10-5 was considered positive. *Results.* Mcl-1 mRNA expression in myeloma cells was seen in 20% of MGUS patients compared to 72% of MM patients (p=0.0008) and all of the HMCL investigated (p=0.003). Among MM patients, there was a trend towards Mcl-1 expression being more frequent in patients with advanced disease defined by Durie-Salmon (DS) stage (64% DS I, 58% DS II, 80% DS III; p=0.049). The median level of mRNA expression did not differ among the groups investigated. Mcl-1 (p=0.03) and especially Blc-2 (p= 0.004) mRNA expression was associated with high age. The MM patients were followed for up to 116 months (median 29 months; range 1 - 116 months), and 74 deaths were registered. Mcl-1, Bcl-2 or Bcl-xL mRNA expression had no impact on overall survival. Forty MM patients receiving intensive therapy were followed for up to 77 months after ASCT (median 28 months; range 0 - 77 months). During this period, 18 patients experienced relapse or disease progression demanding reinstitution of therapy and 3 patients died. Mcl-1 mRNA expression at diagnosis lacked association with event-free survival after intensive therapy. *Summary and Conclusions*. Mcl-1 mRNA expression in myeloma cells rise in frequency through the stages of MM, but lack association with overall and event-free survival.

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0203

MOLECULAR CYTOGENETIC ANALYSIS OF WALDENSTROM'S MACROGLOBULINEMIA IN KOREA

J.W. Seo,[1] S.M. Bang,[2] K.U. Park,[2] S.S. Yoon,[1] D.S. Lee,[1] H.J. Kim,[3] K.H. Kim,[3] S.R. Cho,[4] H.C. Kim,[4] J.W. Song,[5] J.S. Kim,[5] K.W. Kim,[6] J.H. Lee,[6] J.J. Lee,[7] M.K. Shin,[7] C.W. Seo,[8] H.S. Chi,[8] D. Oh,[9] J.H. Won[10]

[1]Seoul National University Hosp., SEOUL; [2]Seoul National University Bundang Hosp., SEONGNAM; [3]Sunkyunkwann University College of Med., SEOUL; [4]Ajou University Hospital, SUWON; [5]Yeonsei University College of Medicine, SEOUL: [6]Gachon University Gil Hospital, INCHEON; [7]Chonnam National University Medical School, GWANGJU; [18]University of Ulsan College of Medicine, SEOUL; [9]Pochon CHA University College of Med., SEONGNAM; [10]Soonchunhyang University Hospital, SEOUL, South-Korea

Background. Waldenstrom's macroglobulinemia (WM) is a malignant lymphoproliferative disorder associated with infiltration of the bone marrow by pleomorphic B-lineage cells and monoclonal IgM production. High incidence of 6q deletion and rarity of IgH rearrangement discriminates WM from other B cell malignancy. *Aims*. To compare the molecular cytogenetic characteristics between WM and multiple myeloma (MM), we performed interphase fluoresecent *in situ* hybridization (FISH) study in Korean patients with WM and MM. *Mehtods*. Thiry nine patients who fulfilled the diagnosis of WM and 132 patients with MM were enrolled for the characterization of cytogenetic changes using conventional cytogenetics and FISH. The sex ratio was 3.9:1.0 (male 31, female 8) and the mean age at the diagnosis was 63 year (range form 40 to 87 year) in WM group. FISH analysis combined with cytoplasmic immunoglobulin light chain staining was performed on bone marrow mononuclear cells or air-dried bone marrow smear, using 6 kinds of probes (6q21, 6q23, IgH rearrangement, p16 gene, RB1 gene, 1q25), which included 2 different regions of long arm of chromosome 6. A cut-off of 10% or greater was used for the interpretation of FISH combined with immunophenotyping.

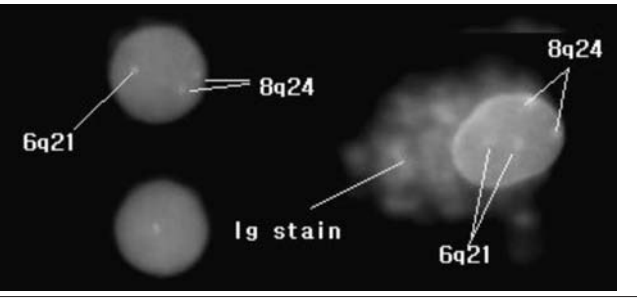

Figure 1. 6q deletion in only small lymphocyte

Results. Out of 23 successful karyotypes, 26% showed abnormal karyotypes, including 1 case of 6q deletion (4%). With FISH method, deletions of 6q23 were observed in 3/29 cases (10%), followed by deletions of 6q21 in 3/31 cases (10%), IgH translocation in 1/29 case (3%), gain of 1q in 1/26 case (4%). Deletion of 6q was hemizygous in all 3 cases. In one WM patient, 6q deletion was observed in only small lymphocytes, but not in plasma cells. In MM group, frequency of 6q deletion was 0% (0/30, FISH for 6q deletion was performed in 30 patients with MM), while RB1 deletion was most frequent change (45%, 59/132), followed by IgH translocation (41.8%, 55/132) , and gain of 1q (38.8%, 50/132). *Conclusions*. In Korean WM patients, deletion of long arm of chromosome 6 (10%) was lower than the reported frequencies of caucacians. WM showed markedly lower frequency of IgH translocation (3%) and trisomy 1q (4%), compared to those of MM (41.8% and 38.8%, respectively), which suggested that MM and WM are the different disease entites.

0204

PHENOTYPE OF PLASMA CELLS IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND MULTIPLE MYELOMA SUBJECTS

L. Kovarova,[1] I. Buresova,[1] R. Suska,[1] L. Pour,[2] L. Zahradova,[1] V. Sandecka,[1] M. Penka,[1] R. Hajek[3]

[1]University Hospital, BRNO, Czech Republic; [2]University Research Center - Czech Myeloma Group, Masaryk University, BRNO, Czech Republic; [3]Dept. of Hemato-Oncology, University Hospital, Brno and Faculty of Medicine, MU, BRNO, Czech Republic

Background. Flow cytometric immunophenotyping can identify $CD38^+CD138^+$ plasma cells (PCs) in monoclonal gammopathies. Monoclonal gammopathy of undetermined significance (MGUS) is the most common asymptomatic plasma cells disorder which eventually progress to symptomatic multiple myeloma (MM). *Aims*. The aim of this study was to find possibilities and limits of multicolour flow cytometry in diagnostics of monoclonal gammopathies and to evaluate whether or not the expression of CD19 and CD56 can distinguish between normal polyclonal and abnormal clonal PCs. *Methods*. Bone marrow of 78 newly diagnosed MM patients (average age 64,0±9,9 years) and 25 MGUS patients (64,0±8,1 years) was analysed. Group of MM patients consisted of 19 patients in clinical disease stage I - according to the D-S system - (MM I), 14 patients in stage II (MM II) and 45 patients in stage III (MM III). Plasma cells were identified by simultaneous expression of CD38, CD138, CD45, CD56 and CD19. Clonality of PCs was confirmed by light chain analysis in specific cases. *Results*. There was found lower number of $CD38^+CD138^+$ PCs in MGUS patients when compared with both whole group of MM patients (p<0,001) and individual MM groups (p<0,047; p<0,001 and p<0,001 respectively). See details in Table 1.

Table 1.

Parameter median (range) %	MGUS (n=25)	MM (n=78)	MM I (n=19)	MM II (n=14)	MM III (n=45)
$CD138^+$ $CD38^+$ cells	0,4 (0,0-4,2)	3,0 (0,0-47,7)	1,0 (0,0-14,6)	3,7 (0,2-18,7)	4,7 (0,0-47,7)
$CD19^+$ PC	15,0 (1,9-75,6)	1,2 (0,0-79,4)	4,7 (0,2-54,8)	0,8 (0,0-79,4)	0,9 (0,0-73,6)
$CD56^+$ PC	23,6 (6,3-91,8)	88,6 (0,1-99,8)	88,3 (0,5-99,4)	65,5 (0,5-99,7)	90,6 (0,1-99,8)

Surface markers CD19 and CD56 were chosen for discrimination between respectively normal residual PCs and abnormal neoplastic PCs. Higher number of polyclonal $CD19^+$ PCs was found in MGUS patients when compared with both whole group of MM (p<0,01) and individual groups of MM (p=0,002; p<0,001 and p<0,001 respectively). However $CD56^+$ PCs were found in predominance over residual $CD19^+$ PCs in 56% (14/25) of MGUS patients. The highest number of residual $CD19^+$ PCs was found in MM I (p=0,048) when all clinical stages of MM were compared with one another. A majority of PCs in MM patients were abnormal $CD56^+$ PCs and no significant differences for CD56 expression were found after comparison of all MM clinical stages with one another. There were found $CD19^+$ PCs in 2,6% (2/78) of patients with MM, these were $CD45^+$ with light chain restriction as well. PCs of 15,4% (12/78) of MM patients expressed neither CD19 nor CD56, but they were monoclonal. *Summary and Conclusions*. Lower number of PCs was found in MGUS patients when compared with MM patients and these PCs can be separated into subpopulations including normal residual $CD19^+$CD56- PCs and abnormal $CD56^+CD19^-$ PCs (alternatively $CD56^+CD19^+$ or CD56-CD19- in MM cases). Surface marker CD19 is a useful parameter for identification of normal residual PCs, but assessment of light chain restriction is recommended for verification of their normality, especially in $CD19^+$ MM cases.

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Multiple myeloma - Clinical I

0205

INDUCTION WITH CYBORD PRODUCES HIGH COMPLETE RESPONSES IN NEWLY DIAGNOSED MULTIPLE MYELOMA

A.K. Stewart,[1] B. Reeder,[1] R. Fonseca,[1] P.L. Bergsagel,[1] A. Pirooz,[1] G. Hentz,[1] E. Boesiger,[1] G. Pisa,[2] V. Kukreti,[2] Ch. Chen,[2] S. Trudel,[2] R. Mikhael,[2] F. Leis,[1] E. Reece[2]

[1]Mayo Clinic, SCOTTSDALE, USA; [2]Princess Margaret Hospital, TORONTO, Canada

Background. The combination of oral cyclophosphamide, bortezomib and dexamethasone (CyBorD) is active in multiple myeloma (MM) producing rapid responses. *Aims.* The goal of this study was to determine the depth of that response in newly-diagnosed patients with multiple myeloma as assessed by CR, nCR and VGPR after 4 cycles of therapy. *Methods*. Patients with newly diagnosed MM were eligible if they had measurable or evaluable disease, were >18 years of age, had an ECOG PS <3 and were able to give informed consent. Treatment consisted of a single arm of cyclophosphamide 300mg/m^2 po days 1, 8, 15, 22, bortezomib 1.3 mg/m^2 IV days 1, 4, 8, 11 and dexamethasone 40mg po days 1-4, 9-12, and 17-20 of a 28 day cycle. A total of 4 cycles was planned with the goal of proceeding on to stem cell transplant. Growth factors were allowed after cycle 1. The primary endpoint was complete response with secondary endpoints of ORR, PFS and toxicity. *Results*. 33 patients have been enrolled and 23 are evaluable for response and toxicity at the time of writing. Patient characteristics included mean age of 60, 43 % female, and ISS stage II/III in 35/26 %. Prior to transplant the ORR is 100 % with 85 % achieving at least a VGPR. 64 % had a CR or nCR. Responses occurred rapidly with a mean reduction in M-Protein of 66 % and 83 % after 1 and 2 cycles. Grade 3 toxicities included neutropenia in 20%, thrombocytopenia in 9 %, hyperglycemia in 17 % and peripheral neuropathy (PN) in 5 %. Gr 1-3 PN occurred in 69%. There was no grade 5 toxicity. All patients that elected to proceed on to transplant were able to undergo successful stem cell harvests. *Conclusions*. The induction regimen CyBorD is highly active in the treatment of newly diagnosed MM and produces very good and complete responses exceeding those seen with other induction regimens, mimicking that seen with high-dose therapy and stem cell transplantation. The main toxicity is peripheral neuropathy.

0206

INCORPORATION OF THALIDOMIDE INTO UP-FRONT DOUBLE AUTOLOGOUS STEM-CELL TRANSPLANTATION FOR NEWLY DIAGNOSED MULTIPLE MYELOMA FAILS TO OVERCOME THE POOR PROGNOSIS IMPARTED BY CHROMOSOME 13 DELETION

M. Cavo,[1] C. Terragna,[1] N. Testoni,[1] P. Tosi,[1] M. Ceccolini,[1] A. Gorgone,[2] S. Annunziata,[2] C. Nicci,[1] M. Renzulli,[1] P.P. Fattori,[2] G. Leopardi,[2] E. Zamagni,[1] P. Tacchetti,[1] G. Perrone,[1] S. Ronconi,[1] A. Lazzaro,[2] M. Offidani,[2] M. Fiacchini,[1] D. Vertone,[2] P. Fabbri,[2] A. Brioli,[1] M. Pallotti,[1] L. Pantani,[1] L. Guardigni,[2] D. Mamone,[2] M. Baccarani[1]

[1]Seràgnoli Institute of Hematology and Medical Oncology, BOLOGNA, Italy; [2]Bologna 2002 Study, ITALIAN MYELOMA NETWORK, Italy

The phase II "Bologna 2002"clinical study incorporated thalidomide-dexamethasone (thal-dex) into melphalan-based (200 mg/m^2) double autologous stem-cell transplantation (ASCT) as up-front therapy for patients with symptomatic multiple myeloma (MM) and less than 65 years of age. By study design, thal (200 mg/d) and dex (40 mg/d x 4d every month, with two added courses on the 1st and 3rd month of therapy) were administered from the outset until the second ASCT. An analysis on an intention-to-treat basis was performed of 311 consecutive patients who entered the study and were followed for a median of 3 years. The rate of ≥ very good partial response (VGPR) increased from 29% after 4 months of primary induction therapy with thal-dex to 63% after the second ASCT. Median durations of relapse-free survival (RFS) and event-free survival (EFS) were 52 and 42 months, respectively. The probability of 5-year projected overall survival (OS) was 70%. A case-match comparison of 135 of these patients with an equal number of pair mates who entered the predecessor "Bologna 96"study and were randomly assigned to receive double ASCT alone showed a significant benefit from added thal to double ASCT in terms of increased ≥ VGPR rate (68% *vs* 49%, respectively; p=0.001), extended 5-year RFS (54% *vs*

32%; p=0.005) and prolonged EFS (median: 52 *vs* 33 months; p=0.01). All 311 patients were screened on purified CD138+ bone marrow plasma cells for the presence at diagnosis of chromosome 13 alterations [del(13)] (47% of cases) and t(4;14) (13% of cases). In a logistic regression analysis, both absence of del(13) (p=0.001) and low beta2- microglobulin (beta2-m) levels (p=0.007) were significantly related to attainment of ≥ VGPR after the second ASCT. In a multivariate analysis of all 311 patients, the most important and independent variable significantly extending time to progression (TTP), EFS and OS was the absence of del(13) (p=0.001, p=0.001 and p=0.007, respectively), along with attainment of ≥ VGPR after the second ASCT. OS was also significantly influenced by both β2-m (p=0.044) and hemoglobin concentration (p=0.05), whereas platelet count was an additional prognostic factor for TTP (p=0.025). In conclusion, in comparison with double ASCT alone, incorporation of thal into double autotransplantation as up-front therapy for symptomatic MM significantly improved the response rate (≥ VGPR), RFS and EFS, without adversely affecting postrelapse OS. The presence of del(13) by FISH analysis was the most important and independent variable adversely influencing attainment of ≥ VGPR, EFS and OS following added thal-dex to double ASCT. Incorporation of thal into double autologous stem cell transplantation failed to overcome the poor prognosis imparted by chromosome 13 deletion.

0207

PROLONGED THERAPY WITH BORTEZOMIB PLUS MELPHALAN-PREDNISONE (VMP) RESULTS IN IMPROVED QUALITY AND DURATION OF RESPONSE IN THE PHASE III VISTA STUDY IN PREVIOUSLY UNTREATED MULTIPLE MYELOMA (MM)

A. Palumbo,[1] R. Schlag,[2] N. Khuageva,[3] O. Shpilberg,[4] M. Dimopoulos,[5] M. Kropff,[6] I. Spicka7, M. Petrucci,[8] M. Delforge,[9] M. Mateos,[10] D. Esseltine,[11] K. Liu,[12] W. Deraedt,[13] H. Van de Velde,[13] P. Richardson,[14] J. San-Miguel[10]

[1]Divisione di Ematologia dell'Universita di Torino, TORINO, Italy; [2]Praxisklinik Dr. Schlag, WÜRZBURG, Germany; [3]SP Botkin Moscow City Clinical Hospital, MOSCOW, Russian Federation; [4]Rabin Medical Center, PETAH-TIQVA, Israel; [5]University of Athens School of Medicine, ATHENS, Greece; [6]University of Münster, MÜNSTER, Germany [7]University Hospital, PRAGUE, Czech Republic; [8]University La Sapienza, ROME, Italy; [9]University Hospital Gasthuisberg, LEUVEN, Belgium; [10]Hospital Universitario Salamanca. CIC, IBMCC (USAL-CSIC), SALAMANCA, Spain; [11]Millennium Pharmaceuticals, Inc., CAMBRIDGE, USA; [12]Johnson & Johnson Pharmaceutical Research & Development, L.L.C., RARITAN, USA; [13]Johnson & Johnson Pharmaceutical Research & Development, BEERSE, Belgium; [14]Dana-Farber Cancer Institute, BOSTON, USA

Background. Results from the phase III VISTA study in previously untreated MM patients ineligible for high-dose therapy demonstrate that VMP is superior to melphalan-prednisone (MP) in terms of response rates (82% *vs* 50%, respectively, by M-protein assessment, including 35% *vs* 5% immunofixation-negative M-protein complete response [CR]), time to progression (median 24.0 *vs* 16.6 months, HR=0.483, p<0.001), time to subsequent therapy (median not reached *vs* 20.8 months, HR=0.522, p<0.001), and overall survival (medians not reached, HR=0.607, p=0.0078). (Off-label use: bortezomib in front-line MM in a novel combination.) *Aims*. Time to response and duration of response (DOR) were secondary end points of VISTA. The analyses presented here compare time to first response, time to best response, and DOR with VMP and MP, and assess the impact of length of VMP therapy on quality of response and duration of CR. *Methods*. Patients were randomized to VMP (N=344) or MP (N=338); all provided written, informed consent. Treatment was administered for up to 54 weeks, and comprised nine 6-week cycles of melphalan 9 mg/m^2 and prednisone 60 mg/m^2, days 1-4, alone or in combination with bortezomib 1.3 mg/m^2 twice weekly (days 1, 4, 8, 11, 22, 25, 29, and 32), cycles 1-4, and weekly (days 1, 8, 22, and 29), cycles 5-9. M-protein analyses were performed by a central laboratory. Response, relapse, and progression in these analyses were determined using European Group for Blood and Marrow Transplantation (EBMT) criteria. Descriptive statistics were provided for responders only; p-values were based on stratified log-rank tests. *Results.* Responses were significantly more rapid with VMP; median time to first response was 1.4 months with VMP *vs* 4.2 months with MP (p<0.001). Nevertheless, late *de novo* responses occurred with VMP; 4% of first responses were seen beyond the initial 24 weeks of therapy (i.e. during cycles 5-9). Median time to best response was 2.3 months with VMP *vs* 4.9 months with MP, and median time to CR was 4.2 months *vs* 5.3 months, respectively (p<0.001), suggesting that quality of response improved with prolonged treatment. Notably with VMP, 28% of CRs as best response occurred during cycles 5-9. Responses were substantially more durable with VMP; median DOR was 19.9 months with VMP *vs* 13.1 months with MP, and median DOR in patients achieving CR was 24.0 months *vs* 12.8 months, respectively. For VMP patients achieving CR, continued treatment beyond initial CR resulted in prolonged CR. In patients who received ≤2 additional cycles, >2 additional cycles but <9 cycles in total, or all 9 protocol-specified cycles, median/25% quantile duration of CR was 16.9/7.4 months, not estimable/9.7 months, and 20.3/16.4 months, respectively. *Conclusions.* VMP results in significantly more rapid and substantially more durable responses than MP. Although many responses occur early with VMP, prolonged therapy results in additional first responses and improves quality of response, in particular maximizing the chance of achieving CR. Continued therapy also improves duration of CR with VMP, indicating that to maximize clinical benefit patients should not discontinue therapy early upon achieving CR.

0208

BORTEZOMIB IN COMBINATION WITH PEGYLATED LIPOSOMAL DOXORUBICIN AND THALIDOMIDE (VDT) FOR THE TREATMENT OF PREVIOUSLY UNTREATED MULTIPLE MYELOMA

S. . Ailawadhi, K.C. Miller, D. Depaolo, A. Whitworth, J. Yu, D.L. Trump, S. Padmanabhan, M. Czuczman, Z.P. Bernstein, F. Hernandez-Ilizaliturri, K. Lee, A. Chanan-Khan

Roswell Park Cancer Institute, BUFFALO, USA

Introduction. Addition of pegylated liposomal doxorubicin (PLD) is reported to enhance the efficacy of bortezomib. To further optimize the clinical efficacy of these agents we included thalidomide to the regimen. In a previous report we described the clinical efficacy of this novel, non-steroidal regimen that incorporated bortezomib (V), PLD (D) and thalidomide (T) in patients with relapsed or refractory multiple myeloma (MM). High clinical responses in heavily pretreated patients, even in the absence of steroids encouraged us to investigate this regimen in previously untreated MM patients. Here we report the efficacy and toxicity profile of the VDT regimen in treatment naive MM patients enrolled on a phase II clinical study at our center. *Methods*. All previously untreated MM patients were eligible for this study. Bortezomib (1.3 mg/m^2) was given on days 1, 4, 15, and 18, pegylated liposomal doxorubicin was given (20 mg/m^2) on days 1 and 15 and thalidomide (200 mg) was given daily continuously throughout the treatment. Patients received treatment on a 4-week cycle. Acyclovir 400 mg PO BID and low-dose warfarin were given for prophylaxis of herpes zoster and venous thromboembolism, respectively. *Results*. A total of 34 patients (median age 60; range 40-82 years), 21 M and 13 F have so far been enrolled. Advance (stage III) disease was noted in 69% of the patients. The median beta 2 microglobulin was 4 mg/L (range1.6-9.7), albumin was 4 g/dL (range 3.1-4.8) and LDH was 333 U/L (range 152-1057). Patients received a median of 5 treatment cycles (range 1-8). Toxicity: The most common grade 3 or 4 hematologic toxicities included lymphopenia (32%) and neutropenia (21%), while the non-hematologic toxicity included infections 21% (n=7). The incidence of grade 3 or 4 plantar palmar erythrodysthesia was 3% (n=1). Venous thromboembolism has not been observed in any patient to date. Grade 3 or 4 peripheral neuropathy was seen in only 1 patient (3%). Response: To date 28 patients are available for response assessment (EBMT criteria), 3 patients are too early for response assessment while 3 patients were not evaluable due to discontinuation of treatment. The ORR of this regimen is 68% with IFE negative CR observed in 4 patients (14%). Another 7 patients (28%) achieved a stable disease for an overall clinical benefit of 96%. Progressive disease was noted in 1 patient on the regimen so far. *Conclusion*. VDT is a novel non-steroidal regimen with clinical efficacy in previously untreated myeloma patients. Overall toxicity is manageable. Several patients remain on treatment and accrual is continuing on the study at present. Detailed and updated results of this study will be presented at the meeting.

0209

A RANDOMISED PLACEBO CONTROLLED STUDY WITH MELPHALAN/PREDNISONE *vs* MELPHALAN/PREDNISONE/THALIDOMIDE: QUALITY OF LIFE AND TOXICITY

N. Gulbrandsen,[1] A. Waage,[2] P. Gimsing,[3] I. Turesson,[4] G. Juliusson,[5] N. Abildgaard,[5] B. Bjørkstrand,[6] K. Carlson,[5] I.M. Dahl,[5] K. Forsberg,[5] L.M. Knudsen,[7] J. Lanng Nielsen,[5] U.H. Mellqvist,[8] I. Nesthus,[9] M. Strandberg,[5] F. Wisløff,[1] P. Fayers[10]

[1]Ullevål University Hospital, OSLO, Norway; [2]St. Olavs Hospital/NTNU, TRONDHEIM, Norway; [3]National Hospital, COPENHAGEN, Denmark; [4]Malmø University Hospital, MALMØ, Sweden; [5]Dep of Hematology, LUND, Sweden; [6]Dep of Hematology Huddinge, STOCKHOLM, Sweden; [7]Herlev, COPENHAGEN, Denmark; [8]Sahlgrenska, GOTHENBURG, Sweden; [9]Haukeland, BERGEN, Norway; [10]NTNU, TRONDHEIM, Norway

Background, methods. Previously untreated patients with multiple myeloma were included in this double-blind randomised trial. Patients not eligible for high dose treatment in Norway, Sweden and Denmark were recruited. The study started in 2002 and accrual of patients stopped 1st of May 2007. Date of analysis was 10th of October 2007. Median follow up is 36 months. Patients were randomly allocated to treatment with melphalan/prednisone/thalidomide or melphalan/prednisone/placebo. Starting dose of thalidomide was 200 mg escalating to 400 mg. *Aims*. Primary endpoint was overall survival, secondary endpoints were event free survival, response, time to progression and quality of life. *Results*. Altogether 363 patients were included and 357 patients were evaluable for analysis. In the thal and placebo arm respectively, the mean age was 75 (49-92) years (both arms), 52 % and 62 % were males, 65 % and 64 % had b2 microglobulin > 3,5 mg/mL, 28% and 32 % had WHO stage 3-4. The response rates were CR/nCR 6 *vs* 3 %, VGPR 9 *vs* 3 %, PR 27 *vs* 22 % at 6 months in the thal arm and placebo arm, respectively. Time to progression were 20 *vs*18 months ($p<0.05$). There was no significant difference in progression free survival (16 *vs* 14 months) and overall survival (29 *vs* 33 months). In patients above 75 years of age there was a tendency to higher early mortality in the thalidomide arm. Self reported quality of life was assessed with the EORTC QLQ-C30 questionnaire, version 3.0. Baseline form was filled in before randomisation, subsequent forms were sent out every 3 months. Registrations up to 39 months were analysed. There was improvement >10 units (considered to be important for patients) during the first 6 months for appetite, insomnia, pain, fatigue, social function, physical function, emotional function and global health status, but no difference between the two arms. There was marked increased constipation in the thalidomide arm. The mean difference was 16 units. There was significantly less diarrhea in the thalidomide arm. For appetite, pain, insomnia, fatigue, cognitive function, physical function, role function, social function, emotional function and global quality of life, there were no or small differences of borderline significance. Questions specifically addressing polyneuropathy symptoms were not included in the questionnaire. The analysis was made on an intention to treat basis, but will also be analysed with respect to the actual treatment periods with thalidomide. The incidence of venous thromboembolism was 8 % in both treatment arms. There was no recommendation for prophylaxis for venous thromboembolism in the study. Approximately 30% of the patients used salicylates, warfarin or heparin for other reasons. *Conclusion*. there was a marked increase in constipation, but not in other symptoms in the thalidomide arm. There was improvement for appetite, insomnia, pain, fatigue, social function, physical function, emotional function and global health status during the first 6 months in both arms.

0210

BORTEZOMIB (VEL) BASED REGIMENS IN MULTIPLE MYELOMA (MM) PATIENTS WITH RENAL IMPAIRMENT (RI): A PRELIMINARY RETROSPECTIVE ITALIAN MULTICENTER STUDY

M. Gentile,[1] S. Ciolli,[2] M.T. Petrucci,[3] S. Galimberti,[4] G Mele,[5] A.F. Casulli,[6] D. Mannina,[7] E. Piro,[8] F. Morabito[1]

[1]Ematologia di Cosenza, COSENZA; [2]Dipartimento di Ematologia, Università e Ospedale Careggi, FIRENZE; [3]Dipartimento di Biotecnologie e Ematologia, Università La Sapienza, ROMA; [4]Dipartimento di Oncologia, Sezione Ematologia, Ospedale Santa Chiara, PISA; [5]Divisione di Ematologia, Presidio Ospedaliero A. Perrino,BRINDISI; [6]Struttura Complessa di Ematologia, Azienda Ospedaliera SS.Annunziata, TARANTO; [7]Divisione di Ematologia, Azienda Ospedaliera Papardo, MESSINA; [8]Divisione di Ematologia Ospedale Pugliese Ciaccio, CATANZARO, Italy

Background. Up to 30% of newly diagnosed MM patients have a RI at diagnosis, remaining a common and severe complication throughout the course of disease. Vel alone or in combination has been shown to be active and well tolerated in both relapsed and newly diagnosed MM with varying degrees of RI. *Aims*. To assess the safety and efficacy of Vel containing regimen in MM patients with different degrees of RI. *Patients Methods*. Between November 2003 and January 2008, 64 patients, either untreated (14 pts), or relapsed/refractory (50 pts) after a median of 2 therapies (range1-4) and with RI (CrCl<80 mL/min) were analysed. Median age was 71 years (range 58-81); 39% were female. Isotypes were IgG 42%, light chain 33%, IgA 23%, IgD 2%. Vel-based regimens utilized were: Vel alone in 7 patients, Vel+Dex in 30, VTD (Vel+Dex+Thal) in 7, PAD (Vel+Dex+Pegylated liposomial doxorubicin) in 6 patients, PAD+Thal in 4, MPTV (Melphalan+Prednisone+Thal+Vel) in 2 and VMP (Vel+Melphalan+Prednisone) in 8. All patients received Vel (median dose 1.3 mg/m^2, range 0.8-1.3) for 4 doses per cycle, administered on days 1, 4, 8 and 11 every 3 weeks in all schedules but VMPT (days 1, 8, 15 and 22 every 5 weeks). RI was evaluated by CrCl, using the Cockcroft-Gault formula, and cases were clustered accordingly into 3 subgroups: CrCl 51-80, 30-50 and <30 ml/min, corresponding to mild (9 pts, 14%), moderate (21 pts, 33%) and severe (34 pts, 53%) RI, respectively. Eight patients required dialysis. *Results*. A total of 332 cycles of Vel were administered with a median number of 5. Seven patients (11%) discontinued therapy (6 refractory and 1 relapsed) for WHO grade 4 neuropathy in 4 pts, diarrhoea in 1, pneumonia in 1, cardiotoxicity in 1. The rate of therapy discontinuation was 18%, 5%, 0% for severe, moderate and mild RI subgroups, respectively (p=ns). Fifteen patients (23%) required a Vel dose reduction (9 with severe and 6 with moderate RI). Overall 65 episodes of WHO grade III/IV toxicity were observed: 17 were non-hematological (neuropathy in 5 pts, gastrointestinal complications in 5, infections in 5 and cardiotoxicity in 2), and 48 were haematological (anemia in 15 pts, neutropenia in 10 and thrombocytopenia in 23). A higher rate of haematological SAEs were observed in severe RI patients than in those with moderate and mild RI (91% *vs* 52% *vs* 66%; p=0.004). ORR (>PR) was 71% (44/62 evaluable pts). Namely, 13 patients (20%) achieved a CR, 4 (6%) a nCR, 9 (14%) a VGPR and 18 (28%) a PR. The ORR was similar across renal subgroups. Reversal of RI was documented in 29/62 cases (47%) after a median time of 2.4 months (range 0.5-7.9). In 2/8 dialysed patients renal replacement therapy was discontinued after 1 and 4 months. After a median follow-up of 12 months, 18 patients died. PFS was 61% at 1 year. *Conclusions*. Vel-based regimens are safe and active in MM patients, inducing an high ORR and a prompt reversal RI in roughly half cases. Thus Vel-based regimens should be considered appropriate treatment options for the MM who have any degree of RI, including those requiring dialysis.

0211

A MULTICENTER COMPARISON OF AUTOLOGOUS STEM CELL TRANSPLANTATION FOLLOWED BY REDUCED-INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION WITH TANDEM AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA :THE KOREAN MULTIPLE MYEOMA WORKING PARTY (KMMWP)

C.K. Min,[1] H. Kim,[2] K. Kim,[3] J.Y. Kwak,[4] S.T. Lee,[5] J.H. Won,[6] S.S. Yoon,[7] J.H. Lee,[8] C. Suh[9]

[1]St. Mary's Hospital, The Catholic University of Korea, SEOUL; [2]Ulsan University Hospital, University of Ulsan College of Medicine, ULSAN; [3]Sungkyunkwan University School of Medicine, SEOUL; [4]Chonbuk National University Medical School, JEONJU; [5]Yonsei University College of Medicine, SEOUL; [6]Soon Chun Hyang University Hospital, SEOUL; [7]Seoul National University College of Medicine, SEOUL; [8]Gachon Medical School, INCHEON; [9]Asan Medical Center, University of Ulsan College of Medicine, SEOUL, South-Korea

Background. Autologous stem-cell transplantation (ASCT) after high-dose chemotherapy is regarded as the standard therapeutic approach for younger patients with multiple myeloma (MM) even if virtually all patients ultimately relapse following this procedure. Recently, tandem ASCT significantly improved overall survival (OS) and event-free survival (EFS) compared with single ASCT. Another strategy is to use reduced-intensity allogeneic stem cell transplantation (RISCT) earlier in the course of disease in chemosensitive patients. *Aims.* we retrospectively compared the outcomes after a planned tandem ASCT or RISCT in the patients who previously underwent ASCT. *Methods*. One-hundred twenty-six patients who received a high dose (140 to 200 mg/m^2) of melphalan as the conditioning regimen of the first ASCT were analyzed. Ninety-six patients (median age, 50.5 years) received a second ASCT, whereas 30 patients (median age, 46.5 years) underwent a RISCT (related in 28 patients and unrelated in 2 patients). The median interval between the first and second transplant was 131 days in ASCT group and 157.5 days in the RISCT group. The conditioning regimen for the tandem ASCT and RISCT consisted of high-dose melphalan±total body irradiation (TBI) and fludarabine + melphalan or TBI, respectively. The two groups were evenly matched with regard to other disease characteristics. *Results*. After a median follow-up of 664 days (range, 143-2904) from the first ASCT, the median event-free survival (EFS) and overall survival (OS) in all 126 patients were 878 days and 1838 days, respectively. The median EFS in the second ASCT *vs* RISCT group were 844 days (95% CI, 714-973) and 1342 days (95% CI, 813-1870), respectively (p=0.262). The median OS in the tandem ASCT *vs* RISCT group were 2160 days (95% CI, 1847-2832) and 1575 days (95% CI, 1202-1947), respectively (p=0.132). Disease-related mortality was not significantly different between the second ASCT *vs* RISCT groups (73.3% *vs* 60.0%, p=0.325) as well as treatment-related mortality between the 2 groups (26.7% *vs* 40%, p=0.358). On multivariate analysis, an achieving a good response (≤ VGPR) after the induction treatment predicted a better EFS compared to a poor response (≤ PR) (RR; 0.245, p=0.01). A good response after first ASCT or the second transplant was associated with a better EFS by univariate analysis but not by multivariate analysis (RR; 0.927, p=0.830 or RR; 0.772, p=0.453, respectively). *Conclusion*. In this retrospective analysis, ASCT followed by RISCT was not superior to the tandem ASCT, either EFS or OS. Disease-related deaths were not different between the 2 groups. Patients whose disease is sensitive to chemotherapy and who obtain a good response after induction treatment benefited the most from this tandem transplant therapy.

0212

INFLUENCE OF GLUCOCORTICOID RECEPTOR POLYMORPHISM G+647C ON EFFICACY OF VAD AND VD THERAPY IN PATIENTS WITH MULTIPLE MYELOMA

N.V. Stepanova, A. Voitovich, V. Larionova, S. Moiseev

St.-Petersburg State Medical University, SAINT-PETERSBURG, Russian Federation

Polymorphisms and mutations glucocorticoid receptor gene may be associated with an altered sensitivity to glucocorticoids in patients with lymphoid neoplasms. Efficacy of VAD therapy in dependency of polymorphisms G+647C glucocorticoid receptor gene (2 intron) was evaluated in 38 patients with multiple myeloma II-III stages. Patients received 4-6 courses of VAD therapy as first line therapy. Polymorphism glucocorticoid receptor gene was evaluated by PCR-RFLP analysis. CC genotype was revealed in 12 (38%), CG genotype - in 21 (55%), GG genotype - in 5 (13%) patients with multiple myeloma. Distribution of these polymorphisms in patients with multiple myeloma did not differ from distribution in population of St-Petersburg. Efficacy of VAD therapy in patients with multiple myeloma was dependent on glucocorticoid receptor G+647C polymorphisms. Uneffectiveness of therapy (progression disease) was revealed in 4 (19%) patients with CG genotype, in 4 (36%) patients with CC genotype and in 5 (100%) c GG genotype. Complete remission (CR) was received in 5 (24%) patients with CG genotype. None of the patients with CC and CG genotype reached CR. Not complete response (near CR, partial remission, minimal response, stabilization disease) was received in 12 (57%) patients with CG genotype, 7 (64%) patients with CC genotype and in none of the patients with GG genotype. 13 patients with multiple myeloma after ineffective VAD therapy as second line therapy received treatment by VD therapy (velcade (bortezomib) 1,3 mg/m^2 at 1, 4, 8, 11 days and dexametazone 20 mg at 1,2, 4,5, 8,9, 11,12 days every over 10 days). Patients received 8 such courses. 6 (46%) patients reached CR, 1(8%) patient - near CR, 2 (15%) - partial remission, 2 (15%) - stable disease, 2 (15%) - progression disease. Efficacy of velcade was not dependent on G+647C polymorphisms of glucocorticoid receptor gene (2 intron). Preliminary results may indicate that G+647C polymorphisms of glucocorticoid receptor gene influence on efficacy of VAD therapy but not influence on efficacy of therapy by velcade + dexametazone.

0213

MELPHALAN, PREDNISONE AND LENALIDOMIDE FOR NEWLY DIAGNOSED MYELOMA: KINETICS OF NEUTROPENIA/THROMBOCYTOPENIA AND TIME TO EVENT RESULTS

A. Palumbo,[1] P. Falco,[1] P. Corradini,[2] C. Crippa,[3] F. Di Raimondo,[4] A. Falcone,[3] N. Giuliani,[5] P. Musto,[6] P. Pregno,[7] G. Aitoro,[8] A. Luraschi,[9] A. Nozza,[10] R. Knight,[11] J.B. Zeldis,[11] M. Boccadoro,[1] M.T. Petrucci[3]

[1]A.O.U. San Giovanni Battista, TORINO, Italy; [2]Divisione Ematologia, Istituto Nazionale Tumori, MILANO, Italy; [3]Italian Multiple Myeloma Network, GIMEMA, ITALY, Italy; [4]Cattedra di Ematologia, Ospedale Ferrarotto, CATANIA, Italy; [5]Cattedra e UO Ematologia e Trapianto Midollo, Università degli Studi di Parma, PARMA, Italy; [6]UO Emat. e Trapianto Cellule Staminali-CROB-Centro Riferimento Oncol. Basilicata, RIONERO IN VULTURE, Italy; [7]Ematologia, Azienda Ospedaliera San Giovanni Battista, TORINO, Italy; [8]DH Ematol.- S.O.C. Immunoematologia e Medicina Trasfusionale, Ospedale Ivrea, IVREA, Italy; [9]U.O.A. Oncologia, VERBANIA, Italy; [10]Dipartimento Oncologia Medica ed Ematologia, Istituto Clinico Humanitas, ROZZANO, Italy; [11]Celgene Corporation, SUMMIT, USA

Background. Melphalan, prednisone and lenalidomide (MPR) has shown significant anti-myeloma activity in newly diagnosed myeloma patients. In a phase I/II study, neutropenia and thrombocytopenia were the most frequent adverse events while non-hematologic toxicities were infrequent. *Aims*. We analyzed the kinetics and risk factors for neutropenia and thrombocytopenia in newly diagnosed myeloma patients who received MPR at the maximum tolerated dose of melphalan (0.18 mg/Kg) and lenalidomide (10 mg/day). Efficacy end-points were also analysed. *Methods*. Twenty-one patients (median age 69 years) received lenalidomide (10 mg/day for 21 days) plus melphalan (0.18 mg/kg for 4 days) and prednisone (2 mg/kg for 4 days) every 4 weeks for a maximum of 9 cycles, followed by maintenance with lenalidomide alone (10 mg/day for 21 days every month). The occurrence of grade-3 neutropenia required G-CSF administration. The occurrence of grade-4 neutropenia despite G-CSF administration or any other grade-4 hematological toxicities required withholding of treatment and subsequent dose reduction at the start of the following cycle. A new cycle was allowed if the neutrophil count was >1x109/L and platelet count >50x109/L. A delay of 2 weeks was allowed, a delay beyond 2 weeks required dose reduction and a delay beyond 4 weeks required therapy discontinuation. *Results*. Grade-3 neutropenia was reported in 38.1% of patients, grade-4 neutropenia in 14.2% of patients, but febrile neutropenia was 9.5%. G-CSF was administered in 42.3% of patients. The mean neutrophil count at the start of each MPR cycle was 2.69×10^9/L (SD 1.4). The mean neutrophil count at nadir (day 15-21) of each cycle was 1.43×10^9/L (SD 1.0). The incidence and depth of neutropenia did not increase with the number of cycles. The mean neutrophil count during maintenance was 2.11×10^9/L (SD 1.0). Grade-3 thrombocytopenia was recorded in 14.2% of patients and grade-4 thrombocytopenia in 9.5%; one patient required platelet transfusion. The mean platelet count at the start of each MPR cycle was 174×10^9/L (SD 63.9). The mean platelet count at nadir (day 15-21) of each cycle was 121×10^9/L (SD 56.3). Thrombocytopenia was more pro-

nounced after the 9 MPR cycles. The mean platelet count at the end of the 9 cycles was 109×10^9/L (SD 53). The mean platelet count after 6 months of lenalidomide maintenance therapy was 158×10^9/L (SD 79.2). One patient reduced the dose of lenalidomide for severe neutropenia. Three patients discontinued therapy for severe thrombocytopenia and neutropenia. Grade 3-4 hematologic toxicity was more frequent in patients with low baseline neutrophil count and in those with Bence-Jones myeloma. More frequent grade-3/4 non-hematologic adverse events were febrile neutropenia (9.5%), cutaneous reaction (9.5%), thromboembolism (4.8%). After a median follow-up of 29.5 months, the median time-to-progression was 28.5 months, the median progression-free survival was 28.5 months and the 2-years overall survival was 90.5%. No death was reported in the first 18 months of treatment. *Conclusions*. Oral MPR is a promising first line treatment for elderly myeloma patients. Hematologic adverse events were frequent but manageable with the use of G-CSF.

0214

A PILOT STUDY TO EXPLORE THE TOLERABILITY AND EFFICACY OF THALIDOMIDE CONTAINING REGIMENS TO REDUCE TUMOUR CELL LOAD PRIOR TO AUTOLOGOUS TRANSPLANTATION IN MULTIPLE MYELOMA (MM)

N. Horvath,[1] D. Joshua,[2] J. Gibson,[2] A. Roberts,[3] J. Norman,[4] C. Underhill,[5] C. To,[1] L.B. To[1]

[1]IMVS, ADELAIDE; [2]Institite of Haematology. Royal Prince Alfred Hospital, SYDNEY; [3]Hepartment of Haematology, Royal Melbourne Hospital, MELBOURNE; [4]The Queen Elizabeth Hospital, ADELAIDE; [5]Hume Regional Integrated Cancer Service, ALBURY-WODONGA, Australia

Background. In multiple myeloma thalidomide has been found to be effective therapy in relapsed/refractory disease. In the early 2000s little was known about the efficacy of thalidomide as part of first line therapy particularly its use as debulking therapy prior to autologous transplantation. *Aims.* To explore the role of thalidomide in pre-transplant induction treatment in MM. *Patients and methods*. Between Sept 2002 and Dec 2006, 48 patients with advanced, de-novo MM were entered into a multicentre, phase 2 study of pre-transplant induction treatment after voluntary informed consent had been obtained. The regimen included DTx3 (pulse dexamethasone 32 mg TDS x 5d every 3 weeks PO and thalidomide 400 mg/d), followed by DT-PACEx2 (thalidomide 400 mg/d, dexamethasone 40 mg/d x 4 PO and cisplatin 10 mg/m^2/d, doxorubicin 10 mg/m^2/d, cyclophosphamide 400 mg/m^2/day, etoposide 40 mg/m^2/d as 4 day infusion administered 4 weeks apart. Stem cells were harvested at recovery from DT-PACE. Paraprotein and BJP responses, rate of CR, VGPR and PR according to international uniform response criteria1, OS and EFS were compared to a historical cohort of 46 unselected patients for whom prognostic and survival data could be retrieved who had been treated with VAD (same dexamethasone scheduling), mobilised with HD cyclophosphamide + G-CSF and proceeded to HDT and ASCT.

Table 1. Comparison of response to trial therapy and VAD+HD

	Median percent paraprotein/BJP present after each phase of treatment		Percent of subjects achieving CR and VGPR after each phase of treatment			
Therapy	Para/ BJP %	p	CR %	p	CR+VGPR %	p
DTx3 all p	12	0.031	2	0.167	44	0.078
DTx3 Tx p n=28	9	0.034	4	0.333	43	0.085
VADx3	30		11		26	
DT-PACEx2	4	0.000	19	0.015	68	0.001
DT-PACEx2 Tx p n=28)	5	0.000	25	0.057	61	0.003
VAD x3	26		9		24	

Results. One patient withdrew himself before commencing therapy and has been excluded from all evaluations. There was no significant difference between age and international staging score of the two groups of patients. 32 patients completed study treatment. 11 patients were withdrawn (9 for adverse events) and 2 died (1 sepsis, 1 haemorrhage) during DT phase. Three patients were withdrawn after DT-PACE 1 (2 for adverse events and 1 for poor response). 29 had successful stem cell harvests. 28 patients were transplanted after completing trial therapy. Table 1 shows the comparison of response to trial therapy and VAD + HD cyclophosphamide 69 serious adverse events included 25 episodes of infection, 3 haemorrhage, 1 pulmonary embolus, 2 deaths and 1 late myelodysplasia. OS and EFS of the 47 evaluable patients at 3 years were 79% and 36% respectively (medians NR and 29 months). OS of the 28 patients who proceeded through all of study therapy and were transplanted *vs* the 46 historical controls were 96% *vs* 76% at 3 years respectively, (medians NR and 76 months, p=0.06) and EFS were 36% *vs* 37% at 3 years respectively (medians 30 months and 28 months, p=0.89). *Conclusion*. 1) Thalidomide plus dexamethasone followed by DT-PACE is associated with tolerable but not insignificant toxicity. 2) Thalidomide plus dexamethasone combination achieves a greater depth of response than VAD. 3) The addition of DT-PACE improves the pre-transplant CR and VGPR rate. 4) In spite of the improved pre-transplant responses, no survival benefit, either OS or EFS, has been demonstrated. 5) We question the validity of pre transplant response as a surrogate marker for outcome in patients treated with novel agents.

Reference

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0215

SEQUENTIAL VAD (VINCRISTINE, ADRIAMYCIN, DEXAMETHASONE) AND VTD (BORTEZOMIB, THALIDOMIDE, DEXAMETHASONE) INDUCTION FOLLOWED BY HIGH-DOSE THERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION AND MAINTENANCE TREATMENT WITH BORTEZOMIB FOR NEWLY DIAGNOSED MULTIPLE MYELOMA: INTERIM RESULTS OF PHASE II TRIAL

S.S. Yoon,[1] H.J. Kim,[1] D.S. Lee,[1] H.S. Eom,[2] J.H. Jang,[3] J.S. Chung,[4] H.J. Kang,[5] C.S. Kim,[6] K.K. Kim,[7] J.H. Lee,[8] S.J. Lee,[9] Y.H. Min,[10] C.M. Seong,[11] S.K. Sohn,[12] C. Suh,[13] J.H. Won[14]

[1]Seoul National University Hospital, SEOUL; [2]National Cancer Center, GOYANG-SI, KYUNGKI-DO; [3]Ajou University Medical Center, SUWON, KYUNGKI-DO; [4]Pusan University Hospital, PUSAN; [5]Korea Institute of Radiological and Medical Science, SEOUL; [6]Inha University Hospital, INCHEON; [7]SungKyungKwan University, Samsung Medical Center, SEOUL; [8]Gachon University Gil Hospital, INCHEON, South-Korea; [9]Chung-Ang University Hospital, SEOUL; [10]Yonsei University Severance Hospital, SEOUL; [11]Ewha Women's University Hospital, SEOUL; [12]Kyungbook National University Hospital, DAEGU; [13]Asan Medical Center, SEOUL; [14]Soon Chun Hyang University Hospital, SEOUL, South-Korea

Background. Effective reduction of myeloma before autologous stem cell transplantation (ASCT) prolongs survival in multiple myeloma patients. Recently, incorporation of novel agents resulted in improved response rate and reduced side effect in newly diagnosed multiple myeloma. *Aims.* We studied efficacy and safety of sequential VAD and VTD as induction chemotherapy. *Methods.* Patients are planned to receive 2 cycles of VAD (vincristine 0.4 mg D1-4, adriamycin 9mg/m^2 D1-4, dexamethasone 40 mg D1-4, 9-12 every 3 weeks), and VTD (bortezomib 1.3 mg/m^2 D1, 4, 8, 11, thalidomide 100 mg daily, dexamethasone 40 mg D1-4, 9-12 every 3 weeks). High dose melphalan (200 mg/m^2) is used as a conditioning regimen for ASCT. Bortezomib (1.3 mg/m^2) as a maintenance treatment is administered weekly x 4 times every 6 weeks for 4 cycles after ASCT. Response was assessed by EBMT criteria, with additional category of nCR. Adverse events were graded by the NCI-CTCAE, Version 3.0. *Results.* At this interim analysis, 60 patients have been entered into the ongoing trial, and efficacy could be assessed in 54 patients. After 2 cycles of VAD, response rate was 63%. After VTD, six patients showed further improvement with additional CR, and an overall response was 94% with 19% CR. Especially, patients with poor prognostic cytogenetics (n=7) all responded after VTD. So far, autologous stem cells were successfully collected in all 35 patients with a median CD34$^+$ count of 6.5×10^6/kg (range, 2.17-44.7$\times10^6$/kg). In

34 patients who underwent autologous stem cell transplantation, seven patients gained additional CR. Seventeen patients completed bortezomib maintenance, and CR rate was 65%. The median follow-up duration was 10 months, and median time to response was 1.4 months, and median overall survival was not reached. Grade 3,4 hematologic toxicity was more frequently observed after VAD than VTD (anemia 15.8%, 4.6%, neutropenia 7.9%, 4.2%), and incidence of grade 2,3 peripheral neuropathy after VAD was lower (VAD 3.5%, VTD 9.6%). There was no thrombosis after VTD. *Conclusions.* Sequential VAD and VTD induction therapy in newly diagnosed multiple myeloma was highly effective, even in patients with poor prognostic cytogenetics, and did not prejudice stem cell collection. VTD could have contributed to increased RR and minimized side effects. An updated results will be presented at the EHA meeting.

0216

LONG-TERM OUTCOMES OF AUTOLOGOUS TRANSPLANTATION IN 141 MULTIPLE MYELOMA PATIENTS: A SINGLE CENTRE EXPERIENCE

M. Krejci,[1] R. Hajek,[2] Z. Adam,[2] A. Krivanova,[2] L. Pour,[2] K. Kresova,[2] M. Holanek,[2] V. Sandecka,[2] M. Sahmani,[2] J. Mayer,[2] J. Vorlicek[2]

[1]IHOK University Hospital Brno and IHOK LF MU Brno, BRNO, Czech Republic; [2]University Hospital Brno, BRNO, Czech Republic

Background. Autologous stem cell transplantation (ASCT) has an important role in the treatment of multiple myeloma (MM) patients (pts). In this report, we describe the long-term outcome of a cohort of 141 pts with newly diagnosed symptomatic MM treated with ASCT in a single institution, median follow-up from transplant was 8.4 years (range: 5.1-12.1). We have specifically analysed those factors that might predict for long-term survival. *Methods*. A total of 141 MM pts were transplanted in our centre between 1996 and 2002. The conditioning regimen was high dose melphalan (200 mg/m^2) in all pts. At diagnosis of MM 75% of pts (105/141) had stage III according to Durie-Salmon, 16% (23/141) II and 9% (13/141) I, clinical stages according to ISS were the following: stage 1 in 58/132 pts (44%), stage 2 in 54/132 pts (41%) and stage 3 in 20/132 pts (15%). Types of monoclonal immunoglobulin were as following: 61% (86/141) IgG, 21% (30/141) IgA, 14% (20/141) light chain, 1% (2/141) IgD, 2% (4/141) non-secretory myeloma. Renal insufficiency was presented in 11% of pts (15/141). Median age at transplantation was 55 years (range: 28-69). When the symptomatic relapse of MM after ASCT was occured, pts were treated by alone chemotherapy (81 cases) or chemotherapy with thalidomide or bortezomib (33 cases). The next transplantation was performed at 59 pts. *Results*. Following ASCT, overall response rate (ORR) was 90% (121/134), 26% of pts (35/134) were in CR, 43% of pts (58/134) were in VGPR, 21% of pts (28/134) in PR, only 10% of pts (13/134) had SD. Median TTP from start of treatment was 40.7 months, median OS was 72.6 months. Nineteen percent of pts (27/141) are alive and disease free, 23% of pts (33/141) are alive with relapse and 57% of pts (81/141) died with median follow-up 8.4 years from ASCT. The subgroup of living pts without relapse had ORR 100% with high CR rate: CR in 70% of pts (19/27), VGPR in 15% of pts (4/27), PR in 15% of pts (4/27). Cytogenetic examination was performed at 59% of pts without relapse (16/27), all pts had normal karyotype. Significant prognostic parameters for poor survival after ASCT were: age at transplant over 60 years (p= 0.003), renal impairment with serum creatinine over 2mg/dL (p= 0.014), clinical stage III according to ISS (P under 0.001) and no achievement of CR after ASCT (P under 0.001). The status of disease before ASCT and type of paraprotein did not significantly affect OS after ASCT. Type of treatment of symptomatic relapse was influenced OS, pts treated with next autologous transplantation had significantly longer survival than others (p=0.032), as well as pts treated with thalidomide or bortezomib in comparing pts treated chemotherapy only (p under 0.001). *Conclusion*. ASCT is effective procedure in MM patients. The achievement CR after transplantation, no presence of stage 3 according to ISS and treatment of posttransplant relapse with thalidomide or bortezomib are the most significant parameters for long-term surviving.

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0217

RETROSPECTIVE COMPARISON OF BORTEZOMIB WITH VINCRISTINE-DOXORUBICIN-DEXAMETHASONE (VAD) AS INDUCTION TREATMENT PRIOR TO AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA

C.K. Min,[1] B,S. Cho,[1] S. Lee,[1] K.S. Eom,[1] J.W. Lee,[1] W.S. Min,[1] C.W. Park,[2] C.C. Kim[1]

[1]St. Mary's Hospital, The Catholic University of Korea, SEOUL, South-Korea; [2]Kangnam St. Mary's Hospital, The Catholic University of Korea, SEOUL, South-Korea

Background. High-dose therapy followed by autologous stem cell transplantation (ASCT) is the standard treatment option for the treatment of younger patients with multiple myeloma (MM). Patients achieving high-quality responses, as reflected by CR (complete response) and VGPR (very good partial response) after transplant benefit from ASCT. Induction pre-transplantation treatment with vincristine, doxorubicin, and dexamethasone (VAD) is currently being replaced by new targeted agents with high antimyeloma activity. The use of this novel agent may increase the CR/VGPR rate before ASCT, which may improve post-transplantation response and survival. *Patients and methods*. We performed a retrospective analysis of 72 patients with MM who consecutively received bortezomib-containing regimens (n=30) or VAD (n=42) before collection of peripheral blood stem cells and ASCT. The median age was 53 (34-65) years and 38 (52.8%) were male. The 2 groups were comparable with respect to the major presenting variables known to potentially affect clinical outcome. Patients with untreated (n=16) or previously treated MM (n=14) received bortezomib alone or in combination with the other chemotherapeutic agents. In 42 patients, vincristine and doxorubicin (by continuous infusion at the doses of 0.4 mg/d and 9 mg/m^2/d, respectively, on days 1 to 4) in combination with dexamethasone were administered every month. *Results*. Objective response (OR, at least PR) prior to ASCT was documented in 27 patients (90%) of 30 who were treated with bortezomib-containing regimens. Among 14 previously treated patients, 7 patients had received only one prior treatment before bortezomib and 7 patients as the third line or more. The corresponding figure among patients who received VAD was 33 (78.6%) of 42. The difference between the 2 groups was not significant (p=.2) However, the high-quality response rate with VGPR or more in the bortezomib group was significantly higher compared to the VAD group (66.7% *vs* 35.7%, respectively, p=.01). The difference between the 2 groups did not reach the level of statistical significance with respect to the response after ASCT (p=.122). After ASCT, all patients had a successful engraftment. There was no significant difference between the 2 groups with respect to the median number of CD34+ cells collected, the median time until neutrophil and platelet regeneration. The severe (grade 3 or more) toxicity of VAD was hematologic, particularly granulocytopenia, and infection; bortezomib-related toxicities were thromobocytopenia and peripheral neuropathy. One patient of the bortezomib group died from severe mucositis and gastrointestinal bleeding 41 days after ASCT; 1 of the VAD group died of penumocytis carinii pneumonia 101 days after ASCT. *Conclusion*. Results of this retrospective comparison of bortezomib-containing regimens with VAD regimen as induction treatment prior to ASCT for MM provided demonstration of the superiority of bortezomib therapy in terms of achievement of high-quality response. PBSC collection and engraftment following ASCT were not affected by bortezomib treatment. Bortezomib may be considered an effective therapy compared to the VAD regimen as front-line therapy for MM patients who are candidates for subsequent ASCT.

0218

VTD (VELCADE, THALIDOMIDE, DEXAMETHASON) REPRESENTS AN ACTIVE INDUCTION REGIMEN FOR PATIENTS WITH MULTIPLE MYELOMA

W. Drach,[1] V. Odelga,[2] J. Ackermann,[2] V. Sagaster,[2] H. Kaufmann,[2] N. Worel,[3] W. Rabitsch,[4] P. Kalhs,[4] C. Zielinski[2]

[1]Medical University of Vienna, VIENNA, Austria; [2]Medical University of Vienna, Dept. of Medicine I, VIENNA, Austria; [3]Medical University of Vienna, Dept. of Blood Group Serology & Transfusion Med, VIENNA, Austria; [4]Medical University of Vienna, Bone Marrow Transplant Unit, VIENNA, Austria

Background. VTD was reported to be an active salvage regimen in patients with relapsed/refractory multiple myeloma (MM). Since initial results with VTD as frontline therapy were also promising, we used

VTD as induction treatment prior to autologous stem cell transplantation in MM patients (pts), particularly in patients with poor prognostic features and/or high tumor burden. *Methods*. Bortezomib (Velcade) was administered at 1.3 mg/m^2 on days 1, 4, 8, and 11; thalidomide was given at a daily dose of 100 mg; dexamethasone (20 mg orally) was given on days 1, 2, 4, 5, 8, 9, 11, and 12. Four to six cycles were scheduled every 3 weeks. Concomitant treatment included prophylaxis against deep vein thrombosis (low dose aspirin, 100 mg daily) and herpes zoster reactivation. *Results*. We here report our experience with 12 pts (7 males, 5 females; median age 50 years, range, 36 - 67 years) with newly diagnosed MM (3 pts had failed prior VAD or thal/dex) treated with VTD. Pts had a high tumor mass at presentation (for example, pt 1: Bence-Jones kappa MM with 95% plasma cell infiltration, serum free-kappa light-chains 4000 mg/l; pt 2: IgG-kappa MM with serum IgG > 9000 mg/dL, bulky plasmacytomas in the iliac bones; pt 3: IgA-lambda MM with 70% plasma cells in the bone marrow, IgA 4500 mg/dL, plasmacytoma in the os sacrum > 10 cm in diameter) and/or cytogenetic abnormalities associated with high-risk disease (deletion of chromosome 13q in 5 pts; amplification of 1q21 (CKS1B) in 4 pts; translocation t(4;14) in 1 pt). VTD resulted in a rapid response already after 1 cycle in 10 of the 12 pts (83%), 7 pts (58%) achieved a complete response (CR)/near CR, and 3 pts had a partial response (including one very good partial response). Only one pt experienced disease progression. Rapid tumor mass reduction was not associated with signs of tumor lysis, and two pts had a significant improvement in renal function (reversal from dialysis in one pt; serum creatinine down from 2.4 to 1.3 mg/dL). 5 pts have already completed G-CSF primed peripheral stem cell collection (2.4 - 7.9 CD34+ cells/kg body weight) and high-dose melphalan (MEL200) plus autologous stem cell transplantation (all 4 evaluable pts achieved a CR after completion of high-dose therapy). Toxicity of VTD was mild (Grade 1-2 gastrointestinal side effects and peripheral neuropathy; one episode of deep vein thrombosis). Updated results will be presented at the meeting. *Conclusions*.Induction treatment with VTD results in rapid tumor mass reduction and a high remission rate (83%) in MM pts even with poor prognostic features. Stem cell collection was not compromised after VTD suggesting that VTD is an effective and safe induction treatment prior to autologous transplantation in MM.

0219

VTD INDUCTION CHEMOTHERAPY FOLLOWED BY MPT MAINTENANCE AS A FIRST LINE TREATMENT FOR THE PATIENTS WITH MULTIPLE MYELOMA WHO ARE NON-TRANSPLANT CANDIDATES

H.S. Eom,[1] Y.K. Kim,[2] J.S. Chung,[3] K.H. Kim,[4] H.J. Kim,[5] J.Y. Jin,[6] Y.R. Do,[7] S.J. Oh,[8] H.Y. Kim,[5] C.W. Suh,[9] C.M. Seong,[10] C.S. Kim,[11] D.S. Lee,[12] J.H. Lee[13]

[1]National Cancer Center, GOYANG-SI, GYEONGGI-DO; [2]Hematology-Oncology Clinic, Chonnam National University Hwasun Hospital, JEOLLANAM-DO; [3]Hemato-Oncology, Pusan National University Hospital, BUSAN; [4]Hematology and Oncology, Samsung Medical Center, SEOUL; [5]Hemato-Oncology, Hallym University Sacred Heart Hospital, GYEONGGI-DO; [6]Hemato-Oncology, Holy Family Hospital, GYEONGGI-DO; [7]Hemato-Oncology, Keimyung University Dongsan Medical Center, DAEGU; [8,9]Hemato-Oncology, Kangbuk Samsung Hospital, SEOUL; [9]Cancer Center, Asan Medical Center, SEOUL; [10]Hemato-Oncology, Ewha Woman's University Hospital, SEOUL; [11]Hemato-Oncology, Inha University Hospital, INCHEON; [12]Laboratory Medicine, Seoul National University Hospital, SEOUL; [13]Hemato-Oncology, Gachon University Gil Hospital, INCHEON, South-Korea

Background. Bortezomib, thalidomide, dexamethasone (VTD) and melphalan, prednisone, thalidomide (MPT) chemotherapies have been known to be active regimens in patients (pts) with multiple myeloma (MM). *Aims*. The objective of this study is to examine response and toxicities and to estimate survival of pts with VTD followed by MPT, who are non-transplant candidates with previously untreated MM. *Methods*. Total of 34 pts were enrolled and informed consent was obtained from March, 2006 through February, 2008 and this study is still ongoing. 14 pts were men and 20 pts were women. The median age was 67 years (range, 61-75 years) and median follow up was 10 months (range, 1-21 months). Pts received bortezomib 1.3 mg/m^2 on days 1, 4, 8, 11, thalidomide 100 mg daily, dexamethasone 40 mg on days 1-4 every 3 weeks for a maximum of 6 cycles of treatment, and thereafter melphalan 4 mg/m^2 on days 1-7, prednisone 40 mg/m^2 on days 1-7, thalidomide 100 mg daily every 4 weeks for a maximum of 12 cycles. *Results*. In 28 pts who completed at least first two cycles of VTD, 96% of them showed responses 18% CR, 4% nCR, 11% VGPR, 64% PR, 3% SD. 24 pts completed 4 cycles of VTD and all of them showed 100% response rates 42% CR, 17% nCR, 33% VGPR, 8% PR. 20 out of 21 who completed 6 cycles of VTD showed 95% responses 57% CR, 10% nCR, 19% VGPR, 10% PR. Also 12 pts who completed 4 cycles of MPT showed 83% responses 76% CR, 8% nCR, 8% VGPR, 8% PR. Among them, 4 pts who had nCR and VGPR at VTD obtained CR. This indicates MPT further improved response rate after VTD. One and half year PFS and OS were 65% and 73%, respectively. 15 pts (44%) stopped protocol therapy because of consent withdrawal (2 pts), death (7 pts), disease progression (2 pts) and severe adverse reaction (4 pts). The causes of death were infection-related in 5 pts who had been in remission. Other 2 pts were unknown etiology. Although peripheral neuropathy affected 88% of pts, only 12% of the pts were grade 3. The most common side effects of the chemotherapies greater than grade 3 were pneumonia (27%), herpes zoster (2%), asthenia (6%), diarrhea (9%), nausea (3%), thrombocytopenia (15%), neutropenia (15%) and anemia (12%). *Conclusions*. As first-line therapy, VTD followed by MPT showed high response rates and manageable toxicities for nontransplant candidates. Although high dropout rate was observed due to complications such as infection and neuropathies, treatment was well tolerated in the majority of the patients. *Protocol Number: ClinTrials.gov. NCT00320476

0220

THADD REGIMEN IS FEASIBLE AND EFFECTIVE IN AN UNSELECTED MULTIPLE MYELOMA POPULATION AGED 75 OR MORE YEARS

M. Offidani,[1] L. Corvatta,[2] C. Polloni,[3] M.-N. Piersantelli,[3] S. Gentili,[3] P. Galieni,[4] M. Catarini,[2] A. Mele,[5] M. Brunori,[2] A. Samori,[2] G. Visani,[4] N. Blasi,[2] M. Ferranti,[2] F. Alesiani,[6] M. Burattini,[2] R. Centurioni,[2] P. Leoni[3]

[1]Clinica Ematologia, ANCONA, Italy; [2]Divisione Medicina, FABRIANO, Italy; 3Clinica Ematologia, Ospedali Riuniti Ancona, ANCONA, Italy; [4]Divisione Ematologia, ASCOLI PICENO, Italy; [5]Divisione Ematolgia, TRICASE, Italy; [6]Unità Oncoematologia, SAN SEVERINO MARCHE, Italy

Background. Although more than 20% of multiple myeloma (MM) patients aged over 75 years, no specific treatment can be considered the golden standard for this population. However, recent data have shown that the MPT combination is able to improve the outcome of very elderly patients with newly diagnosed MM. *Aims*. We conducted a retrospective study to assess the efficacy and toxicity profile with ThaDD combination for very elderly MM patients. *Methods*. Unselected patients 75 or more years old received thalidomide 100 mg continuously, pegylated liposomal doxorubicin 40 mg/m^2 on day 1 every 28 days, dexamethasone 40 mg on days 1-4 and 9-12. They also were given prophylaxis with warfarin 1.25 mg day and ciprofloxacin 250 mg twice daily. Forty patients with MM (27 newly diagnosed and 13 advanced) were considered for this analysis. Median age was 77 years (range 75-91), 30 patients (85%) had ISS equal or higher 2, 13 (33%) PS (ECOG) > 2 and 10 (25%) had renal failure. Unfavourable cytogenetics was present in 34% of patients with a valuable test. *Results*. Overall, 31 patients (77.5%) obtained at least PR. Four patients achieved a CR (10%), 13 VGPR (32.5%), 14 PR (35%). Moreover, 3 patients obtained a MR, 4 progressed and 2 died early. Of note, patients with newly diagnosed MM achieved a higher quality of response if compared with advanced disease (≥VGPR 52% *vs* 23%). With a median follow up of 24 months, median TTP was 19 months for newly diagnosed and 17 months for advanced MM patients, PFS was 17 and 13 months for *de novo* and advanced MM, respectively. Median OS was not reached in newly diagnosed MM patients (75% at 2 years) and 19 months in relapsed/refractory ones. Three patients (10%) stopped treatment due to toxicity (one pulmonary embolism, one cardiotoxicity and one liver toxicity). Thalidomide was withdrawal in 6 patients (15%), mainly due to thromboembolic events. Non hematologic side effects included ≥ grade 2 peripheral neuropathy (12.5%), grade 3 constipation (7.5%) and DVT (20%, one pulmonary embolism). Regarding hematologic toxicity, grade 3-4 thrombocytopenia was reported in 5% of patients and grade 3-4 neutropenia in 12.5% of patients. Grade 3-4 infections occurred in 4 (10%) of patients without related deaths. Compared with younger patients included in the same ThaDD protocol, no significant differences in terms of compliance, non hematologic and hematologic toxicities were observed. *Conclusions*. Our study shows that ThaDD regimen is a suitable therapeutic option also in very elderly MM patients.

0221

SEQUENTIAL THERAPY WITH VAD, BORTEZOMIB AND CYCLOPHOSPHAMIDE AS INDUCTION THERAPY FOR MULTIPLE MYELOMA: A SEQUENTIAL PHARMACOLOGIC COMBINATION TO ACHIEVE COMPLETE REMISSIONS BEFORE STEM CELL TRANSPLANTATION

M. Postorino,[1] L. Pupo,[1] L. Di Caprio,[1] S. Campagna,[1] L. Franceschini,[1] D. Renzi,[1] M. Rizzo,[1] L. Gianni,[1] S. Faccia,[1] R. Cannarsa,[1] M. Ales,[1] F. Buccisano,[1] D. Venditti,[1] M. Cantonetti,[2] S. Amadori[1]

[1]Tor Vergata University, ROMA; [2]Tor vergata university, ROMA, Italy

Background. Multiple Myeloma (MM) remains an incurable disease despite intensive therapy such as high-dose melphalan and autologous stem cell transplantation (ASCT), that remains ,however, the gold standard therapy for patients (pt) without allogeneic donor. In multivariate analysis achieving complete remission (CR) before ASCT is a significant independent variable for Overall Survival and Time to Progression. *Aims.* Following this assertion, in a preliminary and feasibility study, we tried to obtain the best response as soon as possible by the induction therapy with a combinated and sequential therapy with VAD, Bortezomib and Cyclophosphamide. In fact using VAD or Bortezomib based regimen alone we obtained Complete Remision (CR/nCR) in 15% and 35% respectively; instead in our opinion their sequential use could permit a better tumor mass reduction and the exploitation of synergic role of Bortezomib in front of chemotherapy. *Material and methods*. From September 06 to December 07 we enrolled 7 pts with untreated MM; median age was 44 years (34 - 66), M/F was 2/5; the M-protein type was IgG in 3 pts, IgA in 4 pts.; the stage was IIA, IIIA and III B in 2, 4 and 1 pts .respectively; all pts showed bone marrow plasmocitosys > 50% and 5/7 pts showed extensive bone disease. Cytogenetic data were unfavourable in 3/7 pts. Patients underwent to VAD regimen (Vincristine 2 mg and Adriamicine 40 mg/m^2 on day 1 and dexametasone 40 mg day 1-4) for 2 cycles (day 1 -21); 15 days after last therapy, pts started bortezomib regimen (1.3 mg /m^2 on day 1,4,8,11) for 3 cycles and 15 days after last dose pts received Cyclophosphamide 4 g/m^2 and G-CSF for stem cell harvesting. *Results*. All patients achieved the partial remission after VAD scheme, while CR was achieved in 6/7 patients post Bortezomib. After Cyclophosphamide six patients were in CR and one was in near CR . All pts mobilized stem cells and they reached target of CD34+ (10x106/kg) with one and two apheresis in 5 and 2 patients respectively .Toxicity was low and mainly consisted of neutropenia (WHO grade I-II in 15 % of pts) and mild peripheral neuropathy (WHO grade I in 20% of pts). Conclusion. Since our experience was a preliminary and feasibility study, we think that the sequential therapy with combination of VAD, Bortezomib and Cyclophosphamide could be highly effective as up-front therapy in patients with MM; moreover it is associated with low toxicity and results in an excellent stem cell harvesting.

0222

PROMISING THERAPY RESPONSE IN MULTIPLE MYELOMA PATIENTS TREATED WITH INDUCTION CHEMOTHERAPY CONSISTING OF BORTEZOMIB, DOXORUBICIN AND DEXAMETHASONE FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION

W. Zinke-Cerwenka,[1] T. Stojakovic,[2] H. Sill,[1] E. Eibl,[1] W. Linkesch[1]

[1]Division of Hematology, GRAZ; [2]Clinical Institute of Medical and Chemical Laboratory Diagnostics, GRAZ, Austria

Background. Induction chemotherapy followed by high dose melphalane and autologous stem cell transplantation (ASCT) is considered the standard treatment for patients with multiple myeloma. Incorporation of new agents like the proteasome inhibitor bortezomib (PS-341) in combination with doxorubicin and dexamethasone (PAD) increases response rates in induction therapy significantly and may also contribute to improved results after autologous transplantation. *Methods*. After 4 cycles of PAD stem cell mobilisation was performed successfully in 24 patients with newly diagnosed multiple myeloma. All patients but one qualified for ASCT by achieving at least SD. Another patient discontinued therapy before ASCT. Until now, 22/24 patients underwent ASCT after conditioning with melphalane 200mg/m^2. The pre-transplantation remission status (EBMT criteria) of these patients was: 2 CR, 8 nCR, 10 PR and 2 SD. Evaluation of treatment response was performed 3 months after ASCT. Maintenance therapy after transplantation with thalidomide was determined for all patients who achieved only SD or PR before ASCT. *Results*. Twenty-one patients could be evaluated; one did not reach the first evaluation point yet. According to EBMT criteria, 3 patients achieved CR, 12 nCR and 3 PR. One patient died of pneumonia and two patients showed progressive disease. The median follow-up after ASCT is 9 months (range: 3-25). *Conclusion*s. These preliminary data show that improved response rates after PAD induction may translate into improved CR/nCR rates after single ASCT.

0223

A COMPARISON OF TWO CONDITIONING REGIMENS FOR MULTIPLE MYEOLOMA. A SINGLE CENTER EXPERIENCE

J. Peñarrubia,[1] R. Cuello,[2] A. Cantalapiedra,[1] A. Dueñas,[1] E. Fontecha,[1] O. Gutierrez,[1] A. Silvestre,[1] J.M. Martin-Antoran,[1] J. Fernández-Calvo,[2] J. García-Frade[1]

[1]Hospital del Rio Hortega, VALLADOLID, Spain; [2]Hospital Clínico, VALLADOLID, Spain

Background. Different conditioning regimens have been used in myeloma patients treated with autologus stem cell transplantation (ASCT), but none has shown a clear advantage over any other. *Aims.* To assess differences in efficacy and toxicity of two conditioning regimens for multiple myeloma. *Methods*. 36 consecutive myeloma patients (male/female = 19/17; age: median = 54 years, range=33-67) transplanted at our institution between April-96 and June-05 were analyzed. Thirty two patients received the alternating chemotherapeutic regimen VBMCP/VBAD as induction treatment, and the remaining 4 received VAD. All patients were transplanted as front line therapy, immediately after being administered chemotherapy. 21 received melfalan 200mg/m^2 divided in 2 consecutive days (MEL200) and 15 patients received busulfan 12 mg/kg divided in four consecutive days plus melfalan 140 mg/m^2 on the fifth day (BUMEL). *Results*. There were no differences between the 2 groups in patient characteristics both at diagnosis or at transplant. The number of CD34 cells infused (x10^6/kg) in each conditioning group was statistically similar: median (range), BUMEL 2.5 (1.6-4.3), MEL 200 2.35 (1.8-7.8) (p=0.25). After ASCT, granulocyte and platelet recovery were similar for both groups. Time to platelet recovery (20x10^9/L) was 11 days in both groups and time to reach 50x10^9/L/L was 14 days in BUMEL and 15 days in MEL200 (p=0.11). Median hospital stay was 14 days in both groups (p=0.16). No patient died in the first 100 days post-ASCT. Both regimens yielded a similar response in reference to pre-ASCT myeloma status. The 5-year overall survival was 66.7% in BUMEL and 53% in MEL200 (p=0.66). The 5-year event-free survival was 66% in BUMEL and 26% in MEL200 (See Graph). After a median follow-up of 50 months, the median event-free survival was not yet reached in BUMEL, whereas it was 33 months in MEL200. These differences in event-free survival were close to statistical significance (p=0.08). No differences in the type or severity of toxicities were noted. A case of mild veno-oclusive liver disease occured in BUMEL. *Conclusions*. Our data show a trend towards a better anti-myeloma effect for the BUMEL conditioning regimen Vs MEL200 that might reach statistical significance with a larger number of patients. There appears to be no clear-cut differences in toxicity. Further study is warranted to confirm these data.

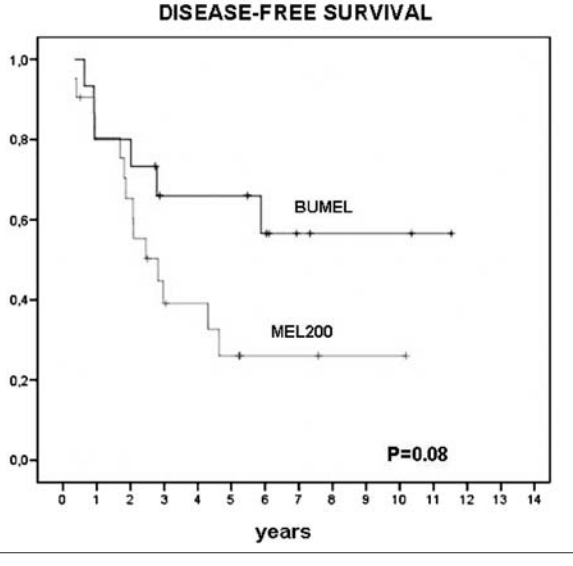

Figure 1.

Myelodysplastic syndromes - Clinical I

0224

EFFECT OF AZACITIDINE (AZA) *vs* LOW-DOSE ARA-C (LDAC) ON OVERALL SURVIVAL (OS), HEMATOLOGIC RESPONSE, TRANSFUSION INDEPENDENCE, AND SAFETY IN PATIENTS (PTS) WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES (MDS)

P. Fenaux,[1] N. Gattermann,[2] J. Seymour,[3] E. Hellström-Lindberg,[4] G.J. Mufti,[5] U. Duehrsen,[6] S. Gore7, F. Ramos8, O. Beyne-Rauzy,[9] H. Dombret,[10] A. List,[11] D. McKenzie,[12] J. Backstrom,[12] A. Allen,[12] C.L. Beach[12]

[1]Hôpital Avicenne, BOBIGNEY, France; [2]Heinrich-Heine-University, DÜSSELDORF, Germany; [3]Peter MacCallum Cancer Centre, EAST MELBOURSE, Australia; [4]Karolinska University Hospital, STOCKHOLM, Sweden; [5]King's College London, LONDON, UK [6]University Hospital Essen, ESSEN, Germany; [7]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, BALTIMORE, USA; [8]Servicio de Hematología, Hospital de León, LEÓN, Spain [9]Chu Purpan, TOULOUSE, France; [10]Hôpital Saint-Louis (APHP), PARIS, France; [11]Moffitt Cancer Center, TAMPA, USA; [12]Pharmion Corporation, OVERLAND PARK, USA

Background. LDAC is often used to treat MDS although no survival advantage has been established, and pts must be monitored carefully for myelosuppression (UK MDS Guidelines Group, Br J Haematol 2003;120:187). The large randomized phase III trial (AZA-001) confirmed AZA as the first MDS treatment to significantly prolong OS in higher-risk MDS pts compared with 3 conventional care regimens (CCR), including LDAC (*Fenaux, Blood 2007;110:Abstract 817*). *Aims*. To assess OS, hematologic response, transfusion independence, and safety in a subgroup analysis comparing pts receiving AZA *vs* LDAC. *Methods*. Higher-risk MDS pts (FAB: RAEB, RAEB-T, CMML; IPSS: Int-2, High) were enrolled. Before randomization, investigators selected 1 of 3 CCR (best supportive care, LDAC [20 mg/m^2/d x 14d every 28 days for ≥4 cycles], or intensive chemotherapy) for all pts. Then, if randomized to AZA, pts received AZA (75 mg/m^2/d SC x 7d every 28 days for ≥6 cycles) regardless of investigator selection; if randomized to CCR, pts received their investigator-selected treatment. All regimens were continued until study end, relapse, progression, unacceptable toxicity, or AML transformation. For this subgroup analysis, OS, hematologic response (IWG 2000), and transfusion independence (≥56 days) were compared between the AZA and LDAC groups This was an intent-to-treat analysis based on all randomized pts using Cox proportional hazard modeling stratified by IPSS and FAB subtypes, adjusting for baseline ECOG, RBC transfusions, FAB subtype, presence of -7/del(7q), LDH, and hemoglobin. Median OS was analyzed using Kaplan-Meier methods. Treatment by investigator-selection interaction was included in the model. Erythropoiesis-stimulating agents were not allowed. All pts gave informed consent.

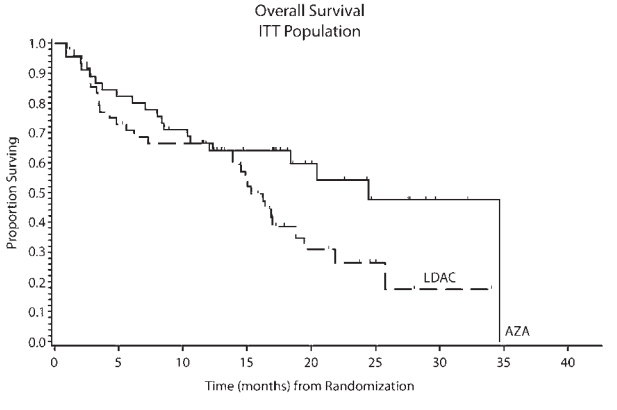

Figure 1.

Results. There were 94 pts selected by investigators to receive LDAC. Per randomization, 45 received AZA and 49 LDAC. Baseline characteristics were similar between the AZA and LDAC groups, respectively, e.g., median age: 69 years (42-82) *vs* 71 years (56-85); cytogenetics: intermediate (16% *vs* 25%), poor (29% *vs* 16%); IPSS: High (42% *vs* 43%); FAB classification: RAEB (60% *vs* 51%), RAEB-T (33% *vs* 39%). AZA was administered for a median of 9.0 (1-39) cycles, LDAC for 4.5 (1-15) cycles. More early discontinuations due to withdrawal of consent, adverse events, or progression were observed in the LDAC group (67%) *vs* the AZA group (39%). Median OS was 24.4 months (95% CI: 12.0-34.7) *vs* 15.3 months (95% CI: 13.9-18.8) in the AZA and LDAC groups, respectively (hazard ratio: 0.38 (95%CI: 0.21-068, p=0.001, Figure 1). The OS advantage with AZA *vs* LDAC, respectively, was supported by CR+PR rates (31% *vs* 12%, p=0.042), HI rates (major+minor: 53% *vs* 25%, p=0.006), and transfusion independence in baseline-dependent pts (45% *vs* 13%, p=0.011). Higher rates of grade 3-4 anemia were seen in the LDAC group (14%) *vs* the AZA group (7%). Rates of thrombocytopenia were similar. Deaths during study were higher in the LDAC group *vs* the AZA group: 59% *vs* 45%, respectively. *Summary/conclusions*. AZA significantly prolongs OS with a 62% reduced risk of death *vs* LDAC. The OS advantage with AZA was supported by significant improvements in hematologic response and improvement, and transfusion independence. AZA should be considered first-line therapy compared with LDAC in higher-risk pts with MDS.

0225

A PHASE II STUDY OF OUTPATIENT ADMINISTRATION OF DECITABINE FOR 5 DAYS EVERY 4 WEEKS TO ADULTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

P. Steensma,[1] M. Baer,[2] J. Slack,[1] R. Buckstein,[3] L. Godley,[4] J. Larsen,[5] M. Cullen,[5] S. Arora,[5] H. Kantarjian[6]

[1]Mayo Clinic, ROCHESTER, MN, USA; [2]Roswell Park Cancer Institute, BUFFALO, NY, USA; [3]Toronto Sunnybrook Regional Cancer Centre, TORONTO, ON, Canada; [4]University of Chicago, CHICAGO, IL, USA; [5]MGI Pharma, BLOOMINGTON, MN, USA; [6]MD Anderson Cancer Center, HOUSTON, TX, USA

Background. Decitabine, a DNA-targeted hypomethylating agent, is FDA-approved for treatment of patients with all FAB subtypes of MDS. An overall improvement rate of 30% in MDS patients (complete responses [CR] + partial responses [PR] + hematologic improvements [HI]) was reported with the approved dosing regimen of 15 mg/m^2 intravenously over 3 hours every 8 hours for 3 days, repeating every 6 weeks. An off-label regimen, in which decitabine is administered 20 mg/m^2 IV over 1 hour once daily for 5 consecutive days repeating every 4 weeks, permits the use of decitabine as outpatient therapy. The 5-day regimen showed promising efficacy in an initial trial (Blood. 2007;109:52), with 39% CR using the International Working Group [IWG] 2006 criteria. *Aims*. This multicenter, open-label, single-arm study evaluated the efficacy and safety of the 5-day decitabine dosing regimen in patients with all FAB subtypes of MDS. *Methods*. Eligible patients were enrolled at 28 sites in the USA and Canada. Inclusion criteria were ≥18 years of age, *de novo* or secondary MDS (including CMML with white count <12,000/mL), and International Prognostic Scoring System (IPSS) score ≥0.5. All patients received decitabine 20 mg/m^2 IV over 1 hour x 5 days, with cycles repeated every 4 weeks. The primary endpoint was overall response, assessed by both the IWG 2000 criteria (available at the time of study initiation and used to aid comparison with the approved 3-day dosing regimen), and with updated IWG 2006 criteria. *Results*. Ninety-nine patients enrolled: median age 72 years, 72% male, 89% *de novo* MDS, median time from diagnosis 22 weeks, and 27% with prior therapy for MDS. FAB classification was: RA, 20%; RARS, 17%; RAEB, 45%; RAEB-T, 6%; and CMML, 11%. This report includes data through 15 months after enrollment of the last patient. Patients received a median of 5 cycles of decitabine (range 1-20), with 38% of patients receiving ≥8 cycles. All 5 doses of decitabine were administered during 98% of cycles. Overall improvement rate in the intent-to-treat population was 43% by the IWG 2000 criteria (CR+PR+HI). Using the IWG 2006 criteria, the improvement rate was 51% overall (17% CR, 15% mCR and 18% HI). Median time to improvement (IWG 2006 criteria) was 1.7 months and 82% of patients that improved did so by Cycle 2. Median survival was 19.6 months and the 1-year survival rate was 66%. The most frequently observed grade 3 or higher adverse events attributed to the study drug were neutropenia (31%), thrombocytopenia (18%), febrile neutropenia (14%), anemia (12%), pneumonia (11%), and fatigue (5%). *Summary and Conclusions*. Administration of decitabine on the alternative 5-day dosing schedule in the outpatient setting demonstrated prompt clinical activity with a manageable toxicity profile, suggesting that both the approved 3-day regimen and this alternative 5-day regimen provide meaningful clinical benefit to patients with MDS.

0226

WELL-DIFFERENTIATED SYSTEMIC MASTOCYTOSIS (WDSM): A NOVEL FORM OF MASTOCYTOSIS

M. Jara-Acevedo,[1] A.C. García-Montero,[1] C. Teodosio,[1] L. Escribano,[2] I. Alvarez,[3] L. Sanchez-Muñoz,[4] C. Akin,[5] D.D. Metcalfe,[6] A. Orfao[1]

[1]Cancer Research Center, SALAMANCA, Spain; [2]Centro de Estudios de Mastocitosis de Castilla La Mancha, TOLEDO, Spain; [3]Centro de Estudios de Mastocitosis de Castilla la Mancha, TOLEDO, Spain; [4]Centro de Estudios de Mastocitosis de Castilla la Manca, TOLEDO, Spain; [5]Allergy and Clinical Immunology, University of Michigan, ANN ARBOR, USA; [6]Laboratory of Allergic Diseases, NIAID, NIH, BETHESDA, USA

Background. Mastocytosis are a heterogeneous group of disorders characterized by an abnormal expansion and accumulation of mast cells (MC) in one or more tissues. In 2001, the World Health Organization (WHO) proposed a combination of major and one minor criteria, or at least three minor criteria, for the diagnosis of systemic mastocytosis (SM). Major criteria was defined as presence of dense infiltrates of >15 MC in bone marrow (BM) and/or other extracutaneous organs. Minor criteria include, 1) dense infiltrates with >25% of MC showing abnormal morphology; 2) presence of KIT mutation at codon 816; 3) aberrant expression of CD2 and/or CD25 in BM MC; and 4) serum total tryptase >20ng/mL. However, a variable percentage of patients that present SM clinical-biological symptoms do not fulfill the SM WHO criteria. In 2004, a case of a novel form of mastocytosis-Well Differentiated Systemic Mastocytosis (WDSM)-was described; but so far the clinical-biological features of this type of SM have not been established. *Aims*. We propose to define the clinical-biological characteristics and the diagnostic criteria of this new subtype of SM. *Methods*. The study was performed at the reference centers of the Spanish Network on Mastocytosis (REMA) in a series of 14 selected patients (3M/11F) that could be included in the WDSM subtype. In addition, data from 3 patients were referred from the University of Michigan. *Results*. Only 9 patients fulfilled the WHO criteria for SM. 16 patients fulfilled the major criteria, but none showed >25% of MC with abnormal morphology. Typical KIT mutation (D816V) was found only in one patient, but 3 patients displayed other KIT mutation (I817V, N819Y and F522C). No aberrant CD2 and/or CD25 expression was found in BM MC, except for a case that displayed a dim CD2 expression in all MC and other 3 patients presenting weak CD25 expression restricted to a MC sub-population. High serum tryptase levels were persistently detected only in 5 patients. Apart from the WHO criteria, all patients showed typical skin lesions and hypergranulated MC. MC clonality was demonstrated by HUMARA test in all 7 female patients analyzed. Comparison of clinical characteristics observed in WDSM patients with those from 132 patients diagnosed with Indolent Systemic Mastocytosis (ISM), showed an earlier -pediatric- onset in WDSM patients (median 5 years old *vs* 29 years old in ISM). Interestingly, WDSM group showed neither anaphylactic episodes nor neuropsychiatry symptoms, whereas in ISM their frequency was 46% and 26%, respectively. Higher BM MC load was found in WDSM (median 0.11%) *vs* ISM (median 0.06%), while serum tryptase levels were lower in WDSM (median 12 ng/mL vs 29 ng/mL). *Conclusions*. WDSM is a subtype of mastocytosis with pediatric onset that typically shows mature large hypergranulate BM MC, absence of D816V KIT mutation, absence or dim expression of CD2 and/or CD25, and low serum tryptase levels. Despite presenting systemic involvement (BM and skin affectation), WDSM patients were not well classified using the actual SM diagnostic criteria (WHO criteria).

0227

5-AZA-2'-DEOXYCYTIDINE CAN INDUCE DIFFERENTIATION OF CLONAL ABNORMAL PROGENITOR CELLS FROM PATIENTS WITH MYELODYSPLASTIC SYNDROMES WITHOUT INDUCTION OF APOPTOSIS

S. Meers,[1] P. Vandenberghe,[1] M. Boogaerts,[1] G. Verhoef,[1] C. Verfaillie,[2] M. Delforge[1]

[1]University Hospital Leuven, LEUVEN, Belgium; [2]Stem Cell Institute, LEUVEN, Belgium

Background. The methyltransferase-inhibitors 5-azacytidine (AZA) and 5-aza-2'-deoxycytidine (DAC) are changing the treatment of high-risk myelodysplastic syndromes (MDS). To date, two major mechanisms of action have been postulated. One mechanism is selective induction of apoptosis in the abnormal progenitor cells allowing outgrowth of remnant normal progenitors. Alternatively, treatment with methyltransferase-inhibitors could (re-)induce differentiation of abnormal progenitors as they have been shown to do in leukemic cell lines. *Aims*. This study was designed to evaluate the *in vitro* effects of DAC and AZA on purified CD34$^+$ cells from patients with MDS and AML. We evaluated the effects of these drugs on (1) the viability and (2) the clonogenic capacity of CD34$^+$ cells. (3) In progenitor cells showing increased proliferation after treatment with DAC or AZA we determined their clonal origin. *Methods*. Bone marrow CD34$^+$ cells from 17 patients (11 MDS, 6 AML) and 11 healthy controls were purified using MACS columns and subjected to different concentrations of DAC (0.5 and 1.0 μM) and AZA (2.5 μM and 5 μM). After 24h, 48h and 72h, the cells were harvested and assessed for viability with trypane blue staining. Subsequently, equal numbers of living cells were cultured in standard clonogenic assays for 14 days. Single hematopoietic colonies were plucked from the methylcellulose medium to be evaluated with FISH. *Results*. (1) Incubation with DAC did not significantly influence the viability of primary bone marrow CD34+ cells from healthy controls. Similar results were found in progenitor cells from MDS patients. In contrast, we noted more apoptotic CD34+ cells from AML patients in the presence of DAC. The concentrations of AZA in this study lead to an increase in cell death in donors as well as in patients. (2) DAC did not significantly influence the number of colonies produced by normal CD34+ cells. In contrast, the concentrations of AZA we used lead to reduced number of colonies and especially the number of BFU-E. The number of colonies in cultures from AML-derived CD34$^+$ cells was low and was not significantly altered by previous culture with AZA or DAC. Only in 1 patients with RARS pre-culture of the CD34$^+$ cells for 72h with DAC resulted in a higher number of mature colonies and this was observed with 2 different concentrations of DAC. (3) This patient with RARS was known with the cytogenetic abnormality 45, X, -Y, DEL(1)(P12P33) (2 of 10 metaphases, others being 46,XY). We randomly picked 10 colonies from the 3 conditions (0, 0.5 μM and 1.0 μM DAC) and analyzed these colonies with FISH. All colonies were found to be derived from the abnormal clone. *Summary/conclusions*. Methyltransferase-inhibitors are increasingly used in the treatment of high-risk MDS. Our *in vitro* study suggests that differentiation-induction of abnormal progenitor cells rather than selective induction of apoptosis of these cells is responsible for their effect in MDS.

0228

IRON CHELATION THERAPY WITH DEFERASIROX (ICL670) REDUCES SERUM FERRITIN (SF) AND LABILE PLASMA IRON (LPI) IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

A.F. List,[1] M.R. Baer,[2] D. Steensma,[3] A. Raza,[4] J. Esposito,[5] J. Virkus,[5] J. Feigert,[6] E. Besa,[7] C. Paley[5]

[1]University of South Florida, TAMPA, USA; [2]Roswell Park Cancer Institute, BUFFALO, USA; [3]Mayo Clinic, ROCHESTER, USA; [4]Univerisity of Massachusetts, MASSACHUSETTS, USA; [5]Novartis Pharmaceuticals Corporation, EAST HANOVER, USA; [6]Arlington Fairfax Hematology/Oncology, ARLINGTON, USA; [7]Thomas Jefferson University, PHILADELPHIA, USA

Background. Transfusion-dependent patients with MDS may benefit from iron chelation therapy to prevent the morbidity and mortality associated with iron overload. The once-daily oral agent, deferasirox, has demonstrated efficacy and tolerability in patients with MDS. Here we report 12-month data from the US03 trial in patients with MDS treated with deferasirox. *Aims.* To evaluate the long-term efficacy and safety of deferasirox in patients with lower-risk MDS in study US03, an ongoing, Phase II, open-label, 3-year trial. *Methods*. Patients enrolled had Low- or Int-1 IPSS risk MDS, transfusional iron overload (SF ≥1000 μg/L and >20 units RBC transfusions), and serum creatinine (SCr) 2-fold the upper limit of normal (ULN). Deferasirox was initially dosed at 20 mg/kg/day, and could be increased to 40 mg/kg/day based on tolerability and response. SF was monitored monthly; LPI, the reactive species of non-transferrin-bound iron, was assessed quarterly. *Baseline results*. In 173 patients (102 men, 71 women) with a mean age of 70 years (range 21-90), IPSS risk groups were Low (46 patients; 27%), Int-1 (123; 71%) and other (four; 2%). Baseline parameters were: SF 3398 μg/L (863-36,280); LPI 0.4 μmol/L (0.0-3.6); mean lifetime transfusions prior to study 63; years of prior transfusions 3.5 (0-34). MDS therapy at study entry included: chemotherapy 22 patients; growth factors 46. Estimated creatinine clearance: normal (>80 mL/min) 77 patients; mild impairment (51-80 mL/min) 68; moderate impairment (30-50 mL/min) 25; severe impairment (<30 mL/min) two. Forty percent of patients had elevated LPI (≥0.5 μmol/L). Efficacy: Fifty-three patients have reached the 12-month treatment milestone in this ongoing trial. Over 12 months, the mean dose was 21 mg/kg/day and mean transfusion rate was 4.1 units/month. Mean SF±SD (μg/L) values were: baseline 3398±3088; 3 months 3065±1743; 6

months 2775±1355; 9 months 2759±1562; 12 months 2603±1336 (Figure 1). Sustained suppression of mean LPI to the normal range was achieved after 3 months' treatment (Figure 1).

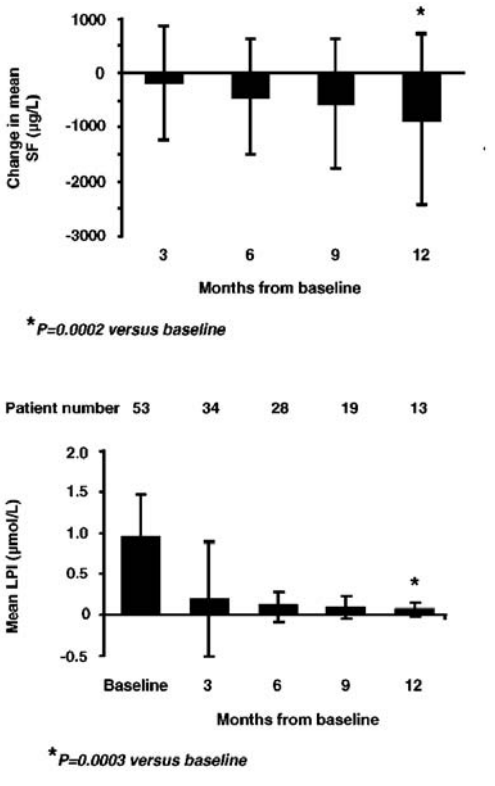

Figure 1.

Safety. Of 165 patients, 10 (6%) discontinued secondary to suspected adverse events (AEs), and seven (4%) serious AEs. There were 11 deaths (7%), all unrelated to deferasirox. Of 140 patients with normal baseline SCr, 35 (25%) increased >ULN (2.2 mg/dL max SCr). SCr increased >33% above baseline in 11 patients (8%) with abnormal SCr at baseline. Thrombocytopenia developed in 22 patients (13%) and neutropenia occurred in 52 (32%). Development of cytopenia was consistent with hematologic progression of MDS. *Summary and Conclusions*. Twelve months' treatment with deferasirox in these patients with MDS and elevated SF decreased mean SF by 859±1548 µg/L and normalized trough LPI in all patients, indicating 24-hour sustained suppression. A high baseline SF may also have contributed to the elevation of SCr. Deferasirox was generally well tolerated. Recent reviews show a 30% increase in hazard ratio for every 500 µg/L increase in SF >1000 µg/L, and NCCN guidelines recommend considering iron chelation therapy in iron-overloaded patients with MDS. Ongoing assessments in this study will evaluate the effect of deferasirox treatment on cardiac, hepatic and endocrine function in these patients with MDS.

0229

TRANSFUSION BURDEN, DISEASE DURATION AND AGE IDENTIFY NON-DELETION 5Q MDS PATIENTS HIGHLY RESPONSIVE TO LENALIDOMIDE TREATMENT

A.F. List,[1] K. Wride,[2] R. Knight[2]

[1]University of South Florida, H. Lee Moffitt Cancer Center and Research Institute, TAMPA, FLORIDA; [2]Celgene Corporation, SUMMIT, NJ, USA

Background. Lenalidomide is an immunomodulatory agent that was shown in the MDS-002 study of patients with non-deletion 5q (del[5q]) myelodysplastic syndromes (MDS) to reduce transfusion requirements in 43% of patients with 26% of patients achieving transfusion independence.[1] Multivariate analysis of this study showed that baseline transfusion burden and duration of disease were co-variates influencing hematologic response to lenalidomide.[1] We hypothesized that lenalidomide targets a disease profile in patients with non-del(5q) MDS that is clinically distinct, and perhaps characterized by an immune pathogenesis.[2] *Aims*. To investigate the demographic profile of patients with non-del(5q) MDS who have the greatest potential for benefit from lenalidomide treatment, we performed a secondary analysis of the MDS-002 multicenter Phase II trial. *Methods*. Transfusion independence rate and the duration of response were analyzed according to features previously linked to erythropoietin (EPO) response and MDS with a potential immune pathogenesis, i.e., transfusion burden (≤4 units RBC *vs* > 4 units in the 8 weeks prior to the study), MDS disease duration (≤2 years *vs* > 2 years) and age (≤60 years *vs* > 60 years). Fisher's exact test and a test for trend were used for the univariate comparisons. The duration of transfusion independence (TI) was estimated by the Kaplan-Meier method. *Results*. Among 214 patients treated with lenalidomide, 60 patients received ≤4 units RBC/8 weeks and had a diagnosis of MDS ≤2 years. Demographics were similar between these patients and those with higher transfusion burden/long-standing disease: median age, 72 years (range, 35-94) *vs* 73 (27-87); male, 60% *vs* 66%; low/int-1 IPSS category, 84% *vs* 77%, respectively. Of the patients who had low transfusion burden and short MDS duration, 43% (26/60) achieved TI with lenalidomide compared with 19% (30/154) of patients with greater transfusion burden or longer disease duration ($p<0.001$). There was a highly significant association between baseline transfusion burden and the frequency of TI response ($p<0.0001$, Figure 1). The frequency of TI to lenalidomide was higher in patients ≤60 years of age (12/29 [41%] *vs* 45/186 [24%], $p=0.069$). Median duration of response was 10.0 months (95%CI, 5.2-not yet reached; range, 1.9-39.5) for patients with low transfusion burden and disease duration ≤ 2 years compared with 9.5 months (95%CI, 4.9-14.0; range, 2.0-37.1) in the other group.

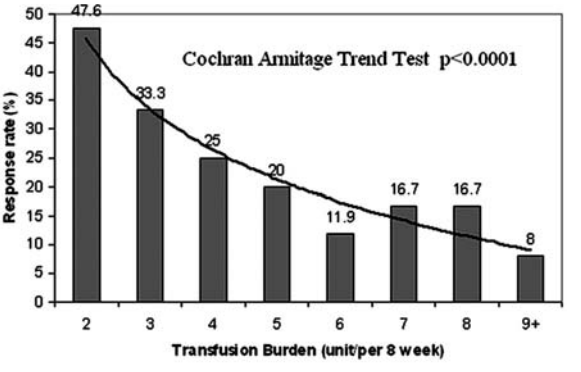

Figure 1. TI response by transfusion burden.

Conclusions. MDS patients with low transfusion burden and disease duration ≤2 years had high rates of transfusion independence with lenalidomide that compare favourably to EPO therapy.[3,4] The age dependent response favoring ≤60 years suggests that lenalidomide may restore T-cell homeostasis and offer an alternative to immunosuppressive therapy in this population. These data suggest that lenalidomide may offer a primary treatment alternative for the management of RBC transfusion dependent MDS patients.

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0230

A NEW EVALUATION OF THE POTENTIAL AETIOLOGICAL ROLE OF SMOKING HABIT IN PRIMARY MYELODYSPLASTIC SYNDROME: INBIOMED HEMA-001/2004 STUDY (PART ONE)

F. Ramos,[1] N. De Las Heras,[1] J.A. Rodriguez,[2] M. Megido,[2] J.R. Gonzalez,[3] L. Florensa,[4] M. Barbon,[1] C. Del Canizo,[3] M. Fuertes,[1] J. Gonzalez-Gallego[5]

[1]Hospital de León, LEÓN; [2]Hospital El Bierzo, PONFERRADA (LEÓN), Spain; [3]Hospital Universitario de Salamanca, SALAMANCA; [4]Hospital del Mar, BARCELONA; [5]INBIOMED, University of León, LEÓN, Spain

Background. Several studies have linked cigarrette smoking habit (SH) to the development of primary myelodysplastic syndromes (MDS). The average smoker is exposed to daily benzene doses 10-times higher than a non-smoker. Tobacco smoke induces oxidative DNA damage but at

least two detoxifying pathways may alleviate this challenge: glutation-transferases M1 and T1 (*GSTM1, GSTT1*) and NAD(P)H quinone-oxidoreductase (NQO1). Polymporphisms of the genes codifying for this enzymes are responsible for a reduction in the detoxifying capacity that has confers a higher risk of AML/MDS. The eventual interaction between SH and these polymporphisms has been analyzed in several human tumours, but it has not been explored so far in MDS. *Aims*. To clarify the role of SH and to check the eventual impact of concurrent polymorphisms in *GSTM1, GSTT1* and *NQO1* in the aetiology of MDS. *Methods*. We have recruited prospectively 241 patients at 3 spanish hospitals, for a case-control study. A case:control ratio of 1:3 was designed, to optimize recruitment. Sample size was estimated with the aim to detect an odds-ratio (OR) of at least 3.0. Sixty one consecutive MDS patients (FAB criteria) and 180 hospital controls participate in this study. Control patients were randomly selected among the patients of the Departments of Traumatology/Surgery. We excluded upfront those with MDS or other blood diseases, abnormal PB counts, previous cancer, previous treatment with radiotherapy, alkylating agents, or azatioprine and SH related-diseases. Life-long enviromental exposures were recalled with the use of self-administered ad-hoc questionnaire. SH has been calculated in *pack-years* and categorized in quintiles. Smoke exposure at enrollment was confirmed by plasma cotinine determination. The presence of a null genotype for *GSTM1* and *GSTT1* was studied by multiplex PCR, while *NQO1* genotyping was performed by RFLP-PCR. Crude and covariate-adjusted odds-ratios, were calculated for SH by single-step logistic regression analysis, after proper identification of target covariates following a hierarchical approach.

Table 1. Odds-ratio for MDS as a function of smoking habit, adjusted for relevant confounders and statistically significant first-order interactions. Q5 stand for 5th quartile of the distribution.

	n	Crude Odds-ratio	Adjusted Odds-ratio	95% CI
Non-smokers	116	1.00		
Smokers (any level)	127	1.38	1.48	0.77 - 2.86
Non-smokers	116	1.00		
Smokers Q4-Q5	48	2.36	3.10	1.38 – 6.96
Non-smokers	116	1.00		
Smokers Q1-Q3	76	0.93	0.91	0.41 – 2.01
Smokers Q4	23	1.53	1.97	0.64 – 6.06
Smokers Q5	25	3.23	4.39	1.49 – 12.99

Results. MDS tends to be a bit more frequent in smokers (OR 1.38; 95% CI 0.77 - 2.47) but a disequilibrium in inactivating polymorphisms of benzene detoxifying enzymes was not obvious (ORs ranged from 0.82 to 1.19). Patient stratification according to SH intensity reveals a clear-cut dose-effect relationship (Chi-square for lineal trend= 5.91, p=0.016) between the SH level and the *riskof* MDS (Table 1), with those exposed to >28 pack-years showing an adjusted-OR of 3.1 (95% CI 1.38-6.96), and those over 52 pack-years reaching 4.4 (95% CI 1.49-12.99). After stratification according to FAB, the effect of SH was limited to advanced MDS. Exposure to automobile exhaust at work showed a synergistic effect with SH, confering a higher risk of MDS (p=0.047). Finally, MDS patients harboring intermediate-risk cytogenetics (IPSS) were smokers more frequently than those with other cytogenetic subgroups (p=0.025, Fisher) and had been exposed to less cigarrette smoke (median exposure 0.0 *vs* 58.5 pack-years, p=0.004 Mann-Whitney). *Conclusions*. Smoking habit over 28 pack-years is a risk factor for advanced primary MDS. Intermediate-risk cytogenetics is more frequent among smokers. Concurrent exposure to automobile fuel exhaust, seems to increase the risk of MDS in smokers. The polymorphisms considered do not make a significant contribution to the problem, and eventual preventive initatives should be addressed to the general population.

0231

5-AZACYTIDINE FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA: RESULTS IN 45 PATIENTS FROM THE ITALIAN PATIENT NAMED PROGRAM

P. Musto,[1] L. Maurillo,[2] A. Spagnoli,[3] M. Genuardi,[3] M. Lunghi,[3] N. Di Renzo,[3] G. Mele,[3] A. Levis,[3] A. D'Arco,[3] A. Gozzini,[3] M. Petrini,[3] M. Mianulli,[3] G. Sanpaolo,[3] A. Santagostino,[3] G. Pietrantuono,[3] F. D'Auria,[3] D. Ferrero,[3] G. Gaidano,[3] F. Ferrara,[3] V. Santini,[3] G. Leone,[3] A. Bosi,[3] A. Venditti[3]

[1]CROB, Centro di Riferimento Oncologico della Basilicata, RIONERO IN VULTURE (PZ); [2]S. Eugenio Hospital, Tor Vergata University, ROMA; [3]On behalf of ad hoc Italian Study Group, AZACYTIDINE IN MDS AND AML, Italy

Background. 5-azacytidine (AZA) is a hypomethylating agent approved in 2004 from FDA for the treatment of all types of myelodysplastic syndromes (MDS), including RAEB-T FAB subtype (marrow blasts between 21 and 30%), which are now classified as AML, according to WHO classification. Indeed, some recent reports have raised the question of a possible efficacy of AZA in selected patients with AML. *Methods*. In September 2007, we started a retrospective study aiming to register and analyse all Italian patients with MDS or AML who had received AZA for the treatment of their disease outside of clinical trials, on the basis of a national patient named program. Among a total of 218 patients treated in 31 different Italian Institutions since 2005, forty-five AML were collected. Most of them were elderly, unfit patients not eligible for more intensive therapies, often with hypocellular AML. Median age was 70 years (range 29-87), twenty-one pts were male. Unfavourable karyotype was present in 59% of assessable pts, while 66% of cases were secondary AML. Median time from diagnosis was 5 months (range 5-27). Thirty pts (66.7%) were pre-treated, 24.4% with one line and 42.3% with two lines of chemotherapy (CT) (12 low dose CT; 16 high dose CT, including autologous or allogeneic stem cell transplantation; 2 growth factors; 11 other therapies). The median number of monthly AZA cycles administered was 3 (range 1-11). Thirty-six pts (80%) received AZA 100 mg/d fixed-dose s.c., nine (20%) 75 mg/d/sqm s.c.. A seven-day per month schedule was employed in 32 pts (71.1%), while 13 pts (27%) received AZA for more than 7 days. One patient received the drug for 5 days. Twenty-four pts (53%) received AZA alone, twenty-one (47%) in various combinations with growth factors (n.1), valproic acid±ATRA (n.18), gentuzumab-ozogamycin (n. 2). *Results*. The most relevant toxicity observed (grade 3-4) was represented by further myelosuppression (15%), infections (24%: in particular, 1 fungal, 3 pneumonia and one septic shock) and gastro-intestinal adverse events (20%). Overall, complete remission (CR) occurred in 3 out of 41 assessable patients (7.4%). Partial response was observed in additional 10 pts (24.2%), so that overall response rate was 31.6%. Median response duration was 2 months (range 1-8). After a mean follow-up of 12 months, seventeen pts (38%) are still alive. A significant difference (p<0.019) in response rate in favour of 75 mg/sqm *vs* 100 mg fixed dose was observed. *Conclusions*. These data confirm, in the practical clinical setting, that AZA could has a role in treating selected patients with *de novo* or pretreated AML. Of interest, in our series the *standard* dose of 75 mg/m^2 for 7 days appeared to be more effective than 100 mg/d (one single vial) fixed dose.

0232

RAPID ONSET OF EFFECTIVENESS WITH THREE ALTERNATIVE AZACITIDINE (AZA) DOSING REGIMENS IN PATIENTS (PTS) WITH MYELODYSPLASTIC SYNDROMES (MDS)

R. Lyons,[1] T. Cosgriff,[2] S. Modi,[3] H. McIntyre,[45] I. Fernando,[4] J. Backstrom,[4] C.L. Beach[4]

[1]Cancer Care Centers of South Texas, SAN ANTONIO; [2]Hematology and Oncology Specialists, METAIRIE; [3]Joliet Oncology-Hematology Associates, JOLIET, USA; [4]Pharmion Corporation, OVERLAND PARK, USA

Background. At a dosing schedule of 75 mg/m^2/day SC for 7 consecutive days every 4 weeks, AZA is an effective and safe treatment for pts with MDS (*JCO 2002;20:2429*). An alternative dosing schedule that eliminates weekend dosing would be more convenient for pts and clinicians. Rapid onset of disease improvement is also important; the time to onset of hematologic improvement (HI) and red blood cell (RBC) transfusion independence (TI) in AZA-treated pts has not been formally studied. *Aims.* To assess the timing of HI or RBC TI using 3 alternative AZA dosing schedules that avoid weekend dosing. *Methods*. In this phase II, multicenter, open-label trial, pts with a diagnosis of FAB-defined RA, RARS, RAEB, RAEB-T, or CMMoL were randomized to 1 of 3 AZA alternative-

dose regimens that were repeated every 4 weeks for 6 cycles: AZA 5-2-2 (75 mg/m²/day x 5 days, followed by 2 days no treatment, followed by 75 mg/m²/day x 2 days), AZA 5-2-5 (50 mg/m²/day x 5 days, followed by 2 days no treatment, followed by 50 mg/m²/day x 5 days) or AZA 5 (75 mg/m²/day x 5 days). Pts with ≥6 days of treatment were evaluable for efficacy. Onset of HI and RBC TI in baseline transfusion-dependent pts, as defined by IWG 2000 criteria (Blood 2000;96:3671), was assessed by treatment cycle. *Results*. In all, 151 pts were randomized to AZA 5-2-2 (n=50), AZA 5-2-5 (n=51), or AZA 5 (n=50). Most pts were RA (43%) or RAEB (30%). Proportions of evaluable pts with HI (major + minor) were 44%, 52%, and 55% in the AZA 5-2-2, AZA 5-2-5, and AZA 5 groups, respectively, and onset of HI occurred within the first 2 treatment cycles for 82%, 58%, and 90% of pts, respectively (Table). Proportions of pts who achieved RBC TI after baseline dependence were 55%, 60%, and 63%, in the AZA 5-2-2, AZA 5-2-5, and AZA 5 groups, respectively, and onset of TI occurred within the first 2 cycles for 92%, 75%, and 75% of pts, respectively (Table 1).

Table 1. Onset of HI or TI by treatment cycle.

	AZA 5-2-2 n (%) pts		AZA 5-2-5 n (%) pts		AZA 5 n (%) pts	
CYCLE	Any HI (n=22)	RBC TI (n=12)	Any HI (n=23)	RBC TI (n=12)	Any HI (n=28)	RBC TI (n=16)
1	9 (41)	6 (50)	6 (26)	4 (33)	15 (54)	9 (56)
2	9 (41)	5 (42)	7 (30)	5 (42)	10 (36)	3 (19)
3	1 (5)	0	7 (30)	3 (25)	2 (7)	1 (6)
4	0	1 (8)	2 (9)	0	1 (4)	2 (13)
5	2 (9)	0	1 (4)	0	0	1 (6)
6	1 (5)	0	0	0	0	0

Onset of HI and TI continued to occur, however, during cycles 3 to 6. All three alternative dosing regimens were generally well tolerated with similar safety profiles to that seen with the approved AZA dosing schedule. *Summary/conclusions*. These data indicate the 3 alternative AZA dosing schedules are effective in the treatment of MDS. Moreover, these data demonstrate the rapid onset of action of AZA, with the majority of pts who achieve HI or RBC TI experiencing onset of effect within the first 2 treatment cycles. Although rapid onset of HI and TI occurred in the majority of pts, onset continued to be observed during later cycles.

0233

TREATMENT-RELATED MYELODYSPLASIA AND SECONDARY AML FOLLOWING FLUDARABINE COMBINATION CHEMOTHERAPY

A. Carney, A. Westerman, S. Tam, A. Milner, H. Prince, M. Kenealy, M. Wolf, E. Januszewicz, A. Ritchie, N. Came, F. Seymour

Peter MacCallum Cancer Centre, EAST MELBOURNE, Australia

Background. Fludarabine combination chemotherapy achieves high response rates in CLL and indolent lymphoma. Fludarabine inhibits DNA repair and augments the cytotoxic effect of DNA damaging agents such as cyclophosphamide and mitoxantrone. This mechanism may also affect marrow progenitor cells to increase the risk of myelodysplasia or secondary acute myeloid leukaemia (MDS/sAML). *Aims*. To investigate the incidence and characteristics of MDS/sAML after treatment with fludarabine in combination (F+) for lymphoproliferative disorders and identify risk factors for its development. *Methods*. Review of the Peter MacCallum Cancer Centre Pharmacy database form 1996-2006 identified 146 patients treated with fludarabine (F) combined with cyclophosphamide (C) and/or mitoxantrone (M)±rituximab (R) who have at least 12 months follow-up since starting treatment. Kaplan-Meier analysis was used to estimate MDS-free survival (MDSFS), defined as the time from first exposure to F+ to onset of MDS. The Mantel-Cox logrank test and Cox proportional hazards regression were used to estimate the effects of patient characteristics on MDSFS, including age, gender, disease type, treatment with alkylators at other times, treatment with radiotherapy, number of F containing treatment episodes, number of prior therapies, and addition of M to first F+ therapy. *Results*. 146 patients (pts) treated with fludarabine in combination (F+) were followed up for a median of 40 months. Ninety-six patients (66%) were male and the median age of all patients was 58 years (range 30-84 years). Disease type was CLL in 69 pts (47%), follicular lymphoma (FL) in 49 pts (34%), Waldenstrom's macroglobulinaemia or marginal zone lymphoma (WM/MZL) in 19 pts (13%) and MCL in 9 pts (6%). Seventeen cases of MDS/sAML have been identified for an overall rate of 11.6% (12 refractory cytopenia with multilineage dysplasia, 2 chronic myelomonocytic leukaemia, 1 refractory anaemia with excess blasts and 2 AML with multilineage dysplasia). Patients included 11 with FL (crude rate 22.4%), 2 with CLL (2.9%) and 4 with WM/MZL (21.1%). Most patients had other treatments but 2 with FL had F+ as their only line of treatment. Ten pts (7%) received M with first F+ treatment and half of these developed MDS. MDS/sAML was diagnosed at a median time of 46 months following commencement of F+ treatment. Karyotypic analysis was typically complex. Median overall survival post-MDS/sAML diagnosis was 11 months. The median MDSFS for this cohort of pts was estimated at 8.2 years (95% CI 8.0 - >9.2 years). Disease type had a significant effect on MDSFS (p=0.029) with the MDS-free rate at 6 years estimated at 100% for CLL, 69% for FL, 100% for MCL and 75% for WM/MZL. Median MDSFS was significantly shorter for those pts treated with F+M (p<0.001). The MDS-free rate at 6 years was 54% for F+M compared to 86% without M. Other variables did not have a significant effect on MDSFS. *Summary and Conclusions.* Fludarabine combination chemotherapy is associated with a moderate risk of MDS/sAML particularly in patients with follicular lymphoma and when combined with mitoxantrone. This complication should be considered when evaluating the potential benefit of this treatment in lymphoproliferative disorders.

0234

TREATMENT WITH VNP40101M (CLORETAZINE?) IN PATIENTS WITH HIGH RISK MYELODYSPLASTIC SYNDROMES (MDS): RESULTS OF A SUBSET POPULATION IN A PHASE II STUDY

G. Mufti,[1] D. Rizzieri,[2] N. Vey,[3] D. Hudak,[4] J. Karp,[5] F. Giles[6]

[1]King's College Hospital, LONDON, UK; [2]Duke University, DURHAM, NC, USA; [3]Institut Paoli-Calmettes, MARSEILLE, France; [4]Vion Pharmaceuticals, Inc., NEW HAVEN, CT, USA; [5]Johns Hopkins University, BALTIMORE, MD, USA; [6]CTRC University of Texas Helath Science Center, SAN ANTONIO, TX, USA

Background. Patients with high risk MDS have few effective therapeutic options. Hypomethylating agents have been approved with complete response rates of approximately 8% in patients with high risk MDS (IPSS score ≥ 1.5). VNP40101M is a novel alkylating agent which preferentially targets the O6 position of guanine resulting in DNA cross-links and has shown activity against AML and high risk MDS in clinical trials. *Aims*. 184 patients were treated in a multi-center Phase II study conducted to investigate the activity and safety of VNP40101M as a single agent in patients with AML or high risk MDS by FAB classification. The primary endpoint, overall response rate (ORR), was defined as CR and CRp according to the AML response criteria proposed by the International Working Group (*Cheson et al. JCO 2003*). A retrospective analysis of the safety and efficacy of VNP40101M treatment in a subset of high risk MDS patients is presented. *Methods*. VNP40101M was administered at 600 mg/m² as a single 30-60 minute IV infusion. A second treatment was allowed for patients who showed hematologic improvement or partial response. Patients who achieved CR or CRp could receive a consolidation course of 400 mg/m². High risk MDS patients enrolled to this study under FAB classification were retrospectively reclassified according to the WHO criteria. Safety and efficacy parameters were analyzed by each classification system. B classification were treated. These patients were categorized as follows: RAEB 12 patients (46%); RAEB-t 9 patients (35%); CMML 3 patients; and *unknown* 2 patients. 8 of 26 patients (31%) had received prior treatment for MDS. The median time from diagnosis to treatment with VNP40101M was 76 days (range 1-808). The median age of patients was 71 (range 59-82) with a median IPSS of 2. Eleven patients (42%) had unfavorable cytogenetics. All 26 patients received a first induction dose of VNP40101M; 8 patients also received a consolidation dose. Response (CR or CRp) was achieved in 10 of 26 patients (38%). Three of the 10 responders had received prior treatment for MDS. Median overall survival (OS) was 3.4 months (range 0.6 - 28.6). One patient died within 30 days of first induction treatment; 1 patient died within 30 days of consolidation. By retrospective reclassification, 17 of 26 patients were identified as having MDS according to WHO criteria. 6 patients were reclassified as AML, and 3 patients as MDS/MPD (CMML). ORR was 53% (9/17). Median OS was 3.9 months (range 0.6 - 28.6). *Summary and Conclusions*. VNP40101M is an active agent for patients with high risk MDS. Efficacy and tolerability is demonstrated regardless of classification by FAB or WHO criteria. Response can be achieved in patients with prior treat-

ment. Further investigation with VNP40101M as a single agent or in combination with other agents in frontline or as salvage treatment is warranted.

0235

PRE-TRANSPLANT SERUM FERRITIN AS AN INDEPENDENT PROGNOSTIC FACTOR IN MDS PATIENTS UNDERGOING REDUCED INTENSITY CONDITIONING HSCT

Z.Y. Lim, V.F. Fiaccadori, S.G. Gandhi, M. Kenyon, A.Y.L. Ho, A.P. Pagliuca, G.J. Mufti

Kings College London and Kings College Hospital, LONDON, UK

Background. Allogeneic haematopoeitic stem cell transplantation (HSCT) is at present the only potentially curative option for MDS. Recent studies have suggested a link between iron overload and post-transplantation liver toxicity, infectious susceptibility, and even overall survival. Iron overload, in terms of ferritin levels, has been studied by several groups and is now known to be an independent prognostic factor for patients undergoing myeloablative HSCT. *Aims*. We performed a retrospective analysis to assess the impact of pre-transplant ferritin on subsequent outcome of MDS patients undergoing reduced intensity conditioning(RIC) HSCT. *Methods*. 99 MDS patients treated between 1999 and 2004 were analysed. All patients were ineligible for conventional myeloablative HSCT due to either age or pre-existing comorbidities. The median age of patients was 51.1 years (range: 19-72). Based on the WHO classification, 3 patients had isolated Del 5q syndrome, 3 RARS, 28 RCMD, 1 RCMD-RS, 16 RAEB-1, 12 RAEB-2, and 36 with AML/MDS. Using WPSS 27 patients were in very high risk group, 50 in high, 16 in intermediate, 6 in low risk group; all patients were transfusion dependent at the time of transplant. We set 1000 ng/mL as the cut off value for pre-transplant serum ferritin, obtaining a group of 26 people with ferritin levels<1000 ng/mL, and a group of 62 with a level over the cut off. Median pre-transplant ferritin was 1991,5 ng/mL (range 6-9580). The RIC protocol consisted of 30 mg/m^2 intravenous (IV) fludarabine daily from day -9 to day -5; 20 mg IV alemtuzumab from day -8 to day -4; and a fixed dose of 4 mg/kg oral busulphan in four divided doses daily from day -3 to day -2. Post-transplant GvHD prophylaxis was achieved with cyclosporine. *Results and discussion*. At 2 years actuarial OS for the cohort was 53%. The DFS, TRM and relapse incidence were 48%, 24%, and 36% respectively. Patients with lower serum ferritin (<1000 ng/mL) showed a better outcome at 2 years in terms of OS (76% vs 43.2%, p=0.007), DFS (64.3% vs 39.5%, p=.015), relapse (23.5% vs 41.9%, p=0.024), but not TRM (15.7% vs 29.5%, p=.250). Serum ferritin correlated with IPSS score: of the 62 patients in high ferritin group, 46 were int-2 or high risk, and only 16 was low or int-1(p=.003); serum ferritin did not correlate with sex, age, cytogenetics, cell source, cell donor, and WPSS. On cox-regression analysis pre-transplant ferritin was an independent prognostic factor in terms of OS (HR=3.151,confidence interval 95%, p=.010), DFS (HR=2.422,CI=95%, p=.018), but not for relapse (HR=2.490, CI= 95%, p=.066) or TRM (HR=1.909,CI=95% p=.251). *Conclusions*. These findings suggest that ferritin levels pre-transplantation can aid in the predicting of subsequent transplantation outcomes in MDS patients undergoing RIC HSCT. Further studies are needed to evaluate the surrogacy of this association and whether iron chelation may play a role in the management of selected patients.

0236

EUROPEAN INTER-COUNTRY TREATMENT SELECTION DIFFERENCES DO NOT ALTER OVERALL SURVIVAL BENEFIT SHOWN WITH AZACITIDINE VS CONVENTIONAL CARE REGIMENS IN HIGHER-RISK MYELODYSPLASTIC SYNDROMES

V. Santini,[1] P. Fenaux,[2] N. Vey,[3] W.K. Hofmann,[4] T. Robak,[5] A. Bacigalupo,[6] L. Silverman,[7] M. Canales,[8] N. Scmitz,[9] P. Muus,[10] I. Fernando,[11] J. Backstrom,[11] L. Zimmerman,[11] C.L. Beach[11]

[1]University of Florence, FIRENZE, Italy; [2]Hôpital Avicenne, BOBIGNY, France; [3]Institut Paoli Calmettes, MARSEILLE, France; [4]Charité - University Hospital, BERLIN, Germany; [5]Medical University of Lodz, LODZ, Poland; [6]S. Martino's Hospital, GENOVA, Italy; [7]Mount Sinai School of Medicine, NEW YORK, USA; [8]University Hospital La Paz, MADRID, Spain; [9]Allgemeines Krankenhaus St. Georg, HAMBURG, Germany; [10]UMCN St. Radboud, NIJMEGEN, Netherlands; [11]Pharmion Corporation, OVERLAND PARK, USA

Background. Recently, a large, international, multicenter, randomized, Phase III trial (AZA-001) demonstrated that azacitidine (AZA) was the first drug treatment to significantly prolong overall survival (OS) in higher-risk pts with myelodysplastic syndromes (MDS) (Fenaux, Blood 2007;110:Abstract 817). The trial was designed to allow comparison of treatment with AZA with a control group receiving one of three frequently used treatments in higher-risk MDS pts. *Aims*. The purpose of this pooled, subgroup analysis was to assess country-specific treatment preselections across eight European countries (France, Germany, Italy, Spain, UK, Sweden, Greece, the Netherlands) to evaluate the effects of pre-randomization treatment selection on the consistency of the AZA-001 OS findings. *Methods*. Higher-risk MDS pts (FAB-defined RAEB, RAEB-T, or CMML; IPSS: Int-2 or High) were enrolled. Prior to randomization, investigators preselected the most appropriate treatment for individual pts from 3 conventional care regimens (CCR, best supportive care [BSC], low-dose ara-C [LDAC, 20 mg/m^2/d x 14d every 28 days for ≥4 cycles], or intensive chemotherapy [IC, 7+3 regimen]). Pts were then randomized to AZA or CCR. Regardless of investigator selection, those randomized to AZA received AZA at 75 mg/m^2/d x 7d, every 28 days for ≥6 cycles; pts randomized to CCR received their investigator-selected treatment. All pts provided informed consent. Investigator selection and OS were compared by practice patterns across the eight highest enrolling European countries. *Results*. Overall, 280 pts were enrolled (78% of the total AZA-001 patient population) across the eight EU countries. Baseline characteristics were consistent between AZA and CCR groups. Investigator selection showed profound selection differences across the countries. Pooled results from France and UK showed the highest preselection for LDAC (74% [62/84]) with 26 patients receiving AZA per randomization to AZA and 36 receiving LDAC per randomization to CCR. Pooled results from Germany, Italy, Spain, Sweden, Greece, and the Netherlands showed the highest preselection for BSC (79% [155/196]) with 80 patients receiving AZA per randomization to AZA and 75 receiving BSC per randomization to CCR. Survival analysis pooling France with the UK (where LDAC selection was highest) showed an OS advantage for the AZA group *vs* the CCR group similar to that observed in the overall AZA-001 OS analysis (Table 1).

Table 1. OS (median months) in France/UK (LDAC driven) and Germany/Italy/Spain/Greece/Sweden/Netherlands (BSC driven) compared with the overall AZA-001 findings.

Country	OS AZA	OS CCR	Difference	HR (95%CI)	Log-rank P
France/UK	24.5	16.4	8.0	0.44 (0.23-0.85)	0.012
Germany/Italy/Spain/ Greece/Sweden/ Netherlands	25.1	15.7	9.4	0.65 (0.43-0.97)	0.032
Overall AZA-001	24.4	15.0	9.4	0.58 (0.43-0.77)	0.0001

Survival analysis pooling results from Germany, Italy, Spain, Sweden, Greece, and the Netherlands (where BSC selection was highest) also showed an OS advantage for the AZA group *vs* CCR that was highly similar to that observed in the overall AZA-001 OS findings (Table 1). Comparison of OS results in the pooled LDAC group (France/UK, n=36) with the pooled BSC group (Germany/Italy/Spain/Sweden/Greece/the Netherlands, n=75) showed no differences: 16.9 months *vs* 17.2 months (HR: 1.01; 95% CI: 0.59-1.73; log-rank p=0.963). *Summary and Conclusions*. Regardless of investigator treatment selection preferences for LDAC (France/UK) or for BSC (Germany/Italy/Spain/Sweden/Greece/the Netherlands), differences in median OS between the AZA and CCR groups remained statistically significant and consistent with results from the AZA-001 trial. Treatment with AZA yielded significant benefit for OS compared with LDAC. LDAC provided no survival benefit *vs* BSC.

0237

TREATMENT OF LOWER RISK MDS WITH DEL 5Q WITH LENALIDOMIDE (LEN): CURRENT RESULTS OF THE FRENCH PATIENT NAMED PROGRAM (ATU)

F. Le Bras,[1] M. Sebert,[2] V. Eclache,[2] T. Lamy,[2] J. Dlaunay,[2] S. Visanica,[2] F. Dreyfus,[2] A. Banos,[2] M. Blanc,[2] C. Leyronnas,[2] C. Besson,[2] B. Derenzis,[2] X. Thomas,[2] C. Rodon,[2] N. Vey,[2] M. Gardembas,[2] M. Faron,[2] F. Bauduer,[2] J.F. Ramee,[2] D. Zajec,[2] A. Delmer,[2] D. Rea,[2] O. Beyne-Rauzy,[2] J. Ceccaldi,[2] P. Beaucournou,[2] J. Dugay,[2] P. Fenaux,[2] L. Ades[2]

[1]AP HP, PARIS, France; [2]Groupe Francophone des Myelodisplasies, PARIS, France for the groupe Francophone des myelodysplasies (GFM)

Background. LEN is very effective in lower risk MDS with del 5q (List *et al.*, 2006). A patient named program was launched by French health authorities to use LEN in low and int 1 risk MDS with del 5q and transfusion dependence (>2 RBC units/ 2 months). *Patients*. Patients received 10 mg of LEN /day,3 weeks on, 1 week off. Hematological response (IWG2006) was assessed after 16 and 32 weeks of treatment. *Results*. 40 pts were evaluable for response as of Feb 1st , 2008. Median age was 73 [range 45-92], M/F 40%, median interval from diagnosis to LEN treatment 44 months (range 4-165). At inclusion, 18 pts had del 5q syndrome, 9 RARS, 4 RCMD and 9 RAEB-1, 16 pts were IPSS low and 24 int-1. Del 5q was isolated, with 1 additional and > 1 additional abn in 27, 8 and 5 pts resp. 8 pts were untreated, while 32 had previously received EPO. Median transfusion requirement was 4,4 units/ 2 months. 30/40 (75%) pts achived erythroid response (IWG 2006), of whom 24 (60%) achieved transfusion independence (TI). Median time to TI was 15 weeks (range 8 - 32). TI was achived in 62%, 54%, 55%, 67%, 40% pts with IPSS low, IPSS int 1, isolated del 5q, del 5q+1abn, del5q + >1abn, resp, and 59% and 62% of EPO pretreated and EPO naive pts. Grade III-IV neutropenia and thrombocytopenia were seen in 68% and 35% pts resp, leading to reduction of LEN dosing in 72% . Other grade III-IV side effects were Lyell's syndrome (n=1), deep venous thrombosis (n=1) Quincke's edema (n=1).One patient died due to CNS bleeding following worsening of thrombocytopenia under treatment, while no pt died from sepsis due to neutropenia. With a still short median FU on treatment (24 weeks), no relapse has occured. No patient has progressed to AML. *Conclusions.* Our results confirm previous findings. An update on the > 100 patients now accrued in the program will be presented.

0238

5-AZACYTIDINE FOR THE TREATMENT OF INTERMEDIATE-2/HIGH IPSS RISK MYELODYSPLASTIC SYNDROMES: RESULTS IN 83 PATIENTS FROM THE ITALIAN PATIENT NAMED PROGRAM

L. Maurillo,[1] A. Spagnoli,[2] A. Gozzini,[2] N. Cecconi,[2] M. D'Argenio,[2] M. Lunghi,[2] S. Rocco,[2] G. Palumbo,[2] F. Rivellini,[2] M. Genuardi,[2] S. Sibilla,[2] F. Ferrara,[2] G. Mele,[2] N. Filardi,[2] G. Sanpaolo,[2] G. Specchia,[2] A. Tonso,[2] A. Santagostino,[2] M.T. Voso,[2] E. Balleari,[2] V. Cassibba,[2] P. Della Cioppa,[2] C. Mazzone,[2] E. Oliva,[2] L. Ciuffreda,[2] D. Russo,[2] S. Galimberti,[2] O. Villani,[2] F. D'Auria,[2] N. Di Renzo,[2] A.M. D'Arco[2]

[1]S. Eugenio Hospital, Tor Vergata University, ROMA; [2]On behalf of ad hoc Italian Study Group, AZACYTIDINE IN MDS AND AML; [3]CROB, Centro di Riferimento Oncologico della Basilicata, RIONERO IN VULTURE, Italy

Background. 5-azacytidine (AZA) is an hypomethylating agent approved in 2004 from FDA for the treatment of all types of myelodysplastic syndromes (MDS) and with still pending approval in Europe. *Methods*. In September 2007, we started a retrospective study aiming to register and analyse all Italian pts with MDS or AML who had received AZA for the treatment of their disease outside of clinical trials, on the basis of a national patient named program. Among a total of 218 pts treated in 31 different Italian Institutions since 2005, eighty-three were int-2/high IPSS risk MDS. Median age was 70 years (range 36-84), M/F ratio 58/25. According to WHO classification, there were 7 RCMD, 16 RAEB-1, 58 RAEB-2, 1 CMMoL, 1 MDS unclassified. Median time from diagnosis was 6 months (range 1-96). Sixty-three pts (76%) were transfusion-dependent, fifty-three (64%) had received prior treatment: approximately 25% had received low dose or AML-like chemotherapy. AZA was administered as single agent in 55 pts (66%), while in the remaining subjects the drug was variously combined with growth factors, valproic acid or other agents. Forty-five pts (55%) received a standard dose of 75 mg/d/sqm s.c., thirty-seven (45%) a fixed dose of 100 mg/d s.c.. Single cycle treatment duration was 7 days in 46 pts (58%), < 7 days in 29 pts (36%), > 7 days in 5 pts (6%), The median number of monthly cycles was 4 (range 1-11), with 46 pts who completed at least 4 cycles (55%). *Results*. The most relevant toxicity observed (grade 3-4) was represented by myelosuppression (54%), infections (18%) and gastro-intestinal adverse events (4%). According to 2006-updated IWG criteria, overall response rate in 74 assessable pts was 38% (43% in 46 pts who had completed at least 4 cycles). In particular, complete response, partial response and haematological improvement occurred in 12%, 19% and 7% of pts (13%, 26% and 4% of those treated with at least 4 cycles), respectively. Disease remained stable in 44% of pts, while progression or failure were observed in 18%. Response duration ranged from 1 to 14+ months. There were no significant differences in response rate according to dose and schedule employed, although a trend in favour of 75 mg/d/sqm for 7 days vs other schedules was observed (52 vs 25-35%, respectively). *Conclusions*. These independent, multicenter study data are consistent with the results recently presented for the phase III international AZA-001 trial and confirm, in the clinical practice, that AZA is a safe and effective drug for int-2/high IPSS risk MDS. An update of this series, including survival data, will be presented at the meeting.

0239

MONOCYTES FROM PATIENTS WITH MYELODYSPLASTIC SYNDROMES ARE MORE RESISTANT TO THALIDOMIDE-MEDIATED TNF-α INHIBITION COMPARED TO MONOCYTES FROM CONTROL SUBJECTS

S. Meers,[1] L. Boon,[2] M. Boogaerts,[1] C. Verfaillie,[3] G. Verhoef,[1] M. Delforge[1]

[1]University Hospital Leuven, LEUVEN; [2]PanGenetics, UTRECHT, Netherlands; [3]Stem Cell Institute, LEUVEN, Belgium

Background. Many factors have been identified that contribute to bone marrow failure observed in patients with myelodysplastic syndromes (MDS). Our group has recently shown that monocytes are activated in patients and can contribute to bone marrow failure via CD40-CD40L interactions with T helper cells (*Meers et al Leukemia 2007;21:2411-9*). We also demonstrated that stimulation of the CD40-receptor on monocytes induces a significantly higher production of TNF-alpha in patients compared to controls. Thalidomide can induce hematopoietic responses in selected patients with MDS and inhibition of TNF-α production is one proposed working mechanism. However, this has never been studied *in vitro* using monocytes from MDS patients. *Aims*. This study was designed to determine (1) the production of the inflammatory cytokines TNF-α, IL-1beta, IL-6 and IL-10 by monocytes from patients after stimulation with either lipopolysaccharide (LPS) or CD40-agonists. (2) In addition, we evaluated the *in vitro* effects of thalidomide on production of these cytokines. *Methods*. With MACS columns, we purified $CD14^+$ cells from peripheral blood of 30 patients with MDS (22 RA, 5 RARS and 3 RAEB) and 28 aged healthy volunteers. $CD14^+$ cells were plated at a density of $3x10^5$ cells/mL in IMDM containing 15% fetal bovine serum. After 1 week, the medium was replaced with fresh medium supplemented with either LPS (at 1 µg/mL) or a mixture of clone 64 (agonist anti-human CD40 monoclonal antibody, PanGenetics, The Netherlands) at 10 µg/mL, and interferon-γ at a final concentration of 1000 IU/mL. Thalidomide was added in various concentrations (5-10-25 µg/mL) immediately thereafter. Supernatant was harvested after 24h and stored at -20°C. Cytokine concentrations were determined with commercially available ELISA-kits. *Results*. (1) We confirmed our previous observation that MDS monocytes produce significantly more TNF-α in response to CD40 stimulation than monocytes from healthy controls ($p<0.05$), whereas LPS induced similar TNF-α production in both groups. In addition, we found that compared to controls, stimulation of CD40 on MDS monocytes induced significantly more IL-1beta and IL-10. (2) Thalidomide inhibited TNF-α production after LPS stimulation in controls as well as in patients. But whereas in control monocytes stimulated with CD40-agonists, any concentration of thalidomide was able to significantly inhibit TNF-alpha production, in patients we observed a significant inhibitory effect only with the highest concentration (25 µg/mL) of thalidomide. We observed no inhibitory effect of thalidomide on the production of IL-1β, IL-6 or IL-10. *Summary and Conclusions*. We present the first *in vitro* data of the effects of thalidomide on cytokine production by monocytes derived from patients with MDS. We confirm that thalidomide is able to inhibit LPS-induced TNF-α production in MDS monocytes as efficiently as in control monocytes. However, the amount of TNF-α that MDS monocytes produce upon stimulation of the CD40 receptor can only be blocked in presence of high concentrations of thalidomide. Since in MDS TNF-α production induced by the CD40-CD40L pathway is probably clinically more important than LPS-induced production, this could explain the rather low response rates in MDS patients treated with thalidomide.

0240

THE ORAL CHELATOR ICL670 REPRESSES SIGNALING THROUGH THE MTOR PATHWAY IN MYELOID LEUKEMIA CELLS BY ENHANCING EXPRESSION OF REDD1

J. Ohyashiki, C. Kobayashi, R. Kurashina, Y. Zhang, R.S. Hamamura, K. Ohyashiki

Tokyo Medical University, TOKYO, Japan

Background. Iron plays a central role in the regulation of many cellular functions. Dysregulation of its metabolism leads an iron overload situation and iron depletion leads to an inhibition of cell proliferation. Recent reports demonstrated that ICL670 (Novartis) acts as a potent NF-kappa-B inhibitor and improves hematological data in a subset of MDS patients (*Cilloni et al, Haematologica, s1: 238, 2007*). However, the precise mechanism of anti-cancer effect of ICL670 is still uncertain. *Aims.* To evaluate the effect of ICL670, and to identify the moleclular pathways responsible for the observed reduced transfusion requirement during chelation therapy, we performed gene expression profiling to focus on the pathway involved in the anti-cancer effect of ICL670. *Method*. A human myeloid leukemia cell line, K562, was incubated with 1-200 μM of ICL670. Fresh leukemia cells were also obtained from patients with *de novo* AML, as well as post-MDS AML, after obtaining an informed consent. Cell numbers at 48 h after incubation with or without ICL670 were assessed with the Cell Counting Kit-8 assay (Dojindo Molecular Technologies, Gaithersburg, MD, USA). Total RNA was extracted after 24 h incubation with or without ICL670 (10 μM and 50 μM), then, gene expression profiling was done using an Affymerix GeneChip (U133 Plus 2.0). Statistical analysis was done by GeneSifter (VizXlab, Seattle, WA, USA). *Results*. Inhibitory concentration (IC50) of ICL670 was 50 μM in K562 cells, while that in fresh leukemia cells was 100 to 200 μM. Gene expression analysis revealed up-regulation of genes related to apoptosis (i.e. BCL6, PHLDA1, and BNIP3L), genes regulating interferon (i.e. IFIT1, IFIT3), genes linked to DNA-damage responses (i.e. REDD1, GADD45B). Based on the results obtained from gene expression profiling, we in particular focused on the REDD1/mTOR pathway in ICL670 treated K562 cells. We found that enhanced expression of REDD1 and its downstream protein, tuberin. Notably, S6 kinase as well as phosphorylated S6 kinase, which is known to be a target of mTOR, was significantly repressed in ICL670-treated K562 cells, in a dose- dependent manner. *Conclusion*. Overall, the data support the conclusion that REDD1 functions up-stream of tuberin to down-regulate mTOR pathway in response to ICL670. ICL670 might have benefit for not only iron chelation but also be an anti-proliferative agent in some MDS patients.

0241

ALL-TRANS RETINOIC ACID (ATRA) COMBINED TO ERYTHROPOIETIN (EPO) FOR THE TREATMENT OF ANEMIA IN LOW RISK MYELODYSPLASTIC SYNDROMES (MDS)

R. Itzykson,[1] S. Ayari,[2] D. Vassilief,[2] E. Berger,[2] B. Slama,[2] N. Vey,[2] F. Suarez,[2] O. Beyne-Rauzy,[2] A. Guerci,[2] S. Cheze,[2] X. Thomas,[2] A. Stamatoullas,[2] M. Gardembas,[2] F. Bauduer,[2] M.C. Chaury,[2] A. Kolb,[2] L. Legros,[2] D. Damaj,[2] F. Hamza,[2] F. Dreyfus,[2] P. Fenaux,[2] L. Ades[2]

[1]Assistance Publique Hopitaux de Paris, Hopital Avicenne, Universite Paris 13, BOBIGNY, France; [2]Groupe Francophone des Myelodysplasies, BOBIGNY, France

Background. First line treatment of anemia in low-risk MDS largely relies on recombinant EPO, +/- G-CSF, but patients with high endogenous EPO levels or requiring frequent RBC transfusions have low probability of response (Nordic score, Hellström-Lindberg E *et al.*, BJH 2003). It has been reported that combining ATRA to EPO a) could yield erythroid responses in MDS with high endogenous EPO level, and b) could also improve other cytopenias (*Stasi R. et al., Blood 2002*). *Aims*. In order to further document this effect, we designed a phase II protocol combining EPO and ATRA in MDS with < 10% blasts and anemia (transfusion dependent or Hb < 10 g/dL) having failed EPO or with low probability of response to EPO or with additional cytopenias. *Patients*. Between 10/2004 and 04/2005, 65 patients (pts) (median age 72y; 16 RA, 21 RARS, 1 RCMD, 24 RAEB-1, 1 5q- syndrome, 1 unclassified; karyotype: 41 fav, 11 interm, unfav 7 NA 6; IPSS: 12 low, 37 int 1, 10 int 2 NA 6) were included, based on (several criteria in some pts): EPO level > 500 U/L (n=19), previous failure of an adequate regimen of EPO alone (ie. 60000 UI/week during 12 weeks; n = 31), other cytopenias (plts < 50 G/L and/or ANC< 1000/mm^3; n=30). 47 pts (72%) were transfusion dependent, 32 (49%) received >2 RBC units/month. Pts received EPO β (Neorecormon® 60 000 UI/week) and ATRA (45 mg/m^2/d every other week). Response was evaluated at 12 weeks. The primary endpoint was HI-E by IWG 2000 criteria (responses were subsequently reclassified per IWG 2006). *Results*. Six of the 65 pts included were not assessable (because of lethal cardiac failure n=1, other cancer n=2, progression n=1, lethal sepsis n=2). Of the remaining 59 pts, 70% achieved HI-E per IWG 2000 (26% Major, 44% minor) and 46% HI-E per IWG 2006. Response rates according to IWG 2000 and 2006 were, respectively, 57 % and 39% in patients with previous EPO failure, 61% and 39% in pts with endogenous EPO >500 U/L, 82% and 61% in pts transfused > 2 RBC/month, 83% and 58% in pts with the last 2 features (who would have expected a response rate to EPO + G-CSF of about 7% according to the Nordic score). Only 1 neutrophil response in 31 neutropenic and no platelet response in 20 thrombocytopenic patients were observed. After 24 weeks of treatment, response was maintained in 87% of responders by IWG 2000 criteria. EPO ATRA combination was well tolerated, with mainly grade ≤ 2 toxicities, the most frequent being skin and mucosal toxicity of ATRA. *Conclusions*. EPO-ATRA combination is safe and yields significant response rates in anemia of low-risk MDS patients having failed EPO alone, or unlikely to respond to EPO because of high endogenous EPO levels or high transfusion needs. However it has no effect on neutropenia and thrombocytopenia.

Non-Hodgkin's lymphoma - Biology and Clinical

P0242

GENOME-WIDE ANALYSIS OF DIFFERENT SUBTYPES OF B-CELL NON-HODGKIN'S LYMPHOMA USING HIGH-RESOLUTION GENOMIC MICROARRAYS

M. Kato,[1] K. Takeuchi,[2] G. Yamamoto,[1] Y. Nannya,[1] M. Sanada,[1] S. Chiba,[1] Y. Ishikawa,[3] S. Mori,[4] Y. Kobayashi,[3] M. Kurokawa,[1] S. Ogawa[1]

[1]The University of Tokyo Hospital, TOKYO; [2]National Cancer Center Hospital, TOKYO, Japan; [3]Cancer Institute Hospital, TOKYO; [4]The University of Teikyo, TOKYO, Japan

Background. B-cell type non-Hodgkin's lymphoma (B-NHL) is a comprehensive group of mature B-cell neoplasm. B-NHLs are categorized into subtypes including diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) and mucosa-associated lymphoid tissue lymphoma (MALT-L), which is characterized by distinct pathological and molecular genetic features. These B-NHLs possess a wide variety of genetic alterations, such as gains, losses, and translocations, as well as abnormalities in allelic composition. These alterations may collectively comprise unique genomic profiles specific to different subtypes of B-NHL, which are tightly linked to the pathogenesis of each B-NHL subtype. Although various genetic abnormalities have been identified in each subtype, the detailed genomic profiles remain to be defined. *Aims*, In this study, in order to obtain a comprehensive registry of genomic abnormalities in B-NHLs, and in order to investigate the unique genomic profiles of different B-NHL subtypes, we performed genome-wide analysis of copy number change and allelic imbalances for 189 B-NHL samples. *Methods*. Recently, we have developed a novel algorithm, CNAG/AsCNAR, to detect copy number alteration and allelic composition using SNP-genotyping microarrays, without dependence on availability of self germline DNA (Nannya *et al.* Cancer Res, 2005). This robust algorithm also enabled sensitive detection of loss of heterozygosity (LOH), even in the primary samples contaminated by 70-80% of normal cells, by finding subtle distortions in allele-specific signals (Yamamoto *et al.* Am J Hum Genet, 2007). In this study, we performed SNP-chip and CNAG/AsCNAR analysis for 189 B-NHL specimens, including 65 cases of DLBCL, 61 cases of FL, and 63 cases of MALT-L. *Results*. A large number of gains and losses of chromosomal/genomic segments as well as allelic imbalances were identified. While individual genomic profiles are substantially variable among different cases, they collectively showed a characteristic genomic profile in each B-NHL subtypes; +1q, +2p and +18 are common to DLBCL and FL, while +3 and +11q were more frequently found in DLBCL and +11p was more characteristic to FL. MALT-L also showed +3 and +18, but rarely had +1q and +2p. CN neutral LOH due to mitotic recombination events are frequently found in DLBC (70%) and FL(54%), but less common in MALT-L. UPD most commonly involves 1p, 1q, 6p, 9p and 12q. In total, 29 loci of high-grade amplification were identified. Among these recurring amplifications were observed at 1q and 2p, which involves FCGR2B, and cRel genes, respectively. These amplifications were detected in both DLBCL and FL, but rare in MALT-L. Total 14 loci of homozygous deletion were also detected and differentially distributed among different B-NHL subtypes. *Summary/conclusions*. SNP-chip with CNAG/AsCNAR analysis revealed characteristic genomic signatures of distinctive B-NHL subtypes, implicating their unique pathogenesis.

0243

CHLAMYDOPHILA PSITTACI (CP) IS VIABLE AND INFECTIOUS IN THE CONJUNCTIVA AND PERIPHERAL BLOOD OF PATIENTS WITH OCULAR ADNEXAL MALT LYMPHOMA (OAML): RESULTS OF A PROSPECTIVE CASE-CONTROL STUDY

J.M. Ferreri,[1] R. Dolcetti,[2] P. Dognini,[1] L. Malabarba,[1] N. Vicari,[3] E. Pasini,[2] M. Ponzoni,[1] M.G. Cangi,[1] L. Pecciarini,[1] A. Giordano Resti,[1] S. Rossini,[1] C. Doglioni,[1] S. Magnino[3]

[1]San Raffaele H Scientific Institute, MILANO; [2]Cancer Bio-Immunotherapy Unit, Centro di Riferimento Oncologico, AVIANO; [3]National Reference Laboratory for Animal Chlamydioses, IZSLER, PAVIA, Italy

Background. Some lymphomas are linked to specific bacterial infections. Confirmation of these associations by bacteria isolation from patients' (pts) samples (second Koch's postulate) has been achieved for H. pylori, but not for other lymphoma-related bacteria. OAML is linked to Cp infection, but the viability and infectivity of this microorganism in OAML pts has not been investigated yet. *Aims*. A single-center prospective trial was conducted to assess the prevalence of Cp infection in 20 OAML pts and 42 healthy blood donors referred to our Institution in a 6-month period, and to define whether the Cp DNA and antigens previously detected in OAML pts correspond to a viable and infectious microorganism. *Methods*. 20 OAML pts and 42 healthy blood donors referred to our Institution in a 6-month period were intervisted for epidemiological and clinical features. Biological samples (conjunctival swabs and peripheral blood mononuclear cells - PBMC) of pts and donors were collected during interview. The presence of Cp on conjunctival swabs and PBMC was assessed by TETR-PCR and *in vitro* cultural methods. The presence of Cp was assessed also in lymphoma tissue. Differences in epidemiologic features and infection rates were compared by Fisher exact test. *Results*. Donors were more commonly young males living in urban areas, whereas OAML pts frequently reported a history of chronic conjunctivitis and prolonged contact with household animals (85% *vs* 38% of donors; p=0.00001). Cp was detected in lymphoma tissue of 15 (75%) pts. Cp DNA (TETR-PCR)was detected in conjunctival swabs and/or PBMC from 10 (50%) OAML patients and in PBMC from one (2%) donor (p=0.01). Viability and infectivity of Cp, demonstrated by growth in cell cultures, were confirmed in conjunctival swabs and/or PBMC from 5 (25%) OAML pts, but not in donors (p=0.002). *Conclusions*. This prospective case-control study demonstrates, for the first time, that Cp is viable and infectious in conjunctival swabs and/or PBMC of OAML pts. The association between Cp and OAML is now supported by a second Koch's postulate level. Epidemiological features in OAML pts are consistent with increased risk of Cp exposure.

0244

PATTERN OF ANGIOGENESIS-RELATED GENE EXPRESSION DURING IN VIVO GROWTH OF MANTLE CELL LYMPHOMA CELL LINE JEKO-1

J.M. Molinsky,[1] S.L Leahomschi,[1] M.H. Hulova,[1] T.S. Simonova,[1] R.P. Pytlik,[1] M.T. Trneny,[1] T.S. Soukup,[2] J.M. Mokry,[2] E.N. Necas,[1] J.Z. Zivny,[1] P.K. Klener[1]

[1]Charles University in Prague, 1st Faculty of Medicine, PRAHA; [2]Charles University in Prague, Faculty of Medicine in Hradec Kralove, HRADEC KRALOVE, Czech Republic

Background. The significance of angiogenesis for disease progression is well established in solid tumors. Alternatively, other mechanisms such as vasculogenic mimicry and vessel cooption were described to provide oxygen supply. The contribution of these processes and their molecular mechanisms in hematologic malignancies remains to be elucidated. *Aims*. To analyse expression profile of angiogenesis-related genes in human mantle cell lymphoma cell line JEKO-1 growing *in vivo* after subcutaneous xenotransplantation into immunodeficient mice in comparison to the JEKO-1 cell line cultured *in vitro*. *Methods*. We injected 5 millions of JEKO-1 cells subcutaneously into sublethally irradiated NOD/LtSz-Rag1null mice (n=4). Animals were killed when the tumors reached 2 cm in diameter and total RNA was isolated from the tumors. After reverse transcription, real time PCR analysis of angiogenesis-related gene expression was performed by using low density arrays (human angiogenesis panel). The results were normalized to GAPDH expression and two independent RNA isolations from *in vitro* growing JEKO-1 cells were used as calibrator sample. Flow cytometry analysis revealed that tumors consisted of 20-40 % of human CD45 positive cells. To rule out possible nonspecific detection of mouse genes, we separated human lymphoma cells from murine cells on magnetic column by anti human CD45 microbeads. CD45 positive and CD45 negative cell fractions were analysed as positive and negative controls, respectively. *Results*. The positive and negative controls confirmed the specificity of the array for human genes. The selected CD45 positive tumor cells showed similar expression levels as nonselected cells (i.e. the whole tumor cell population). No gene expression was detected in CD45 negative tumor cells. The most upregulated gene in all JEKO-1 tumors compared to *in vitro* growing JEKO-1 cells was platelet-endothelial cell adhesion molecule 1 (PECAM-1/CD31; 25-40 - fold increase). Other upregulated genes included brain-specific angiogenesis inhibitor 1 (BAI-1), fibulin 5 (FBLN5), vascular endothelial growth factor receptor 1 (VEGFR-1), angiogenin (ANG), connective tissue growth factor (CTGF), hepatocyte growth factor (HGF) and lymphangiogenic markers prospero-related homeobox 1 (PROX1) and CD44. Proangiogenic vascular endothelial growth factor (VEGF) and midkine were downregulated and expression of VEGFB didn´t change. No expression of VEGFC, angiopoietin-1,

angiopoietin-2 was detected as well as expression of receptors TIE-1, VEGFR-2, VEGFR-3 and fms-related tyrosine kinase 3 (FLT3). *Summary and Conclusions*. Our results revealed significant changes in gene expression of angiogenesis-related genes in JEKO-1 mantle cell lymphoma cell line after xenotransplantation into immunodeficient mice. Interestingly, the most upregulated gene was platelet-endothelial cell adhesion molecule 1, CD31/PECAM-1. Furthermore, we detected differential expression of several well-known proangiogenic factors (VEGF, ANG, CTGF, HGF) as well as inhibitors of angiogenesis (BAI-1, FBLN5 and VEGFR-1). The results suggest that regulation of angiogenesis by lymphoma cells might represent an important factor impacting growth and propagation of mantle cell lymphomas.

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0245

CRYPTIC REARRANGEMENTS OF THE ALK GENE IN ALK-EXPRESSING DIFFUSE LARGE B-CELL LYMPHOMA

K. Van Roosbroeck,[1] J. Cools,[1] D. Dierickx,[2] J. Thomas,[3] P. Vandenberghe,[1] P. Marynen,[1] J. Delabie,[4] C. De Wolf-Peeters,[4] I. Wlodarska[1]

[1]Department of Human Genetics, Catholic University of Leuven, LEUVEN; [2]Department of Hematology, Catholic University of Leuven,LEUVEN; [3]Department of Oncology, Catholic University of Leuven, LEUVEN; [4]Department of Pathology, Catholic University of Leuven, LEUVEN, Belgium

Background. ALK$^+$ DLBCL represents a recently described subtype of B-cell lymphoma, expressing the anaplastic lymphoma kinase (ALK). An aberrant expression of this protein tyrosine kinase is typically found in T/null-cell derived ALK$^+$ anaplastic large cell lymphoma (ALCL) which are characterized by 2p23/ALK rearrangements. So far, less than 40 ALK$^+$ DLBCL cases have been published. The majority of them is characterized by the t(2;17)(p23;q23)/ALK-CLTC rearrangement. *Aims*. The aim of our study was to identify and characterize ALK rearrangements in two DLBCL cases showing expression of ALK. *Methods*. Immunohistochemistry (IHC) was used to assess the expression pattern of ALK in two CD20-/CD138$^+$ DLBCL cases. Conventional cytogenetics, followed by FISH, was applied to identify and characterize the underlying 2p23 aberrations. The putative ALK partner was identified by 5' RACE PCR and RT-PCR followed by sequencing. *Results*. Case 1: cytoplasmic expression of the ALK protein, found by IHC, suggested a variant t(2p23). Cytogenetics revealed complex aberrations not involving 2p23, while FISH showed translocation of the 3'ALK region to 3q27 and translocation of the entire BCL6 gene (3q27) to 2pter. Further 5' RACE PCR detected an in-frame SEC31A-ALK fusion transcript in which exon 24 of SEC31A was fused to exon 20 of ALK. Interestingly, the 4q21 region harbouring SEC31A looked normal in the present case. Further multicolour karyotyping and FISH analysis identified the cryptic SEC31A-ALK fusion at 3q27. This rearrangement probably occurred by a t(2;3)(p23;q27) associated with a cryptic insertion of the 5'end of SEC31A upstream of the translocated 3'ALK. In addition, a duplication of the SEC31A-ALK fusion was found on a der(20). Case 2: IHC showed cytoplasmic and nuclear expression of ALK. Cytogenetics identified a t(2;2)(p23;q31) as a part of a complex karyotype. FISH with LSI ALK showed translocation of the 3'end of ALK to a normal-looking 5q35. This region harbours the NPM gene involved in a classical t(2;5)(p23;q35) which typifies approximately 75% of the ALK$^+$ ALCLs. Further FISH analysis showed a cryptic insertion of the 3'ALK into the NPM locus. This is the first ALK$^+$ DLBCL case in which a t(2;5)-independent NPM-ALK fusion has been detected. *Summary and Conclusions*. In summary, we identified two new rearrangements of ALK in ALK$^+$ DLBCL and confirmed the key role of this gene in the pathogenesis of this rare subtype of B-cell lymphoma. In both reported cases, ALK rearrangements were submicroscopic and generated by an insertion of either the partner gene, SEC31A, into the 3' end of ALK, or by an insertion of the 3' end of ALK into the partner gene (NPM/5q35). These cytogenetically cryptic rearrangements of ALK have never been described in ALK$^+$ DLBCL.

0246

EXPRESSION OF CXCR4 AND CD26 ON DENDRITIC LYMPHOPLASMACYTOID LYMPHOMA: A KEY FOR INTERPRETING SKIN HOMING OF NEOPLASTIC CELLS

L. Del Vecchio,[1] R. Franco,[2] A. De Renzo,[3] G. Cerciello,[3] F. Perna,[3] C. Lo Pardo,[4] V. Mettivier,[5] A.M. D'Arco,[6] G. Corazzelli,[7] P Mirabelli,[8] G. Abate,[8] M. Gorrese,[8] B. Rotoli[3]

[1]CEINGE Institute, NAPLES, Italy; [2]Istituto Dei Tumori Pascale - Anatomia Patologica, NAPOLI; [3]Ematologia Università Federico II, NAPOLI; [4]Immunoematologia Ospedale A. Cardarelli, NAPLES;[5]Ematologia Ospedale A. Cardarelli, NAPLES; [6]Ematologia Ospedale Umberto I, NOCERA INFERIORE (SA); [7]Ematologia Istituto dei Tumori Pascale, NAPLES; [8]Ceinge Institute, NAPLES, Italy

Background. An unusual form of leukemia/lymphoma expressing HLA-DR, CD4, CD56 and CD123 (IL3R) has been recently described. Surface antigen mosaic and functional properties of these cells are similar to a fraction of normal dendritic cells, the DC2 or lymphoplasmacytoid subset. This type of neoplasia perfectly overlaps to CD4$^+$CD56$^+$ lymphoma previously named in WHO classification as *blastic NK* lymphoma. To date, two main types of patients affected by dendritic lymphoplasmacytoid lymphoma (DLPL) have been described: (i) cases with an acute leukemia-like picture and (ii) cases with initial skin involvement and subsequent leukemic or lymphomatous dissemination. *Aims*. The aims of this study were: (i) to define the exact immunophenotype of this rare type of neoplasia; (ii) to assess the real incidence of these cases; (iii) to correlate immunophenotype with clinical behavior. *Methods*. In this study we characterized 10 cases of DLPL by using an extended panel of monoclonal antibodies, flow cytometry (FCM) and immunohistochemistry (IH). Nine cases were studied by FCM and 8 by IH. The panel utilized for FCM characterization was the following: CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11a, CD11b. CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD23, CD24, CD25, CD26, CD29, CD33, CD34, CD36, CD37, CD38, CD40, CD41a, CD42b, CD43, CD44, CD45, CD56, CD57, CD58, CD61, CD69, CD71, CD80, CD86, CD103, CD184. *Results*. FCM characterization on bone marrow aspirates in 7 cases with heavy bone marrow involvement consistently evidenced the phenotype HLA-DR$^+$, CD4$^+$, CD56$^+$, CD36$^+$, CD103$^+$. Three cases with massive involvement of bone marrow and no cutaneous infiltration clearly showed CD26. All cases with bone marrow involvement were initially referred to our institution as acute myeloblastic leukemia (AML). During the same period in which we observed the cases reported here, we overall studied 2551 cases of acute myeloblastic leukemia. IH was performed on cutaneous biopsies in 5 patients, all of which expressed CD4, CD56, CD123 and TCL1, along with variable expression of CD68 and CD43. We studied also CXCR4 (CD184), since this receptor and its ligand CXCL12/SDF1 seem to drive metastasis in several cases of neoplastic disease. CXCR4 is antagonized by CD26, trough its enzymatic action on SDF1. CXCR4 was observed by IH in all cases, while convincing expression of CD26 was never found. One cutaneous fragment was also studied by FCM, confirming the CXCR4$^+$CD26$^-$ pattern. *Conclusions*. In conclusion, immunophenotype of this rare disease seems to be stable as regards HLA-DR, CD4, CD56, CD36 and CD103 display. CD26/DPPIV seems to counterbalance CXCR4 function based upon its activity on CXCL12, abundantly produced in the skin, which seems to represent the main destination of dendritic lymphoma spreading. CXCR4 and CD26 appear to be associated with tissue distribution of neoplastic cells, with CXCR4$^+$CD26$^-$ pattern corresponding to cases characterized by initial cutaneous involvement and metastatic potential, CD26 bright expression being restricted to acute leukemia-like disease.

0247

FACS SORTING IS AN EFFICIENT, FLEXIBLE AND CONVENIENT CELL TARGETING STRATEGY: APPLICATIONS IN ROUTINE MOLECULAR DIAGNOSTICS OF MATURE B-CELL MALIGNANCIES

B. Maes, K. Hensen, F. Hillen, V. Peeters, J.-L. Rummens

Virga Jesse Hospital, HASSELT, Belgium

Background and aims. In non-Hodgkin lymphoma and multiple myeloma, the malignant cell populations are often situated within a background of benign lymphoid and non-lymphoid cells. In lymphoid tissues, lymphoma involvement may only be partial and/or reactive B- or T-cells may be abundantly present. Bone marrow or peripheral blood may show only minimal infiltration by lymphoma or myeloma. Detection of chromosomal or gene aberrations on any of these sample types, may be hampered by the abundance of non-malignant cells. A cell targeting strat-

egy may therefore be required, to allow a sufficient sensitivity of any genetic or cytogenetic method such as, FISH or gene- or expression array applications. Methods and *Results.* Within the past years we have successfully validated and implemented in routine diagnostics, the combined approach of FACS imunophenotyping, simultaneous cell purification by FACS directly on glass slides, followed by interphase FISH. FACS is a very flexible purification technique, as all populations of interest can be simultaneously sorted. Only small amounts of material and low numbers of the desired cell type are required for FACS. In our hands, purity of FACS sorting was significantly higher compared to magnetic bead sorting (95-99% *vs* 80 %). Efficiency was also substantially better for FACS sorting. Subsequent interphase FISH analysis can be applied for any genetic aberration of interest on FACS sorted cells. It is fast and non-laborious thanks to the purity of the investigated cell population and the absence of background cells or substances, and with reduced reagent costs as only a small amount of probe should be applied on the small cell spot generated by FACS. In CLL, we have demonstrated that the sensitivity of detecting deletion 17p positive CLL cells by FISH on FACS sorted CD19+CD5+ cells is 1 in 10exp5 to 1 in 10exp6 white blood cells. This sensitivity is as high as that of allele-specific oligonucleotide PCR and allows this methodology to be used for assessing MRD negative status. We have shown that plasma cells present in as low as 1 % of WBC in the bone marrow can be successfully investigated for the occurrence of IGH-translocations, deletion 13q and deletion 17p by this FACS-FISH approach. In addition, we have also shown that array-CGH and metaphase FISH is feasible on FACS purified plasma cells. In follicular lymphoma and mantle cell lymphoma, respectively FISH for the IGH/BCL2 fusion and FISH for the *IGH/CCND1* fusion is successfully performed on respectively $CD19^+CD10^+$ and the $CD19^+CD5^+$ cell populations sorted by FACS. Either blood, bone marrow or a cell suspension of an involved lymph node can be used for diagnosis or staging, or to sensitively detect residual bone marrow involvement after therapy. *Conclusions*. FACS-sorting is an efficient, flexible and convenient cell targeting strategy that allows adequate combination of immunophenotyping and detection of genetic aberrations by FISH in different types of B-cell malignancies. In addition, it is a promising technique in view of novel micro-array applications for B-cell malignancies, which will benefit from the high cell population purity obtained by FACS sorting.

0247

REPEATED RADIOIMMUNOTHERAPY (RIT) WITH I-131 RITUXIMAB IN PATIENTS WITH RELAPSED OR REFRACTORY B-CELL NON-HODGKIN'S LYMPHOMA (NHL); PRELIMINARY RESULTS

H.J. Kang,[1] S.M. Lim,[1] G.J. Cheon,[1] C.W. Choi,[1] S.S. Lee,[1] I.I. Na,[1] B.Y. Ryoo,[1] W.S. Kim,[2] S.H. Nam,[3] Y.H. Park,[2] C. Suh,[4] H.S. Eom,[5] S.H. Yang[1]

[1]Korea Cancer Center Hospital, SEOUL, South-Korea; [2]Samsung Medical Center, SEOUL; [3]Seoul Veterans Hospital, SEOUL; [4]Asan Medical Center, SEOUL, South-Korea; [5]National CancerCenter, SEOUL, South-Korea

Background and Aims. Previously, we reported the results of single treatment with I-131 rituximab in relapsed or refractory B-cell non-Hodgkin's lymphoma (12th EHA abstract 0300). The study results showed modest response rate (35.0% in total patients; 55.6% in low-grade lymphoma, 18.2% in DLBCL.) with relatively short duration of response (median, 2.4 months; range, 1.0-31.6). Therefore, we designed a new protocol to investigate that the efficacy of RIT could be increased by repeated administration of I-131 rituximab as done with chemotherapy. *Methods*. Inclusion criteria were as follows: B-cell NHL with relapsed or refractory to primary standard therapy, measurable disease, adequate hematologic, renal, and hepatic function, informed consent. The rituximab (Mabthera, Roche) was radiolabeled with iodine-131 (I-131) using a modified chloaramine T method. All patients received unlabeled rituximab of 70 mg immediately prior to administration of therapeutic dose of I-131 rituximab (median 7.4 GBq; range, 4.44-9.73 GBq), and 1 months later tumor response was evaluated by contrast enhanced 18F-FDG PET/CT. RIT with I-131 rituximab was repeated in patients who did not progress after single treatment, and continued until disease progression or up to maximum six cycles. *Results*. 16 patients were enrolled (7 Mantle cell, 3 Marginal zone B-cell, 2 follicular cell, 4 diffuse large B-cell; median age, 61 years). Patients had been received one more than prior chemotherapy regimens (range, 1-4) and median treatment cycles of 6 (range, 5-15). A total of 50 cycles of I-131 rituximab (median 3; range, 1-6) were administered, and all patients were evaluated for response and toxicities. The total objective response rate was 68.8%, and 66.7% (5 CR, 3 PR) in low-grade lymphoma, 75% (3 PR) in DLBCL. The median duration of response was 6.6 months (range, 0.6-24.7 months). Adverse events were primarily hematologic toxicities; the incidence of grade 3 or 4 neutropenia and thrombocytopenia was 12.0% (23/50 cycles) and 18% (31/50 cycles), respectively. There was no treatment-related death. *Summary and Conclusions*. Compared to single treatment, repeated administration of I-131 rituximab can increase the duration of response and response rate in patients with relapsed or refractory B-cell NHL.

0248

THE HAEMOPHAGOCYTIC SYNDROME (HPS) RE-VISITED: A SOUTHEAST ASIAN PERSPECTIVE

C. Tan, W. Hwang, Y. Loh, C. Chuah, H.J. Ng, L.H. Lee, S.L. Tien, L.C. Lim, Y.T. Goh, M. Koh

Singapore General Hospital, SINGAPORE, Singapore

Background. HPS is a clinicopathologic entity characterized by fever, cytopenia, liver dysfunction and coagulopathy due to dysregulated activation of non-neoplastic histiocytes. It is associated with a myriad of different causes, with geographical variations, and commonly results in an unfavorable outcome. Data pertaining to its approach is scarce. Advances in immunophenotyping have allowed better delineation of lymphoma-associated HPS. We report the clinical spectrum and outcome of 55 cases of HPS in an adult Asian population from a single institution. *Methods*. Patients(pt) with findings of haemophagocytosis on bone marrow morphology from year 2002 to 2007 were identified and clinical records of such patients were reviewed in this retrospective analysis. Only patients who fulfill the criteria of having HPS were included in the study. *Results*. There were 37/55 cases (67%) associated with malignant lymphoma. According to WHO classification, these include NK/T cell lymphoma, nasal-type (n=11), peripheral T-cell lymphoma (NOS) (n=8), anaplastic large T-cell lymphoma (ALTCL)(n=2), hepatosplenic Á‰ T-cell lymphoma (n=2), angioimmunoblastic T-cell lymphoma (n=1), diffuse large B-cell lymphoma(DLBCL) (n=9), indolent B-cell lymphoma (n=3) and Advanced Hodgkin's disease (n=1). Compared to pt with T or NK-cell lymphomas, pt with B-cell lymphoma were older (median age 69yrs vs 48yrs, p=0.02). Of 11 pt with NK/T-cell lymphoma, nasal-type, 9 had primary extra-nasal presentation, while 3 were extra-nasal relapses, with prior history of nasal involvement. Common to both the T and NK-cell lymphomas were the demonstration of EBV involvement by in-situ hybridization for EBV-encoded early small RNA or PCR for EBV DNA, absence of serological evidence of an active EBV infection and unremarkable tumour masses. Except for 2 cases of ALTCL, marrow infiltration by lymphoma was evident in all cases of T or NK-cell lymphomas. Only 4/9 cases of DLBCL had evidence of marrow involvement. Other causes of HPS (33%) included disseminated TB (n=5), autoimmune disease (n=3), viral infection (1 each for HIV, disseminated variecella, and dengue virus infections respectively), bacterial infection (n=1) and marrow metastasis (n=1).

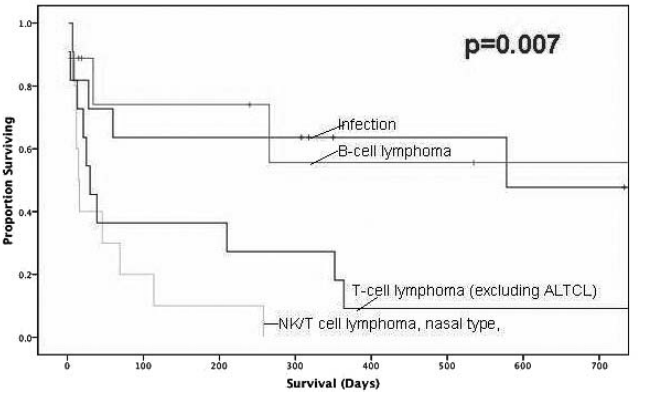


Figure 1. Overall survival by underlying causes of HPS.

Etiological cause of HPS could not be elucidated in 5 cases. Despite similar presenting features, prognosis of non-malignant causes of HPS was good once appropriate treatment was instituted. Two patients with disseminated TB who did not receive anti-TB treatment due to a delay in the diagnosis succumbed to HPS. Median overall survival for NK/T cell lymphoma, nasal-type, T-cell lymphoma (excluding ALTCL), B-cell

lymphoma and infective causes were 15 days, 30 days, 578 days and not reached respectively (p=0.007). Underlying etiology of HPS was a more important prognostic factor than age or performance status on multivariate analysis. *Conclusions*. Vigorous search for an underlying etiology is crucial. Infectious disease screening has to be guided by epidemiological data. A delay in diagnosis may compromise the outcome of a treatable infection like TB. EBV associated NK/T or T-cell lymphoma accounts for a disproportionate number of cases unique to the Asian perspective and prognosis remains dismal. HPS is the common end-point and contributory cause of mortality for all pt with NK/T lymphoma, nasal-type . This has implications in clinical trials.

0249

TRANSCRIPTOME DEFINED PATHOGENESIS AND CLASSIFICATION OF B-CELL NON-HODGKIN LYMPHOMAS IDENTIFIES SIGNATURES THAT PROVIDE AVENUES FOR PROGNOSTIC MARKERS AND GENOMIC DRIVEN TARGETED THERAPIES

M. Aggarwal,[1] M.A. Piris,[1] E. Kimby[2]

[1]*Spanish National Cancer Center, MADRID, Spain;* [2]*Karolinska Institutet, STOCKHOLM, Sweden*

Background. The knowledge so far accumulated about B-cell lymphoma pathogenesis has not been sufficient to facilitate the development of targeted therapies. We analyzed a large series that includes all the major B-cell lymphoma types. *Aims*. Identification of commonly dysregulated pathways in B-cell non-Hodgkin's lymphomas. Identify heterogeneity in B-cell lymphomas in terms of functional pathways. To find out key driving mechanisms in the pathogenesis of B-cell lymphomas. To investigate the prognostic potential of biological markers of pathogenesis. To translate genomic profiling into discovery of targeted therapie. *Methods*. We used a combination of microarray; gene set enrichment analysis, tissue microarrays, gene silencing and multiple bioinformatics tools for our analysis.

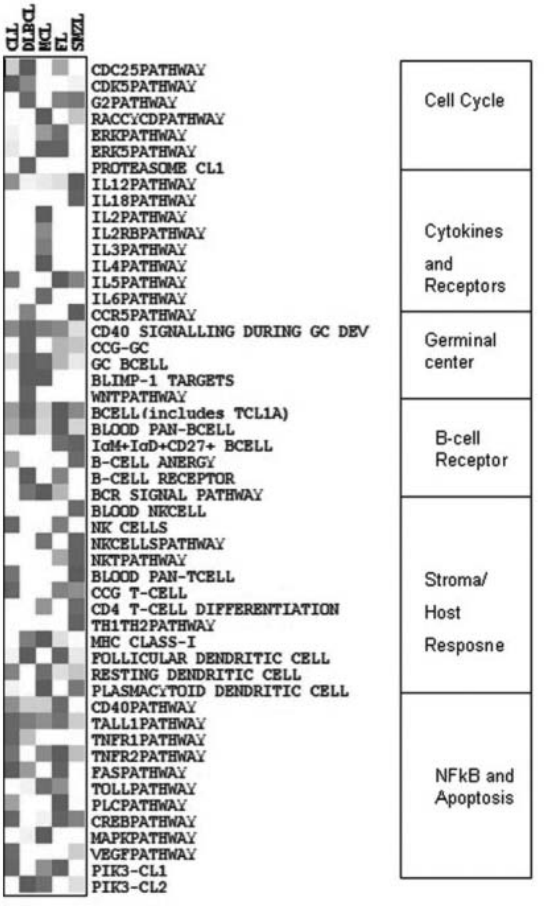


Figure 1. Functional pathways defining heterogeneity

Results. The functional signatures determining lymphoma classification were cell-cycle, apoptosis, cytokine-cytokine receptor interaction, T-cell receptor, B-cell receptor (BCR), cell adhesion, and NF-kB activation. These signatures reveal subclasses of diagnosed lymphoma types, suggesting the existence of a distinct functional heterogeneity within most lymphoma types, and particularly among CLL, MCL, and DLBCL. Functional signatures in B-cell lymphomas allow a better understanding of common mechanisms, and at the same time establish the biological basis of the clinical variability. The results identify the main pathways driving lymphoma growth, and reveal the existence of common survival mechanisms (B-cell receptor pathway) and genes (TCL1a oncogene) that are essential for lymphoma pathogenesis. We explored the functional pathways that define prognostic variance in CLL and other lymphomas. In CLL, using chemical genomic profiling techniques, we identified drugs that can potentially reverse the progression of disease. *Summary and Conclusions*. We identify lymphoma to be a result of multiple oncogenic lesions and deregulated pathways, the key drivers being altered BCR signaling and TCL1A expression acting via the calcium signal pathway in B-cells and T-cells. These findings helped us to explore targeted drugs using chemical genomic profiling in B-NHL.Additionally, we found markers with prognostic potential in MCL an CLL from profiling studies.

0250

FOLLICULAR LYMPHOMA (FL) DIFFERS FROM FOLLICULAR HYPERPLASIA (FH) ALSO AT THE EPIGENETIC LEVEL

J. Paz Carreira,[1] R. Losada,[1] A. Garcia-Rivero,[1] M. Varela-Pérez,[2] A. Alvarez,[3] F. Bal,[2] J. Alba,[4] M. Gonzalez-López[3]

[1]*Centro Oncologico de Galicia, LA CORUÑA;* [2]*Xeral-Calde Hospital, LUGO;* [3]*Juan Canalejo Hospital, A CORUÑA;* [4]*POLUSA, LUGO, Spain*

Introduction. The distinction between benign follicular hyperplasia (FH) and follicular lymphoma (FL) can sometimes be challenging. Germinal centers (GC) are unique sites in peripheral lymphoid tissue where clonal selection of B cells takes place. This occurs as a response to stimulation by various antigens originating, sometimes, follicular hyperplasia (FH). GC have been known to be a major source of B-cell lymphomas including follicular and diffuse large cell. DNA methylation of tumor-suppressor genes is a mechanism of gene silencing involved in the pathogenesis of FL. Much less is known about the role of methylation in FH. We determined the methylation status of 5 tumor-suppressor genes in 20 patients with FL and 30 patients with BFH in order to see any differences between the pathological and the physiological state. Material and Methods. Genomic DNA extracted from paraffin-embedded samples of 20 FL and 30 BFH were analyzed by methylation-specific polymerase chain-reaction to determine promoter hypermethylation of DAP-k, SHP1, Rarβ, p14 and p15. FL samples were obtained from lymph nodes. BFH samples were obtained from lymph nodes and tonsils. Diagnosis was based on morphology and immunohistochemistry analysis. All cases were matched for age, sex and ethnic origin. *Results*. DAP-k, SHP1, p14, p15 and RARb promoters were methylated in FL and BFH. DAP-k and RARb promoter methylation occurred with higher frequency in FL (89% and 72%) than in BFH (44% and 39%) (p=0.004 and p=0.024). SHP1 is methylated in 89% FL vs 35% BFH (p=0.000). p14 was more frequently methylated in FL (100%) than in BFH (36%) (p=0.01), but only five samples were analyzed for this gene. p15 was methylated more frequently in FL than in BFH (39% vs 25%) but the difference was not statistically significant (p=0.388). *Conclusions*. Inactivation of DAP-K, SHP1 and Rarβ is present in FL with significantly higher frequency than in BFH. Therefore, it may have pathogenic significance. With our data, methylation of the cyclin dependent kinase inhibitor p15 is not a differential pathogenic event in FL with respect to FH. However, in the case of p14, in spite of the small size of the sample, it may represent a complementary route to disease progression.

0251

CUTANEOUS CCL27 EXPRESSION IN EARLY-STAGE MYCOSIS FUNGOIDES: ITS ROLE IN THE BALANCE BETWEEN THE TUMORAL INFILTRATE AND ANTI-TUMORAL IMMUNE RESPONSE, AND PROGNOSTIC UTILITY AS A PREDICTOR OF RECURRENCE

G. Goteri,[1] S. Rupoli,[2] A. Campanati,[3] S. Sabato,[1] L . Canafoglia,[2] G. Ganzetti,[3] P. Picardi,[2] A. Costagliola,[1] D. Stramazzotti,[1] S. Pulini,[4] A.R. Scortechini,[2] S. Serresi,[5] M. Ottaviani,[6] M. Nicolini,[6] A. Cellini,[3] G. Fabris,[1] A.M. Offidani,[3] P. Leoni[2]

[1]Institute of Pathology, ANCONA; [2]Clinic of Hematology, ANCONA; [3]Clinic of Dermathology, ANCONA; [4]Department of Hematology, PESCARA; [5]Division of Dermathology, I.N.R.C.A.-I.R.C.C.S., ANCONA; [6]Division of Dermatology, FABRIANO, Italy

Background. Recruitment of neoplastic T-cells in skin is a critical step in the pathogenesis of Mycosis Fungoides (MF) lesions. The chemokine CCL27 attracts memory CCR10-positive T cells to the skin. Increased CCL27 serum levels and skin epidermal expression have been observed in MF patients compared to normal controls, but the interactions between neoplastic cells and the skin immune system needs to be further elucidated. *Aims*. We investigated whether CCL27 expression in MF is related to the density of dendritic cells (DC), CD8+ lymphocytes and CD4+ neoplastic lymphocytes; moreover, whether it influences the clinical response to therapy, it is modifiable by therapy itself and if the changes eventually observed can predict the frequency of recurrences and event-free survival. *Material and methods*. CCL27 epidermal immunohistochemical staining was performed in 52 early stage-MF patients, 34 M/18 F, treated by PUVA plus interferon α for 14 months, with a complete follow-up data. Specimens were obtained at diagnosis and at the end of treatment. Formalin-fixed/paraffin-embedded tissue sections were immunostained with anti-CCL27, CD1a, CD8, CD4 antibodies. CCL27 immunoreactivity was graded as + (faint/moderate staining, limited to epidermal basal layer, as in normal skin); ++ (faint/moderate staining in the suprabasal layers; strong staining up to the lower two-thirds of epidermis); +++ (strong staining of full-thickness epidermis). Density of CD1a-positive DC and CD8+ lymphocytes was graded as + (few/isolated cells), ++ (small, ≤5 cell clusters), +++ (larger, >5 cell clusters). Density of CD4+ lymphocytes was graded as + (perivascular infiltrate), ++ (discontinuous subepidermal band), +++ (continuous band). Statistical analysis was also performed (chi2 test for tables of contingency; event-free survival curves (EFS) by Kaplan-Meier method). *Results*. At diagnosis, CCL27 expression was similar to normal skin in 16 cases and abnormal/suprabasal in 36, with 12 +++ cases. A normal/basal CCL27 expression tended to be correlated with a high DC density and with a low neoplastic infiltrate density (although p=NS). CCL27 at diagnosis was not correlated with clinical response (50 complete remission/CR, 2 partial remission). Treatment induced a significant CCL27 increase (suprabasal in 42 cases with 24 +++ cases; χ^2 test: p=0.004). Skin CCL27 expression at the end of treatment, but not at diagnosis, was related to recurrence and influenced EFS. During follow-up (median, 92.5 mo.s; range, 43-165), 33 patients relapsed (median EFS, 46 mo.s). A normal/basal CCL27 expression at the end of treatment, was correlated with a lower incidence of disease recurrence (3/9 compared to 30/41 with suprabasal expression; χ^2 test: p=0.022) and a longer median EFS (111 mo.s vs 39 mo.s with suprabasal expression; log rank test: p=0.031). *Conclusions*. Increased CCL27 expression in early-stage MF lesions might be related to a balance between neoplastic cells and immunomodulant dendritic cells. CR induction in almost all patients by treatment is not correlated with a CCL27 reduction, but a normal CCL27 expression after treatment designates a subset of patients (about 20%) with a favourable behaviour. The mechanisms involved in the increased CCL27 expression after therapy in the remaining 80% patients, which have a higher probability to recur, need to be further investigated

0252

MUTATION ANALYSIS OF IGVH GENES IN 18 SPLENIC MARGINAL ZONE LYMPHOMA (SMZL) CASES AND CORRELATION WITH CLINICAL AND IMMUNOPHENOTYPIC CHARACTERISTICS

C. Kalpadakis,[1] E. Dimitriadou,[2] T. Vassilakopoulos,[2] M. Angelopoulou,[2] M. Siakantaris,[2] F. Kontopidou,[2] H. Papadaki,[1] P. Panayiotidis,[2] G. Pangalis[2]

[1]University of Crete, HERAKLION; [2]University of Athens, Laikon General Hospital, ATHENS, Greece

Background. SMZL is a distinct entity. Current data suggest that there is an heterogeneous pattern of immunoglobulin (Ig) somatic hypermutation. The prognostic significance of these molecular findings is yet controversial. *Aims.* To evaluate the mutational status in a series of 18 SMZL patients and to correlate it with patients' features and outcome. *Methods.* DNA was extracted from mononuclear cells of spleen tissues and bone marrow. Polymerase chain reaction (PCR) was performed using 5' leader region specific primers for each one of the six VH families (VH1-VH6) and a 3'J consensus primer. Resultant PCR products were next ligated and cloned and plasmid DNA was isolated from overnight cultures of randomly selected colonies. At least five plasmids per case were sequenced for both orientations. Clinical, laboratory and outcome data of these patients were recorded and evaluated in relation to mutational status of IgVH genes. *Results.* In 9 of 18 cases (50%), IgVH genes were found mutated (<98% homology to the nearest germline configuration). Sixteen cases used the VH3 and the other two the VH1 family segments. Correlation of mutation status with clinical, laboratory and outcome characteristics of our pts disclosed no statistical significant findings. Sixteen cases (9 mutated and 7 unmutated) used the VH3 family, while the other two unmutated used the VH1-2 family. In order to determine whether mutations in IgVH regions were antigen driven, we further analyzed the mutation pattern of somatic hypermutation in the mutated cases (Table 1). p values of ≤0,05 according to the multinomial distribution model were considered statistically significant of antigen-selection, as it is currently accepted. In two of the nine mutated cases the R/S ratio in CDR was greater than expected by chance only, suggesting antigen selection. In three mutated cases the excess of R over S mutations targeted the FR regions rather than the CDR. In the remaining four mutated cases the pattern of R/S mutations in FR or CDR showed no evidence of antigen selection. *Summary and Conclusions*. Our results indicate that there are two types of SMZLs, one with mutated VH genes and one with unmutated. VH3 family was the most commonly used. In the mutated cases the ratio of R/S mutations displayed an heterogeneous pattern and in 4 of them antigen selection process could not be clearly supported. Prognostic significance of the VH family usage and mutational status has not been confirmed in this study.

Table 1.

VDJ gene in mutated patients (% numb. of mutations)	FR R/S	P value FR	CDR R:S	P value CDR
VH3-11 J4 D5-5 (97.57%)	3/0	0.28	3/0	0.022
VH3-23J4D6-6 (67.36%)	5/1	0.55	5/1	0.19
VH3-23J4D2-2 (97%)	3/2	0.01	5/1	0.004
VH3-15J4D3-22 (96%)	4/5	0.008	3/2	0.23
VH3-13J1D3-10 (91.58%)	6/1	0.97	0/0	0.93
VH3-7J6D6-19 (84.03%)	18/0	0.99	2/0	0.65
VH3-4J2D2-21 (90.28%)	15/0	0.00	3/0	0.34
VH3-48J2D7-27 (97%)	7/1	0.52	3/0	0.12
VH3-74J4D4-17 (88.5%)	14/0	0.00	0/0	0.94

0253

LONG-TERM SURVIVAL OF PATIENTS DIAGNOSED WITH NON-HODGKIN LYMPHOMA AFTER A PREVIOUS MALIGNANCY

D. Pulte,[1] A. Gondos,[2] H. Brenner[2]

[1]Weill Cornell Medical College, NEW YORK, USA; [2]German Cancer Research Center, HEIDELBERG, Germany

Background. An increase risk of non-Hodgkin lymphoma (NHL) has been observed in survivors of several malignancies. Survival for patients with primary NHL has improved in the 1990s and early 21st century, but population-based survival data for patients diagnosed with NHL after a prior malignancy are sparse. One prior study of NHL after HD treated between 1981 and 1999 found generally poor outcomes, with overall survival of about 30% in this patient population, a much lower rate than that for patients with primary NHL treated during the same time period. *Aims*. To disclose changes in survival in patients with NHL after a previous malignancy between the 1990s and the early 21st century. *Methods*. We estimated trends in age specific 5- and 10-year relative survival

of NHL patients with prior malignancy in the United States from 1990-1994 to 2000-2004 from the 1973-2004 data base of the Surveillance, Epidemiology, and End Results (SEER) Program. Period analysis of survival was employed to disclose recent developments with minimum delay. *Results*. 10,605 patients were diagnosed with a NHL after a prior malignancy during the periods studied. The most common preceding malignancies were non-epithelial skin cancers other than melanoma (representing 35% of prior malignancies in this sex and age group) and prostate cancer (12%) for men aged 15-64, prostate (43.5% and 47.4%, respectively) and colon/rectum cancer (12.2% and 16.5%, respectively) in men 65-74 and 75+ years of age. Breast (33.1%) and cervical (19.4%) cancers were most common for women aged 15-64 while breast (41.9% and 42.0%, respectively) and colon/rectum cancers (13.3% and 20.2%, respectively) were most common among women aged 65-74 and 75+. Five and 10-year relative survival has strongly improved for NHL patients with prior malignancies between 1990-94 and 2000-04, from 38.0% to 54.1% and 24.4% to 41.0%, respectively. The increase was most pronounced among men below the age of 65 (from 17.7% to 58.6% and from 12.7% to 44.4%, respectively) but major increases were also seen in older age groups and among women. Despite a strong increase in relative survival, patients with prior malignancy continued to have a worse prognosis compared to those with no prior malignancy (Figure 1), although the gap in survival has lessened over time. *Conclusions*. NHL after a prior malignancy is common, with 1 in 7 cases of NHL occurring in patients with a history of prior malignancy. The prognosis of patients with secondary NHL has strongly improved in recent years, particularly among male patients below 65 years of age. The use of antibody therapy, active treatment for HIV, and other advances are likely to contribute to the improvements observed. A history of prior malignancy continues to be a marker of relatively poor prognosis in patients with NHL, but survival has improved greatly in patients with and without prior malignancy.

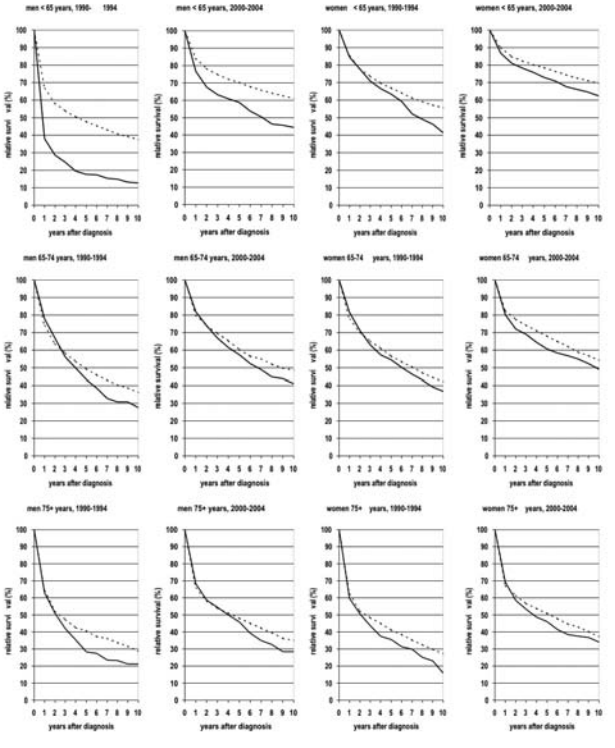


Figure 1. Relative survival rates after in patients diagnosed with NHL. Solid lines represent patients with prior malignancy, dashed lines patients without prior malignancy.

0254

NT-PROBNP IS A POWERFUL AND INDEPENDENT PROGNOSTIC FACTOR FOR SURVIVAL IN ANTHRACYCLIN-TREATED LYMPHOMA PATIENTS

A. Salar, R Guerri, J Comín, A. Alvarez-Larrán, M Gomez, E. Gimeno, B Sanchez-Gonzalez, C. Pedro, E. Abella, L Molina, T. Gimenez, J Bruguera, C. Beses

Hospital del Mar, BARCELONA, Spain

Introduction. N-terminal pro-brain natriuretic peptide (NT-proBNP) has been shown to be a sensitive indicator of cardiac abnormalities. High blood levels of NT-proBNP have been associated with poor clinical outcome in patients with amyloidosis treated with conventional chemotherapy and/or autologous transplantation. However, its clinical value in lymphoma is unknown. *Aims.* To assess the value of NT-proBNP as a prognostic variable for survival in patients with lymphoma receiving anthracyclin-containing chemotherapy. *Patients and methods*. patients with Hodgkin´s (HL) and non Hodgkin´s lymphoma (NHL) candidate to receive anthracyclin-containing chemotherapy were included. Clinical heart examination, evaluation of cardiovascular risk factors, determination of NT-proBNP blood levels and high resolution echocardiography were done at diagnosis, at the end of therapy and annually thereafter. NT-proBNP levels were measured with electrochemiluminescence sandwich immunoassay (ECLIA; Roche) on an Elecsys System 2010. Clinical variables related with patient characteristics, lymphoma risk and cardiovascular risk were analyzed. *Results*. 81 patients have entered onto the study and 79 pts have been analyzed (2 early lost of follow-up). Patient characteristics: median age: 61 years (range: 18-85); male: 58%; NHL: 79%; HL: 21%; ECOG/PS ≥2: 25%; Ann Arbor III-IV: 57%; B-symptoms: 24%, bulky: 17%; LDH >1 nv: 34%; β2-microglobulin >1 nv: 53%; albumin <35 g/L: 32%; haemoglobin <105 g/L: 20%; extranodal disease ≥2: 17%; comorbidities ≥2: 44%; previous cardiac disease: 11%; median NT-proBNP: 100 pg/mL (9-9102); median left ventricular ejection fraction (LVEF): 65% (range 41-83). High blood levels of NT-proBNP were associated with: age, Ann Arbor stage, ECOG/PS, LDH, β2-microglobulin, albumin, haemoglobin, comorbidities and previous cardiologic history (but not with LVEF). Median follow-up from starting treatment was 18 m (0-45). Ten patients died. Overall survival (OS) at 12 and 24 months were 87% (95% CI: 80-94%) and 84% (95% CI: 80-88%) respectively. Table 1 shows sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficacy for several cut-off levels of NT-proBNP. Factors associated with worse OS in the univariate analysis were: NT-proBNP >900, ECOG/PS ≥2, LDH >1 nv, β2-microglobulin >1 nv, albumin <35 g/L, haemoglobin <105 g/L, number of comorbidities ≥2, previous cardiologic history, number of extranodal sites ≥2, B-symptoms, age >60. In multivariate analysis, the strongest factor predicting for survival was NT-proBNP >900 (HR 22, 95% CI: 4.1-119) (p<0.0001). *Conclusions*. NT-proBNP >900 is a strong and independent prognostic factor for survival in patients with lymphoma treated with anthracyclin-containing regimens. 2. Levels of NT-proBNP are not associated with LEFV in this study. 3. NT-proBNP is an easy, fast, widely available and well-standardized parameter that could be useful in addition to other well-established prognostic indexes.

Table 1.

Cut-off of NT- proBNP	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Efficacy (%)
> 330	90	78.3	37.5	98.2	79.8
> 500	80	82.6	44.4	96.7	84.8
> 900	80	82.6	72.7	97	93.7

0255

T-CELL LARGE GRANULAR LYMPHOCYTE LEUKAEMIA: CLINICAL COURSE AND RESPONSE TO TREATMENT

F. Fortune,[1] A. Fortune*,[1] K. Kelly*,[1] J. Sargent,[2] S. McGuckin,[1] F. Quinn,[1] K. Haslam,[1] D. O'Brien,[1] E. Conneally,[1] P. Browne,[1] P. Thornton,[2] E. Vandenberghe[1] *Co first author

[1]St. James Hospital, DUBLIN, Ireland; [2]Mater Hospital, DUBLIN, Ireland

Background. Large Granular Lymphocytic Leukaemia (LGL) is an indolent T lymphoproliferative disorder. Molecular analysis of the T cell receptor (TCR) gene to define clonal T populations has made LGL diagnosis robust and thus clarified its natural history. *Aims.* We have reviewed the clinical course and treatment outcome of patients with LGL attending 2 large haematology services to clarify its natural history and assess response to treatment. *Methods*. The clinical features, laboratory findings and treatment response. Of 20 patients diagnosed between 2003-2007 with a clonal proliferation of T-LGL confirmed by morphology, flow-cytometry and molecular analysis. *Results*. Twenty patients with a median age of 66 (range 27-78) were referred to a haematology unit by their general practitioner with non-specific symptoms of fatigue (55%), recurrent infections (45%) and minor blood count abnormalities. The median time from symptom onset to diagnosis was 5 months. Thirteen (65%) patients were neutropenic, 6 had an absolute lymphocytosis (>5×10^6 /μL) and 7 (35%) were anaemic. Physical examination was normal in 60% of patients with splenomegaly noted in 15%. An excess of LGLs was seen on blood film with the following immunophenotype: $CD3^+$, CD7 weak, CD4:CD8 ratio reversal and CD56-. Clonality was confirmed using TCRβ, γ and δ Biomed probes, of which 18 had β, 18 γ and 9 δ gene rearrangements. Twelve (60%) patients needed treatment for symptoms or a neutrophil count <0.5×10^9/L, with a median time from diagnosis to treatment of 11 months. Six patients received cyclosporine as first line therapy, resulting in a temporary improvement in blood count in 3 patients. Methotrexate or Chlorambucil was used in 4 patients with no improvement. Eight patients were treated with the purine analogue 2'deoxycoformycin (Pentostatin); 4 receiving it as first line therapy. The overall response rate was 62.5% with a complete haematological response in 3 patients (including 2 molecular remissions) and a partial response in 2 patients. Two patients relapsed 18 months after Pentostatin, with 1 patient achieving a further remission with alemtuzamab and a second multiply treated patient with a 20-year history of LGL proceeding to allogeneic bone marrow transplantation. *Conclusions*. This case series confirms the clinically heterogeneous nature of this disorder, and suggests that it may be under-diagnosed resulting in non-specific morbidity in the community. Accurate diagnosis using molecular techniques is now possible. Pentostatin is an effective, well-tolerated treatment for symptomatic LGL. Clarity on indications for treatment and a logical treatment algorithm might improve the morbidity of this disease.

0256

HYPOGAMMAGLOBULINEMIA AND NON-NEUTROPENIC INFECTION AFTER CHEMOTHERAPY COMBINED WITH RITUXIMAB. PREDICTIVE FACTORS AND CLINICAL SIGNIFICANCE

K. Filanovsky, L. Shvidel, M Shtalrid, A. Duek, M. Haran, E. Sigler, A. Berrebi

Kaplan Medical Center, REHOVOT, Israel

Rituximab is a chimerical human/ mouse monoclonal anti-CD20 antibody successfully applied in B-cell lymphoproliferative disorders. The suppression of B-lymphocytes may induce hypogammaglobulinemia and increased risk for infection. These complications are rarely observed as a result of monotherapy with rituximab but have been often noted following combination with aggressive chemotherapy or in the setting of autologous stem cell transplantation (ASCT). In an attempt to study the role of rituximab in induction of hypogamma, we analyzed the frequency of this phenomenon, its possible causes, infectious complications and infection-related mortality in lymphoma patients treated with different chemotherapy regimens with or without rituximab. We analyzed 182 patients with lymphoma; 136 of them received rituximab concurrently with chemotherapy (R-Chemo) and 46 patients treated with chemotherapy only, with follow-up period of up to five years. Following R-Chemo, 17 cases (12.5%) of severe hypogamma (defined as <60% of normal IgG range) were diagnosed, 21 patients (15.5%) had mild and moderate hypogammaglobulinemia, and 16 (12%) were found with decreased IgM alone. Multivariate analysis showed a significant increase of severe hypogamma when >6 doses of rituximab were given. Moreover, rituximab in >8 doses leaded to severe hypogamma in 50% of patients compared to 11.8% in patients treated with ≤8 doses (p .006) associated with 3 times more infections. The type of chemotherapy regimen didn't influence development of the hypogamma. Even myeloablative chemotherapy used in combination with rituximab before ASCT slightly increased the rate of severe hypogamma (p .065). Only the combination of fludarabine and rituximab (FR) compared to other Chemo-R, induced non-significant increase of severe hypogammaglobulinemia (21% vs 11%; p=0.02), and severe non-neutropenic infection 19.4% vs 5.0% (p .005) and infection-related mortality 24% *vs* 6% (p. 004). Multivariate analysis showed that patients post FR treatment were 6.4 times more prone to have severe infections than post other Chemo-R regimens (95% CI 1.49-27.0). Age and gender of patients, type of lymphoma, bone marrow involvement didn't affect the immunoglobulin level. We found a strong correlation between the IgG level and frequency of severe infections and mortality from infection (p<0.01). Finally, the pre-treatment hypogamma was not found as a risk factor for infection. However, the duration of this phenomenon was found important: patients with severe hypogamma lasted >6 months were 4.5 times more likely to have severe infection (95%CI 1.19-18.5). In conclusion, hypogammaglobulinemia is an important phenomenon leading to increase in infection and infection-related mortality rate in lymphoma patients receiving chemotherapy with or without rituximab. Rituximab given for >6 cycles significantly increases the incidence of severe hypogammaglobulinemia. Fludarabine-based chemotherapy combined with rituximab and the duration of hypogammaglobulinemia are independent predictive factors for severe infection and infection-related mortality.

0257

INTERLEUKIN 10 (IL10) PROMOTER POLYMORPHISMS ARE ASSOCIATED TO THE EPSTEIN-BARR VIRUS STATUS IN CHILDHOOD BURKITT'S LYMPHOMA: PATHOGENIC CLUES FROM AN INTERMEDIATE RISK REGION

C. F. M. Fonseca Minnicelli, M.H.M. Barros, I.Z. Zalcberg, R.H. Hassan

National Institute of Cancer, RIO DE JANEIRO, Brazil

Background. IL10 is an immunoregulatory cytokine that exerts B cell proliferation effects. IL10 expression is influenced by several of EBV proteins. Moreover, EBV susceptibility seems to be related to IL10 promoter polymorphisms. *Aims*. To investigate the role of IL10 as a susceptibility factor in BL, related to EBV status. *Methods.* A case-control study was performed including 57 BL patients (1-16 years, median 6; M40:F17) and a group of 270 healthy controls (M148:F122), including 20 umbilical cord blood samples (age 0), 51 children (median age, 8 years) and 199 adults (median 26 years). EBV association was determined in BL samples by EBER-ISH and PCR. Genotyping was performed by allele specific (AS)-PCR and fluorescent STR-PCR for positions -1082(A/G) and -592(A/C) of the proximal haplotype and the IL10.G microssatelite, respectively. Analysis of the proximal haplotype -1082(A/G), -819(C/T),-592(A/C) was performed in 106 controls and 48 BL cases. *Results.* In the control group, genotypic frequencies were AA= 44.3%, GG= 9.9% e GA= 45.8% for the -1082 position and AA= 6.8%, CC=28.4% and AC= 64.8% for -592. In BL patients, -1082 genotypic and genic frequencies were GG=26.3%, GA=40.3% and AA=33.4%; G allele 0.46, and A allele 0.54. The -1082 GG genotype and G allele, belonging to the high IL10 expression GCC haplotype, were over-represented in BL compared to the control group (p= 0.006 and p=0.043, genotypic and genic respectively). When BL patients were grouped in EBV+ (n=31) and EBV- (n=24), the frequency of -1082 G allele was significantly higher in the EBV-group in relation to control (OR 2.38; CI95%: 1.34-4.22; p=0.0043). Analysis of the -592(A/C) position in BL group showed frequencies of AA=2.3%, AC=50% and CC=47.7%; A allele 0.27 and C allele 0.73. Genotypic frequencies were significantly different from controls, due to an over-representation of CC genotype (p=0.04). Such differences were not related to EBV status. The proximal IL-10 haplotypes for high, intermediate and low cytokine production, GCC, ACC and ATA, respectively were detected in control and BL groups. In BL, ATA/ATA (1%) and GCC/GCC (42%) were the less and most frequently found genotypes, compared to controls, with ACC/ACC (4.7%) and GCC/ACC (24.5%) as the less and most frequent genotypes. Statistical comparison showed a higher-than-expected frequency of homozygous GCC genotype in BL (p=0.066). The IL10.G CA tandem repeat polymorphisms showed a range of 18 to 25 in the BL group and 18 to 26 in control group, without significant differences. *Conclusions*. Our results suggest that IL10 inter-individual genetic variations have a role in BL predisposition and patho-

genesis. Differences between BL cases and controls were accounted by an over-representation of the GCC haplotype. -1082G allele and GG genotype were over-represented in EBV- BL. This study has the characteristic of evaluating similar number of EBV+ and EBV- cases originated in the same socio-geographical context, allowing to propose a differential effect of IL10 polymorphisms according to the virological status of BL. In EBV+ cases, genetically determined IL10 expression variation might be obscured by the potent EBV-mediated IL10 induction.

0258

RISK OF FEBRILE NEUTROPENIA AND USE OF G-CSF PRIMARY PROPHYLAXIS IN NON-HODGKIN'S LYMPHOMA PATIENTS RECEIVING R-CHOP-21

C. Haioun,[1] U. Jaeger,[2] P. Lugtenburg,[3] R. Pettengell,[4] F. Principe,[5] F. Rossi,[6] A. Salar,[7] M. Schwenkglenks,[8] G. Verhoef,[9] S. Edmundson,[10] P. Bacon[11]

[1]CHU Henri Mondor, CRETEIL, France; [2]Universitatsklinikum, VIENNA, Austria; [3]Erasmus Medical Center, ROTTERDAM, Netherlands; [4]St George's University of London, LONDON, UK; [5]Hospital San Joao, PORTO, Portugal; [6]CTMO Fondazione OM PoMaRe IRCCS, University of Milan, MILAN, Italy; [7]Hospital del Mar-IMAS, BARCELONA, Spain; [8]European Center of Pharmaceutical Medicine, University Hospital, BASEL, Switzerland; [9]University Hospital Gasthuisberg, LEUVEN, Belgium; [10]Amgen Ltd, UXBRIDGE, UK; [11]Amgen Europe (GmbH), ZUG, Switzerland

Background. Three-weekly CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy with rituximab (R-CHOP-21) is the standard of care for aggressive Non-Hodgkin's Lymphoma (NHL) in many countries. CHOP regimens are myelotoxic, carrying a substantial risk of febrile neutropenia (FN). Current guidelines recommend granulocyte colony-stimulating factor (G-CSF) primary prophylaxis for patients at overall ≥20% risk of FN due to their chemotherapy regimen and patient-related factors. IMPACT NHL is an observational study assessing neutropenia and anaemia management with 2- and 3-weekly CHOP regimens in Europe and Australia. *Aims.* In this analysis, we report physician-assessed risk of FN at baseline in patients with aggressive and indolent B-cell NHL receiving R-CHOP-21 and the use of G-CSF primary prophylaxis. *Methods*. For this observational study of NHL management, data are collected both retrospectively and prospectively. This interim analysis focuses on retrospective data from consecutive NHL patients treated with R-CHOP-21 at 62 centres in 10 countries. Eligible patients were the five who had most recently completed treatment prior to the centre joining the prospective study. Patients received 1-8 cycles of R-CHOP-21. Physicians were asked to review patient records and assess FN risk prior to therapy using criteria from the EORTC guidelines on G-CSF use (i.e. total FN risk derived from R-CHOP-21 and any patient-related factors such as older age and advanced disease stage). Here, we present the proportion of patients assessed to be at ≥20% baseline FN risk and the use of G-CSF primary prophylaxis (defined by current practice as initiation up to Day 7 of cycle 1). The subsequent incidence of FN and the incidence of FN-related hospitalizations are also shown. *Results*. In total, 174 patients who began treatment with R-CHOP-21 between 01/2005 and 05/2007 were included in this analysis. About two thirds (61%) were male and the mean age was 61.2 years (range: 18-83; 83 [48%] were aged ≥65 years). Forty percent of patients had Stage IV disease; 12% had received prior treatment. Diffuse large B-cell lymphoma was the most common histological type (59%), followed by follicular lymphoma and mantle cell lymphoma. Ninety-one patients (52%) were judged to have been at ≥20% FN risk from R-CHOP-21 and patient-related factors; 44% of these patients received G-CSF primary prophylaxis (Table 1). The actual incidence of FN in this group of patients was 23%. Overall, 29% of patients received G-CSF primary prophylaxis (pegfilgrastim or daily G-CSF [mean±SD daily G-CSF prophylaxis doses in cycle 1: 4.55±1.80]). Twenty percent of patients eventually experienced FN. There were 38 hospitalizations primarily related to FN. *Conclusions*. In this analysis, physicians estimated over half of NHL patients receiving R-CHOP-21 to be at high (≥20%) risk of FN at baseline (according to guideline criteria). Less than half of these patients received G-CSF primary prophylaxis and 23% of them subsequently experienced FN. The overall incidence of FN for all patients was 20%. FN led to a substantial number of hospitalizations. This preliminary analysis suggests G-CSF primary prophylaxis should be considered for NHL patients receiving R-CHOP-21.

Table 1. Physician-assessed baseline FN risk, G-CSF primary prophylaxis and actual incidence of FN in NHL patients receiving R-CHOP-21.

	Assessed baseline FN risk <20%	Assessed baseline FN risk ≥20%	All patients
	N = 69	N = 91	N=174
G-CSF primary prophylaxis	10 (14%)	40 (44%)	50 (29%)
FN cycle 1	7 (10%)	7 (8%)	14 (8%)
FN in any cycle	13 (19%)	21 (23%)	34 (20%)
NB. No FN risk was assigned for 14 patients			

Non-Hodgkin's lymphoma - Clinical (indolent + miscellaneous)

0259

THE (R)VAD+C REGIMEN IS A GOOD PREPARATIVE REGIMEN BEFORE PBSC TRANSPLANTATION FOR MCL PATIENTS TREATED IN FIRST LINE AND KI67 APPEARS AS AN EFFECTIVE INDEPENDANT FACTOR OF SURVIVAL: TEN YEARS EXPERIENCE OF THE FRENCH GOELAMS GROUP

R. Gressin,[1] S. Caulet Maugendre,[2] E. Deconinck,[2] O. Tournilhac,[2] M. Dartigeas,[2] M.P. Moles,[2] J. Dugay,[2] D. Guyotat,[2] J.F. Rossi,[2] S. Le Gouill,[2] G. Lepeu,[2] G. Damaj,[2] T. Lamy,[2] P. Solal Celigny,[3] H. Maisonneuve,[4] B. Corront,[4] J.P. Vilque,[2] P. Casassus,[2] J.L. Dutel,[4] J.P. De Jaurreguyberry,[5] F. Maloisel,[2] P. Rodon,[4] A. Thyss,[6] P. Feugier,[2] C. Le Maignan,[2] S. Cailleres,[4] V. Lucas,[4] i. Grulois,[4] A. Bellange,[4] M. Colonna,[7] P. Colombat[2]

[1]CHU Michallon, GRENOBLE, France; [2]CHU, RENNES; [3]Clinique Victor Hugos, LE MANS; [4]CHR, LA ROCHE SUR YON; [5]CH Sainte Anne TOULON; [6]Centre Lacassagne, NICE; [7]Cancer Register of Isère, GRENOBLE, France

Background. Consolidative PBSC transplantation appears as the best strategy for MCL patients who have responded to a first line treatment. Today there is no consensus for that first regimen. The overall survival (OS) of these patients depends on independent factors described in the recent MIPI score. Nevertheless this index doesn't integrate the proliferation index which has been often underlined in the literature as a potential factor for survival. *Aims.* to confirm the real efficacy of the (R)VAD+C regimen before PBSCT in MCL patients (Gressin Ann Oncol 1997) and to analyse the value of the MIB1 (KI67) on survival we present the results of two consecutive prospective phase II studies of the Goelams. *Methods.* Following informed consent, 113 patients were enrolled in one of the two studies opened between 1996 and 2005. MCL diagnosis was centrally reviewed and ki67/MIB1 expression systematically counted on 1000 cells (2x500). From 1996 to 2000, 74 patients received the VAD + C regimen (classical VAD followed by ten days of chlorambucil 12 mg/D from D20 to D29. Delay between each cycle was 35 D). After 4 cycles, the responders over 60 years received 4 additional cycles and those under 60 two additional cycles followed by PBSCT. Between 2001 and 2005, 39 patients aged less than 65 years received RVAD+C (rituximab 375 mg/m^2 D1 of each VAD) followed by PBSCT. The conditioning regimen associated alkeran 140 and a 8 grays TBI. Treatment response was defined according to the Cheson criteria after 4 cycles of (R)VAD+C. *Results.* the two patient series showed similar clinical and biological features at diagnosis and both overall and complete response rates (ORR 73% and CRR 46%). Significantly, grade ? haematological toxicity occurred in less than 5% of patients. 62% (49/79) of the young patients were transplanted. The response to the (R)VAD+C depends on 3 independent factors: lymphocytosis> 5G/L, LDH>N and B symptoms. With less than 2 factors, the ORR and CRR of 70% of the patients were 80 and 60%. OS was better in transplanted population (30 months vs 65 m). 4 independent factors of OS were determined by a cox regression : LDH>N, PS> 2, MIB1 > mean value 25 and B symptoms. 4 prognostic groups were defined with a median OS respectively at 112 m (no factor), 55 m (1 f), 40 m (2 f) and 11 months (3-4 f). The MIPI score distinguished three groups as defined in literature (low, intermediate and high risk) with a median OS of respectively 75 m, 42 M and 20 m. *Conclusion*. The (R)VAD+C regimen offers a good efficacy/tolerability ratio before PBSCT for MCL treatment. It is especially relevant for 70% of patients which appear with few factors of response at diagnosis. Despite the relevance of the new MIPI score for these patients treated by a less toxic regimen than the RCHOP, we believe that the MIB1/KI67 could better qualify these survival prognosis factors.

0260

PROLONGED SURVIVAL IN REFRACTORY/RELAPSED MANTLE CELL LYMPHOMA FOLLOWING EARLY-INTENSIFIED CHEMOTHERAPY WITH MULTIPLE AUTOLOGOUS HEMATOPOIETIC STEM CELL SUPPORT

S.C. Cortelazzo,[1] M. Mian,[1] T.C. Tarella,[2] A. Rossi,[3] M.M. Magni,[4] A.M.G. Gianni,[4] P.C. Patti,[2] M.S. Mirto,[2] B.F. Benedetti,[2] P.G. Pizzolo,[2] R.A. Rambaldi[3]

[1]Division of Hematology and [2]BMT, BOLZANO; [3]Hematology, TURIN, Italy; BERGAMO, Italy; [4]Oncology INT, MILANO, Italy

Background.Patients (pts) with Mantle Cell Lymphoma (MCL) resistant/relapsed after front-line therapy, generally had a short survival even though they were treated with high-dose chemotherapy supported by autologous peripheral blood progenitor cell (PBPC) transplantation (ASCT). *Aims*. To evaluate if an early intensified chemotherapy (HDS) with or without rituximab with multiple PBPC support could improve the clinical outcome of this poor-risk subset of pts. *Methods*. A cohort of 41 MCL pts, median age 55 years (range 36-69), M/F 26/15, resistant (N=19) or relapsed (N=22) after anthracyclin or fludarabine containing regimens with or without rituximab is the subject of this intention to treat retrospective analysis. The adverse features at enrolment in this salvage program were: blastoid variant (17%), bulky disease (23%), BM involvement (73%), age>60 yrs (32%), B symptoms (17%), elevated LDH (35%), advanced stage (90%), poor ECOG-PS (10%), >1 extranodal sites (42%), IPI>2 (33%). After 2-3 cycles of cisplatin-containing chemotherapy, pts received high dose (hd) cyclophosphamide (CTX) 7g/sqm, and hd-Ara-C (2g/sqm every 12 hours for 6 days) with PBPC support. Following HDS a conditioning program with hd-melphalan (180 mg/sqm) supported by PBPC and/or hd-mitoxantrone plus hd-melphalan (60 and 180 mg/sqm) with ASCT was planned. Rituximab (375 mg/sqm) was given to 66% of pts for a total of 6 doses, twice after hd-CTX and hd-AraC, as *in vivo* purging before CD 34$^+$ cell harvest and twice after ASCT. *Results*. 73% of pts completed the planned program. All pts mobilized enough cells CD 34$^+$ to support hd Ara-C and hd-melphalan and ASCT after mitoxantrone+melphalan. Following HDS 62% of pts achieved a CR. At completion of treatment the CR rate was 71%, and TRM at 100 days was 7%. After a median follow-up of 44 months (range 10-123) relapse occurred in 10/29 remitters (34%). By Chi-square test only BM involvement was associated with relapse. The rate of 2nd CCR was 49 % and the 5-year estimate of OS, EFS and DFS was 51, 46 and 59%, respectively. It is noteworthy that resistant and relapsed pts were equally sensitive to HDS in term of CR rate (63.vs 77%) and duration (5 year DFS, 62 *vs* 70%). Pts who received rituximab had a superior CR rate (78 *vs* 57%) and duration (5 year DFS, 62 *vs* 42 %) even if the small number of pts did not allow the achievement of statistical significance. Cox multivariate analysis failed to identify within potential prognostic markers factors predictive for OS and EFS. *Conclusions*. The present study showed that poor risk pts with resistant/relapsed MCL may benefit of early intensified chemotherapy and multiple PBPC support. It should be investigated whether the implementation of the use of rituximab in induction and/or maintenance could improve these promising results or if other active agents might be considered.

0261

ANALYSIS OF OUTCOMES AND FOLLOW-UP IN NON-HODGKIN LYMPHOMA PATIENTS TREATED WITH 90Y-IBRITUMOMAB TIUXETAN FOR RECURRENT OR REFRACTORY DISEASE. DATA OBTAINED FROM RADIOIMMUNOTHERAPY REGISTRY

P.G. Giraldo,[1] J. Gómez Codina[2]

[1]H. U. Miguel Servet, ZARAGOZA, Spain; [2]H. U. La Fe, VALENCIA, Spain

Background. 90Y Ibritumomab tiuxetan (90Y-RIT) is approved for the treatment of refractory or recurrent non-Hodgkin follicular lymphoma (NH FL). *Aims.* To analyze retrospectively the aggregate data of NHFL patients, from the Spanish-Portugal registry of patients treated with 90Y-RIT in clinical practice setting in terms of effectiveness and tolerability in order to provide a pragmatic approach to experimental data. *Methods.* Effectiveness endpoints retrospectively studied were: objective response rate (ORR), time to progression (TTP) overall survival (OS) and safety. Clinical prognostic factors were collected to asses their possible influence upon treatment effectiveness, by multivariate analyses. *Results.* 169 p from 51 centres (treated since commercial availability in Spain and Portugal until August 2007) were registered: M/F 57.7%/42.3%; mean age 59.6 years (19-83); ECOG 0-1 79.7%; 113 had FL (66.9%) and 56 non-

FL (33.1%). ORR was 66.9% (95%CI: 59.8, 74.0). According to FLIPI, ORR was: LR: 86.8%; IR: 65.5%; HR: 58.6%. According to IPI, ORR was: LR: 52.2%; ILR: 57.1%; IHR: 44.4%; HR: 33.3%. Median follow-up time was 6.6 months, median TTP was 10.1 months (95% CI:6.8-13.3) and median OS has not been achieved, with an estimated OS at 1 and 2 years of 74.1% and 51.0% respectively. Intermediate and high FLIPI score were significantly associated with worse response rate and more risk for progression than lower score: Odds Ratio 0.29 (95%CI: 0.09-0.88) and 0.25 (95%CI: 0.08-0.77), respectively; Hazard Ratio 3.01 (95%CI: 1.36-6.66) and 4.33 (95% CI: 1.98-9.49), respectively. More than 2 previous treatments, was also related with a worse response rate for FL p: OR 2.49 (95% CI: 0.87-7.08). Safety analysis is summarized in Table 1.

Table 1. Safety analyses.

Haematological % (G3-4)	
▪ Anemia	56.2 (23.7)
▪ Neutropenia	62.7 (45.6)
▪ Leucopenia	67.5 (37.3)
▪ Thrombocitopenia	85.2 (50.3)
▪ Febrile Neutropenia	10.1 (10.1)
Non haematological toxicity % (G3-4)	
▪ Asthenia	36.1 (8.3)
Treatment/interventions required (%)	
▪ Hospitalization	26.6
▪ G-CSF	43.8
▪ Red blood cell transfusions	29.0
▪ Platelet transfusions	30.8

Conclusions. Despite the limitations of the retrospective design of the Registry, these results obtained with 90Y-RIT for lymphoma patients treated within the clinical practice setting are similar to that obtained in clinical trials. Updated data will be presented at the meeting.

0262

LONG TERM OUTCOMES OF PATIENTS WITH ADVANCED-STAGE CUTANEOUS T CELL LYMPHOMA AND LARGE CELL TRANSFORMATION

S. Arulogun,[1] H.M. Prince,[1] J. Ng,[2] S. Lade,[1] G.F. Ryan,[1] O. Blewitt,[1] C. McCormack[2]

[1]Peter MacCallum Cancer Centre, EAST MELBOURNE; [2]St Vincent's Hospital, MELBOURNE, Australia

Background. Cutaneous T cell lymphoma (CTCL) is a rare disease, and only a small number of long-term follow-up studies worldwide have been performed on large cohorts of patients. Although typically an indolent disease, approximately one-third of patients present with, or progress to, advanced-stage disease (stages IIB-IVB). *Aims and Methods*. We aimed to define the outcome of patients with CTCL and determine the risk of progressing to advanced-stage disease. A 31-year retrospective analysis of our cutaneous lymphoma database, containing 297 patients with mycosis fungoides (MF) and Sezary syndrome (SS), of whom 92 patients had advanced-stage disease (ASD) and 22 patients had large cell transformation (LCT), was undertaken to study long-term outcomes and identify clinical predictors of outcome. LCT was diagnosed if at least one skin biopsy showed a population of large cells that were greater than 4 times the size of small lymphocytes exceeding 25% of the total lymphoid infiltrate. *Results*. The median age at diagnosis of ASD was 60 years. Two-thirds of patients with ASD presented with *de novo* ASD; the remaining one-third had a preceding diagnosis of early-stage MF. The median follow up was 4.4 years (0.1-33 years) for the entire MF/SS population. The median overall survival (OS) for ASD was five years with a 10-year predicted OS of 32%. Age at initial diagnosis (p=0.01), tumour stage (p=0.01) and clinical stage (p=0.0003) were found to be significant predictors of outcome of ASD. Patients who presented with *de novo* advanced-stage MF/SS demonstrated a non-statistically significant trend to better outcomes than those with a prior diagnosis of early-stage MF. Large cell transformation was diagnosed in 22/297 MF/SS patients (7.4%), and present at the time of initial MF/SS diagnosis in 7/22 patients (32%). With respect to patients who progressed to LCT, the rate was only 1.4% in patients with early-stage disease, compared with stage IIB (27%) and stage IV (56%-67%) disease, with a median time from initial diagnosis of MF/SS to LCT of 2.3 years (0.1-29 years). At the time of diagnosis of LCT, patients had plaque lesions (4), tumour lesions (16) and erythroderma (2). The median OS following the diagnosis of LCT was 2 years. *Conclusions*. In this group of patients with long-term follow-up, we demonstrate that the incidence of LCT is strongly dependent on tumour stage at diagnosis and demonstrate a much lower overall risk of LCT than previously reported, which likely reflects the community-based referral pattern of our cutaneous lymphoma clinic.

0263

RESULTS FROM A PHASE II STUDY INVESTIGATING THE EFFICACY AND SAFETY OF LENALIDOMIDE ORAL MONOTHERAPY IN RELAPSED OR REFRACTORY INDOLENT NON- HODGKIN'S LYMPHOMA

H.W Peter,[1] J.M. Vose,[2] T.D. Moore,[3] C.B. Reeder,[4] C.E. Cole,[5] G. Justice,[6] H.P. Kaplan,[7] M. Voralia,[8] D. Pietronigro,[9] K. Takeshita,[9] A. Ervin-Haynes,[9] J.B. Zeldis,[9] T.E. Witzig[4]

[1]New York Medical Center, NEW YORK, USA; [2]University of Nebraska, OMAHA, NE, USA; [3]Mid Ohio Oncology/Hematology Inc., COLUMBUS, OH, USA; [4]Mayo Clinic, SCOTTSDALE, AZ, USA; [5]Gundersen Clinic, LA CROSSE, WI, USA; [6]Pacific Coast Hematology/Oncology Medical Group, FOUNTAIN VALLEY, CA, USA; [7]Swedish Cancer Institute, SEATTLE, WA, USA; [8]Saskatoon Cancer Center, SASKATOON, SASKATCHEWAN, Canada; [9]Celgene Corporation, SUMMIT, NJ, USA

Background. Indolent non-Hodgkin's lymphoma (NHL) has a median survival of 10 years but is rarely cured. Lenalidomide (Revlimid®), an immunomodulatory drug of the IMiDs® class, is approved by the FDA and EMEA in combination with dexamethasone, for treatment of multiple myeloma in patients who have received at least 1 prior therapy, and by the FDA as a monotherapy for the treatment of transfusion-dependent anemia in patients with myelodysplastic syndromes associated with a deletion 5q cytogenetic abnormality. Lenalidomide also has activity in chronic lymphocytic leukemia and cutaneous T-cell lymphoma. *Aims*. This study was designed to assess the efficacy and safety of oral lenalidomide monotherapy in patients with relapsed or refractory indolent NHL. *Methods.* Patients with relapsed or refractory indolent NHL with measurable disease after at least 1 prior treatment regimen were eligible. Patients received 25 mg lenalidomide orally once daily on Days 1-21 every 28 days and continued therapy for 52 weeks as tolerated or until disease progression. Response and progression were evaluated using the IWLRC methodology. *Results*. Forty-three patients were enrolled in the study and included in this analysis. The median age was 63 (range 43-89) years and 17 patients were female. Median time from diagnosis to lenalidomide treatment was 4.5 (0.4-24) years and the median number of prior treatment regimens was 3 (1-15). Histologies included small lymphocytic lymphoma [SLL] (n=18), follicular center lymphoma [FCL] grades 1-2 (n=22), nodal marginal B-cell lymphoma (n=2), and extranodal marginal zone B-cell lymphoma of MALT type (n=1). Eleven of the 43 patients (26%) exhibited an objective response, including 2 complete responses, 1 complete response unconfirmed, and 8 partial responses. Fifteen patients had stable disease, 13 had progressive disease and 4 were not evaluable. Responses included 4/18 SLL patients (22%) and 7/22 FCL (32%) patients. Median time to response was 3.6 (range 1.7-4.1) months. Median progression free survival was 4.6 months for all patients, and at least 7.7 (range 4.4->13.5) months for responding patients and still ongoing. Sixteen patients (37%) required at least one dose reduction with a median time to first dose reduction of 2 (0.5-9.6) months. The most common grade 4 adverse event was neutropenia (14%), and the most common grade 3 adverse events were neutropenia (21%), and thrombocytopenia (12%). *Summary/conclusions*. Lenalidomide oral monotherapy is active in relapsed or refractory indolent NHL, resulting in a response in 26% of patients, with manageable side effects.

0264

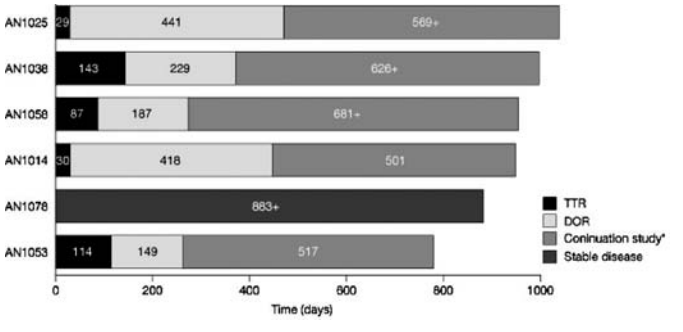
VORINOSTAT PROVIDES PROLONGED SAFETY AND CLINICAL BENEFIT TO PATIENTS WITH ADVANCED CUTANEOUS T-CELL LYMPHOMA (CTCL)

M. Duvic,[1] E.A. Olsen,[2] D. Breneman,[3] T.R. Pacheco,[4] S. Parker,[5] E.C. Vonderheid,[6] J.L. Ricker,[7] S. Rizvi,[7] C. Chen,[7] K. Boileau,[7] A. Gunchenko,[7] C. Sanz-Rodriguez,[7] L.J. Geskin[8]

[1]MD Anderson Cancer Center, HOUSTON; [2]Duke University, DURHAM; [3]University of Cincinnati Medical Center, CINCINNATI; [4]University of Colorado Health Sciences Center at Fitzsimons, AURORA; [5]Emory University, ATLANTA; [6]Johns Hopkins University, BALTIMORE; [7]Merck Research Laboratories, UPPER GWYNEDD; [8]University of Pittsburgh, PITTSBURGH, USA

Background. Vorinostat, an orally active histone deacetylase inhibitor, was approved in October 2006 by the FDA for the treatment of the cutaneous manifestations of CTCL in patients with progressive, persistent or recurrent disease on or following two prior systemic therapies. *Aims*.

To evaluate the activity and safety of at least 2 years' treatment with vorinostat in patients with advanced CTCL. *Methods*. Patients with stage ≥IB CTCL who had received at least two prior systemic therapies and recovered from all prior treatment-related toxicities were eligible for inclusion in an open-label, multicenter Phase IIb trial. All patients provided written informed consent before enrollment. Patients were treated with oral vorinostat 400 mg once daily for 7 days per week and remained on study medication until intolerable toxicity or progressive disease (PD). The primary endpoint was objective response rate measured by the severity weighted assessment tool. Secondary endpoints included time to response and duration of response. Safety and tolerability were also evaluated. Patients could continue to receive vorinostat in an extension study after the time of data cut-off. This post hoc subset analysis reports results from patients who had received vorinostat therapy for ≥2 years as of November 1, 2007. *Results*, Six of 74 patients enrolled in the original study had received vorinostat therapy for ≥2 years as of November 1, 2007 (Figure 1). This includes five responders (one complete responder, four partial responders) and one patient with prolonged stable disease. The median age was 65 years (range 57-74), the median number of prior therapies was 2.5 and the median time from diagnosis of CTCL to enrollment was 1.8 years (range 0.7-5.9). The most common drug-related adverse events (AEs) as determined by the study investigator were diarrhea (100%), nausea (83%), fatigue (67%), and alopecia (50%). One patient had a serious AE of pulmonary embolism, which resolved in 7 days and this patient is continuing therapy as of Day 955. One patient who experienced serious AEs of increased creatinine phosphokinase (CPK) [Day 490] and rash (Day 645) remained on therapy until Day 948. The only other grade ≥3 AEs were anorexia (n=1) and thrombocytopenia (n=1). One patient discontinued due to PD, one patient discontinued due to PD, rash, and increased CPK, and four are continuing therapy as of November 1, 2007 (one complete responder, two partial responders and one with stable disease). Three of the four patients continuing therapy had baseline CTCL stage IIB, while the fourth patient had stage IVA CTCL at study entry. Updated data on these patients will be presented. *Conclusions*. Vorinostat is a novel anticancer agent that has demonstrated prolonged safety and clinical benefit in these patients with advanced CTCL.



*During the follow-up study, patients who benefited from vorinostad continued on theraphy with ongoing clinical responses without formal mSwat assessment.

Figure 1. Time on study of patients on vorinostat therapy for ≥2 years (available data as of November 1,2007)

0265

FIRST-LINE TREATMENT WITH RITUXIMAB COMBINED WITH INTRAVENOUS OR ORAL FLUDARABINE FOR PATIENTS WITH EXTRANODAL MUCOSA ASSOCIATED LYMPHOID TISSUE (MALT) LYMPHOMA

A. Salar,[1] E. Domingo-Domenech,[2] C. Estany,[3] M.A. Canales,[4] C. Montalban[5]

[1]Hospital del Mar, BARCELONA; [2]ICO-Bellvitge, BARCELONA; [3]Mutua de Terrasa, TERRASA; [4]Hospital La Paz, MADRID; [5]Hospital Ramón y Cajal, MADRID, Spain

Background. Fludarabine as a single agent has shown activity in MALT lymphoma. The addition of rituximab to other drugs has improved outcomes in several types of NHL without a significant addition of toxicity. Our aim was to evaluate the safety and efficacy of rituximab combined with fludarabine in first-line therapy for extranodal MALT lymphoma. We also explore whether an early response evaluation could allow us a reduction from 6 to 4 cycles of treatment. *Methods*. This study enrolled adult patients with untreated extranodal MALT lymphoma who were candidate to receive systemic treatment. Patients received rituximab 375 mg/m^2 intravenously (IV) on day 1 and fludarabine 25 mg/m^2 (IV) given on days 1-5 (days 1-3 in > 60 years), every 4 weeks; after the first cycle, oral fludarabine was allowed to be given orally at 40 mg/m^2 with the same schedule. After three cycles, a work-up was done. Patients in CR received an additional cycle and, if PR, a total of 6 cycles was recommended. *Results*. 22 patients have started on therapy. Characteristics of the first 18 pts: median age: 59 years; 7 male, 11 female; PS 0 (94%); site of origin: stomach (61%), skin (16%), lung (11%), parotid gland (11%); stage: I (66%), II (16%) and IV (16%). A total of 82 cycles of R-F were administered; 2 pts received 2 cycles, 9 pts 4 cycles, 7 pts 6 cycles. 17 pts are evaluable for response. Overall response rate was 100% with 94% achieving CR. In the pt in PR, symptoms resolved and received no further treatment being in PR at last follow-up. One pt with parotid gland involvement at dx relapsed at 6 m from the end of treatment in two unaffected areas at dx (breast and bone marrow). Median follow-up from starting treatment is 15 m (range: 17-27). PFS rate is 93% (CI95%:79-100%) at 12 m and OS rate is 100% at 12 m. Tolerance to oral fludarabine was excellent with most patients preferring this formulation. Mild neutropenia was the most common toxicity, usually presenting after the third cycle. 1 pt developed a prolonged grade 4 neutropenia after the 6th cycle. No blood transfusions were required. 3 pts developed grade 2 respiratory infection, but none pt had to be admitted. *Conclusions*. These preliminary data indicate that the RF regimen, either with intravenous or oral fludarabine, was well tolerated even in elderly patients. This combination is very active for the treatment of untreated extranodal MALT lymphoma, even with fewer cycles than initially planned. Updated data will be presented.

0266

PRIMARY EXTRANODAL FOLLICULAR LYMPHOMA OF HEAD AND NECK (PEFLHN): A RARE DISEASE WITH A POOR CLINICAL OUTCOME (IELSG 23)

S.C. Cortelazzo,[1] M. Mian,[1] R. Tsang,[2] A. Rossi,[2] M.E. Cabrera,[2] F.M. Federico,[2] Z.E. Zucca,[2] M. Busetto,[2] R.A. Rambaldi,[2] C.F. Cavalli[2]

[1]Division of Hematology and BMT, BOLZANO, Italy; [2]TORONTO, Canada

Background. PFLHN is a rare disease whose clinical characteristics, treatment and outcome are scarcely known. *Aims.* To compare the clinical features and outcome of PFLHN patients (pts) with those of most frequent and well known primary extranodal MALT of head and neck (PEMLHN). *Methods*. From May 1986 to January 2007, 72 MALT and 44 FL were referred to 7 international cancer centers. The main site of presentation in MALT was the parotid and salivary glands (49%) followed by the Waldeyer's ring (32%), whereas in the FL-group prevailed the location of Waldayer's ring (58%) and less frequently parotid and salivary glands (27%). Both groups had comparable median age (60 yrs),age >60 years (53 *vs* 50%) and a low frequency of B symptoms (6 *vs* 5%), However, more PFLHN pts had the following: advanced stage (61 *vs* 42 %), elevated LDH (19 *vs* 2%), poor ECOG-PS (7 *vs* 3%), bulky disease (7 *vs* 3%) and MIPI>1 (49 *vs*30%). Regarding therapy more PEMLHN pts received radiotherapy in comparison with PFLHN (79 *vs* 54%; p=0.002), alone (16 *vs* 3) or with anthracyclin-containing chemotherapy (10 *vs* 6) and/or surgery (10 *vs* 15). *Results*. 93% of pts in both groups achieved a complete remission, 4.4 % a partial remission and 3% were resistant to therapy. Acute toxicity (G2-3) mostly consisted of xerostomia and none died of treatment-related mortality. Among 106 responders, 36 pts eventually relapsed (MALT 30%; FL 39%). More than sixty percent of relapses occurred in sites different from presentation. After a median follow-up of 44 months (range 2-152) the 1st CCR-rate was 71% in the MALT *vs* 61% in the FL pts. Forty-three per cent of PEMLH died of disease *vs* 61% in PFLHN. The projected 5-year estimate of OS, EFS, and DFS in MALT was 81%, 56% and 65% compared to 62%, 44% and 51% in FL pts, without plateau in both groups. Cox multivariate analysis failed to identify within potential prognostic markers factors predictive for OS and EFS. *Conclusions*. The present study showed that pts with primary indolent lymphoma of head and neck had an high relapse rate comparable to that of cases with advanced disease, without any evidence of cure. Moreover. PFLHN pts had more adverse features at presentation and a worse outcome than PEMLHN. The less intensive use of radiotherapy in FL pts could in part explain the different relapse rate in the two groups. These results suggest that a combined treatment including radiotherapy could improve the outcome of pts affected by this rare disease.

0267

A PHASE II INTERNATIONAL, MULTICENTER STUDY OF ORAL PANOBINOSTAT (LBH589) IN PATIENTS WITH REFRACTORY CUTANEOUS T-CELL LYMPHOMA (CTCL)

M.G. Bernengo,[1] F. Vanaclocha,[2] M. Duvic,[3] T. Kuzel,[4] F. Kerdel,[5] L. Pinter-Brown,[6] A. Bosly,[7] C. Okada,[8] D. Breneman,[9] P.L. Zinzani,[10] J. Becker,[11] L. Hughey,[12] M. Ardaiz,[13] J. Zain,[14] L. Zhang,[15] S. Hirawat,[15] G. Laird,[15] D. Johnson,[15] H.M. Prince[16]

[1]San Lazzaro University of Turin, TURIN, Italy; [2]Hospital 12 de Octobre, MADRID, Spain; [3]MD Anderson Cancer Center, HOUSTON, TX, USA; [4]Robert H. Lurie Comprehensive Cancer Center, CHICAGO, IL, USA; [5]Florida Academic Dermatology Centers, MIAMI, FL, USA; [6]UCLA Medical Center, School of Medicine, LOS ANGELES, CA, USA; [7]UCL Mont Godinne, YVOIR, Belgium; [8]Oregon Health and Science University, PORTLAND, OR, USA; [9]University Dermatology Consultants, CINCINNATI, OH, USA; [10]University of Bologna, BOLOGNA, Italy; [11]Klinik der Universitat Wuerzburg, WUERZBURG, Germany; [12]University of Alabama at Birmingham, BIRMINGHAM, AL, USA; [13]Hospital J.M. Ramos Mejia, BUENOS AIRES, Argentina; [14]Columbia University, NEW YORK, NY, USA; [15]Novartis Pharmaceutical Corp., FLORHAM PARK, NJ, USA; [16]Peter MacCallum Cancer Centre, MELBOURNE, Australia

Background. Panobinostat (LBH589) is a potent deacetylase inhibitor (DACi) which induces apoptosis of tumor cells at nanomolar levels. Prince *et al*. noted encouraging panobinostat activity in patients with CTCL (*ASCO, 2007*). This phase II study seeks to confirm the efficacy in refractory CTCL patient population. *Methods*. This open label study is enrolling patients with stage IB-IVA CTCL from ~40 sites globally. Eligibility criteria include diagnosis of mycosis fungoides (MF) or Sezary syndrome (SS), treatment with ≥2 prior systemic regimens, adequate organ function and ECOG PS ≤2. Patients are excluded for significant cardiovascular abnormalities or QTcF >450 msec. Patients receive oral panobinostat on days 1, 3, and 5 weekly in 28 day cycles until disease progression or intolerance. Patients are being accrued to two groups. Group 1 includes patients previously treated with oral bexarotene, while group 2 are patients who are bexarotene naïve. Each group follows a Simon 2-stage design independently. The primary endpoint is response rate measured using a composite score. Intensive ECG monitoring for QTcF is incorporated in the study. *Results*. Sixty patients (group 1 - 36; Group 2 - 24) have been treated to date (median age 57 years [range 25-88]; 36 males, 24 females; 46 MF, 14 SS). Median number of prior treatment regimens is 4.5 for group 1, and 3 for group 2. Twenty-five patients (69.4%) in group 1 and 19 patients (79.2%) in group 2 had ?stage IIB disease at study entry. Patients have received 1-14+ cycles of treatment. Efficacy was evaluated by the composite score (modified Severity-Weighted Assessment Tool [mSWAT] to assess skin disease, and CT scan for evaluation of visceral and lymph node involvement, and circulating SS counts/flow cytometry of peripheral blood mononuclear cells for SS patients). In group 1, 3 of 27 patients with at least 2 post-baseline efficacy assessments have achieved an overall partial response, and 7 patients have achieved a skin response, including 1 complete response, assessed by mSWAT. In group 2, 2 of 15 patients with at least 2 post-baseline efficacy assessments have achieved a skin response. With most patients enrolled recently, many patients in both groups have not yet had disease evaluation for response. Twelve patients have progressed on treatment and ten patients discontinued treatment due to AEs. There has been no treatment-related death. The most common adverse events (all grades, regardless of drug causality) have been diarrhea (~53.3%), thrombocytopenia (~37%), nausea (~32%), fatigue (~27%), pruritus (~23%), asthenia, abdominal pain, and blood triglycerides increased (~20% each), headache (~18%), neutropenia, hyperglycaemia, and dysgeusia (~17% each). The most common Grade 3 or 4 adverse events (regardless of drug causality) have been neutropenia (~8%), thrombocytopenia (~8%), hypophosphataemia (5%), diarrhea (5%), and hyperglycaemia (5%), Among 2838 ECGs analyzed thus far, there has been no ECG with QTcF >500ms, two with QTcF >480ms, and three with QTcF >60ms increase from baseline. *Conclusions*. Encouraging activity is being observed in this ongoing study. Panobinostat has been generally well tolerated with no major safety concerns. Enrollment to the study is currently ongoing for both groups.

0268

90Y-IBRITUMOMAB TIUXETAN TREATMENT FOR RELAPSED AND/OR REFRACTORY B CELL NON-HODGKIN`S LYMPHOMA. MULTI-INSTITUTIONAL ARGENTINIAN STUDY

R. Cacchione,[1] J. Milone,[2] E. Nucifora,[2] J. Bordone,[2] J. Dupont,[1] S. Rudoy,[3] M. Brown,[4] D. Lafalce,[5] L. Palmer,[6] D. Argentieri,[7] P. Negri,[8] M. Bartomioli,[9] G. Milone,[10] A. Trouboul,[11] M. Ardaiz,[12] M. Iabstrebner,[13] G. Pombo,[14] A. Basso,[15] A. Diaz,[16] R. Tur,[17] G. Saidon,[18] H. Krupitzki,[1] G. Garay,[1] D. Riveros,[1] A. Carrasco,[19] C. Chiattone,[20] F. Bezares[16]

[1]CEMIC, BUENOS AIRES, Argentina; [2]Hospital Italiano, DE LA PLATA, Argentina; [3]Instituto Santogiani, BUENOS AIRES, Argentina; [4]Sanatorio Parque Rosario, SANTA FE, Argentina; [5]Hospital Tornu, BUENOS AIRES, Argentina; [6]Hospital Churruca, BUENOS AIRES, Argentina; [7]Clinica Junin, PROV BUENOS AIRES, Argentina; [8]Hospital Provincial, PARANA, Argentina; [9]Clinica Bahia Blanca, BUENOS AIRES, Argentina; [10]FUNDALEU, BUENOS AIRES, Argentina; [11]Clinica Chivilcoy, BUENOS AIRES, Argentina; [12]Hospital Ramos Mejia, BUENOS AIRES, Argentina; [13]OSECAC, BUENOS AIRES, Argentina; [14]Favaloro, BUENOS AIRES, Argentina; [15]Rosario, SANTA FE, Argentina; [16]Hospital Alvarez, BUENOS AIRES, Argentina; [17]La Plata, PROV BUENOS AIRES, Argentina; [18]Capital, BUENOS AIRES, Argentina; [19]CLARO, MEXICO CITY, Mexico; [20]Hospital Santa Casa, SAO PAULO, Brazil

Background. 90Y-ibritumomab tiuxetan (Zevamab® ; ZEV; Bayer-Schering Pharma- Argentina) has been approved for the treatment of relapsed, refractory and transformed (high grade) follicular lymphomas. *Methods*. Between Sep. 2005 and Feb. 2008, 45 patients (pts) [22 F & 23 M; median age 62 yrs (45-83)] with refractory/relapsed lymphoma were enrolled. Diagnoses: 37 follicular lymphoma (FL), 5 were mantle cell lymphoma (ML) and 3 transformed lymphoma (TL); 18 pts had bulky disease, 8 had bone marrow involvement and 21 had stage III-IV disease. Median time from diagnosis was 5 yrs (0.5-29). Twenty two pts had received 1-2 previous treatments, and 23 pts had received 3-5 previous treatments including 5 pts with autologous bone marrow transplantation. All had previously received anti-CD20 monoclonal antibody therapy. Six pts received previous radiotherapy. ZEV was administered at 0.3 or 0.4 mCi, based on initial platelet count. Seven days before, and the same day of the ZEV administration, pts received standard rituximab 250 mg/m^2. In 3 pt, ZEV was part of the conditioning regimen of autologous bone marrow transplantation. *Results*. Forty pts were evaluable for response: 34 (86%) pts responded - 19 CR (48%), 15 PR (38%). Overall survival and PFS for the entire group at 18 months was 63% and 37% respectively, with a median follow-up of 12 months (1-29 months). Of the 45 patients, 5 pts (11%) had died before third month and response was not assessed, 6 pts (13%) did not respond, 3 (7%) died with response from other causes, 14 pts (31%) responded and subsequently relapsed. Finally 17 pts (38%) continue to be in response, 9 (20%) lasting more than twelve months (long lasting responders). Slight differences in duration of response and survival were observed between FL vs ML and TL favouring FL (RR 2.047). Forty six per cent of pts required filgrastim for neutropenia, 24% required platelet transfusions, 22% had neutropenia plus fever and were admitted for complicated pancytopenia, and 20% required red blood cells transfusion. Two patient died 30 & 40 days after treatment with hypoplastic bone marrow complicated with sepsis in the post autologous bone marrow transplantation period. Four pts with previous bone marrow transplantation required filgrastim, transfusions and 2/3 had febrile neutropenia. *Conclusions*. Our experience with ZEV in relapsed and refractory FL shows 48 % CR. Even heavily treated pts that had previous bone marrow transplantation were able to receive ZEV, although they required extra support. Our experience supports the use of ZEV in relapsed and refractory lymphomas even after autologous bone marrow transplantation.

0269

BORTEZOMIB AND INTERFERON IN ASSOCIATION IN THE TREATMENT OF ADVANCED OR REFRACTORY SÉZARY SINDROME: A SINGLE CENTER EXPERIENCE

M. Postorino,[1] L. Pupo,[1] L. Di Caprio,[1] L. Franceschini,[1] S. Campagna,[1] D. Renzi,[1] M. Rizzo,[1] L. Gianni,[1] S. Faccia,[1] R. Cannarsa,[1] M. Ales,[1] F. Buccisano,[1] D. Venditti,[1] G. Lombardo,[2] G. Baliva,[2] M. Cantonetti,[1] S. Amadori[1]

[1]Tor Vergata University, ROMA; [2]Istituto Dermatopatico dell'Immacolata, ROMA, Italy

Background. Sézary Sindrome (SS) is a chronic, lymphoprolipherative cutaneus disease. The conventional treatments as photoapheresis, alpha interferon, polichemoterapy and immunotherapy with monoclonal antibodies can induce a short partial remission with decreasing of tumor burden. The most recent data demonstrated a possible role of allogenic hemopoietic stem cells transplantation to obtain a continuous complete remission. The majority of patients with advanced or refractory disease receive palliative cares due to age, performance status and matched donor availability. The mechanisms responsible for the resistance of Sézary cells to a wide range of death-inducing agents remain largely unknown. Recently, the antitumor effects of proteasome inhibitors, mostly bortezomib, have been reported in different types of cancer. Several lines of evidence indicate that proapoptotic and antiproliferative effects of bortezomib on cancer cells result from an inhibition of the NFk-B pathway; this effect is enhanced by use of Interferon alfa (INF). *Aims.* The evaluation of efficacy and toxicity of a long-term treatment with bortezomib associated with alfa IFN is the main aim of our study. *Methods*. From November 2006 to dicember 2007 Six patients with SS were enrolled in our protocol: they were treated with bortezomib at the dose of 1.3 mg/m^2 weekly and Interferon 3x10^{6}UI twice a week for 12 weeks(induction phase).The patients, who achieved a complete remission,received the same dose of bortezomib every 2-3 weeks and continued IFN therapy(maintenance phase). In case of near Complete Remission (nCR: absence of cutaneous symptoms and very low count of Sezary cell) or good partial response (gPR: absence of cutaneous symptoms and reduction >60 % of circulating Sezary's cells) the bortezomib and Interferon dose remained weekly until the disease progression. Median age of patients was 53 years (range 43-67). All patients were resistant to first-line-therapy and to Interferon. At the enrolment, one patient showed hyperleukocytosis (WBC > 50000/uL) and unfavourable cytogenetic. Two patients, with matched donor, received the therapy to reduce the cutaneous and circulating Sezary's cells before the conditioning treatment; the other patients were candidated to long-term therapy. *Results*. All patients responded to the bortezomib-IFN association: two patients were in CR, at 3 and 16 months after the induction therapy and now they are in maintenance. Two patients were in nCR and one was in gPR at 14, 11 and 12 months respectively (one of the patients in nCR is going to allogeneic SCT). The patient with hyperleukocytosis obtained a partial remission but after 2 months he relapsed. All responder patients showed an important restore of normal clones of T lymphocytes and nobody presented infectious diseases. The protocol was well tolerated and any toxicity has been experienced. *Conclusions*. The association bortezomib-IFN seems effective and safe in reducing tumor burden in advanced SS patients. A longer follow up and a higher number of treated patients are requested for the response evaluation.

0270

PHASE I TRIAL OF ORAL VORINOSTAT IN COMBINATION WITH BEXAROTENE IN ADVANCED CUTANEOUS T-CELL LYMPHOMA

R. Dummer,[1] K. Hymes,[2] W. Sterry,[3] M. Steinhoff,[3] C. Assaf,[3] H. Kerl,[4] J. Ahern,[5] S. Rizvi,[5] J.L. Ricker,[5] S. Whittaker[6]

[1]Universitätsspital Zürich - Dermatologische Klinik, ZÜRICH, Switzerland; [2]NYU Medical Center, NEW YORK, USA; [3]Campus Charité Mitte des Universitätsklinikums Charité, BERLIN, Germany; [4]University Clinic of Dermatology, GRAZ, Austria; [5]Merck Research Laboratories, UPPER GWYNEDD, USA; [6]St John's Institute of Dermatology, LONDON, UK

Background The histone deacetylase inhibitor vorinostat and the RXR-selective retinoid bexarotene are each clinically active and safe as monotherapy in the treatment of cutaneous T-cell lymphoma. *Aims.* This study was performed to test whether the combination of vorinostat and bexarotene could be safely administered to patients with advanced cutaneous T-cell lymphoma. *Methods*. A Phase I trial of vorinostat (200, 300, or 400 mg daily) in combination with bexarotene (150, 225, or 300 mg/m^2 or 150 mg, daily) was conducted. Cycles were repeated every 28 days for ≤ 6 cycles until progressive disease or intolerable toxicity. Adult patients with stage ≥IB progressive, persistent, or recurrent cutaneous T-cell lymphoma refractory to ≥1 systemic therapy were eligible. Patients must not have received prior bexarotene within 3 months or prior histone deacetylase inhibitor treatment and must have adequate hematologic, hepatic, and renal function. The primary objective was to determine the maximum tolerated dose. Activity and safety of the combination regimen were also assessed. *Results*. Nineteen patients have been enrolled: median age, 58 years (range 33-77), median number of prior systemic therapies, 3 (range 1-14). Eighteen patients have received ≥1 dose and were evaluable for safety as of 11/1/07. Two patients (Cohort 2) experienced dose-limiting toxicity (Table 1).

Table 1.

Cohort	Vorinostat (mg)	Bexarotene	N	CTCL Stage	# of Cycles	Dose limiting toxicities	Best Response
1	200	150*	3	IB, IVA (2)	2, 5, 6	-	PD, SD (2)
2	300	150*	5	IIB (3), IVA, IVB	1, 1, 2, 3, 6	Grade 3 fatigue,Grade 3 hypertriglyceridemia (3)	PR, SD (4)
2a	200	225*	3	IIB, IVA (2)	3, 4, 6	-	CR, SD (2)
2b	200	300*	3	III, IVA (2)	6, 6, 6	-	PR, SD (2)
3	400	150†	5	IB, IIB (2), III, IVA	1, 1, 2, 5, 6	-	PR, SD (2), PD, NE

NE = not evaluable; PD = progressive disease; PR = partial response; SD = stable disease. *mg/m^2/day; †mg/day

The maximum tolerated dose has not been reached. The most common drug-related toxicities were hypertriglyceridemia (50%), hypercholesterolemia (28%), hypothyroidism (28%), and lethargy/fatigue (28%). Grade 3 drug-related adverse events included hypertryglyceridemia (17%) and neutropenia (11%). Two patients had drug-related serious adverse events (skin necrosis; lymphangitis and lymph node abscess) and both recovered. Twelve patients have discontinued treatment: only 2 due to adverse events (skin necrosis, fatigue). Preliminary data indicate that of 18 evaluable patients, 1 had a complete response, 3 had partial responses, 12 had stable disease, and 2 had progressive disease. *Conclusions*. Accrual continues to determine the maximum tolerated dose. The combination of vorinostat and bexarotene is tolerable and active in patients with advanced cutaneous T-cell lymphoma.

0271

RITUXIMAB MAINTENANCE SIGNIFICANTLY PROLONGS TIME TO PROGRESSION IN PATIENTS WITH RELAPSED FOLLICULAR LYMPHOMA AND ALSO ACHIEVES A LONG TERM DISEASE CONTROL IN *DE NOVO* FOLLICULAR LYMPHOMA

A. Salar, S. Saumell, C. Pedro, A. Alvarez-Larrán, E. Gimeno, E. Abella, T. Gimenez, A. Ferrer, B. Bellosillo, C. Beses

Hospital del Mar, BARCELONA, Spain

Background. Rituximab (R) maintenance has recently demonstrated survival improvement compared with observation in patients with relapsed or refractory follicular lymphoma (FL). The aim of this study was to retrospectively evaluate our experience with rituximab maintenance in *de novo* or relapsed FL. *Methods*. We have retrospectively evaluated 42 patients with FL who received R maintenance. Only patients in CR or PR were candidates for R maintenance. Treatment consisted of R 375 mg/m^2/week x 4 consecutive weeks every 6 months during 2 years (Maintenance type 1) or R 375 mg/m^2 every 3 months during 2 years (Maintenance type 2). Overall and progression free survival were calculated from the day of the first R infusion. Time to progression after R maintenance was compared to the time to progression of the immediately previous treatment between the same patients, using the non parametric Wilcoxon test. *Results* Forty patients were included, with 2 pts receiving rituximab maintenance in two different episodes. Median age: 64 years (range: 27-85); male 18 (43%). First line R in 24 pts (57%) and in second or third relapse in 18 pts (43%). Number of previous treatments: 1 in 24 pts (57%), 2 in 14 (33%) and 3 in 4 (10%). Immediately previous treatment: CVP±R in 5 pts (12%), CHOP± R 14 (33%), fludarabing-containing±R 9 (21%), R 5 (12%) and others±R 9 (21%). Status previous starting maintenance: CR in 36 (86%), PR in 6 (14%). Among pts in CR, 5 progressed and 1 died (pneumonia + progressive disease) during maintenance. Among pts in PR, 2 progressed and 4 pts converted to CR during maintenance. At a median follow-up since first R maintenance infusion of 38 months (1-70), 13 pts (31%) have relapsed. Overall and progression free survival at 3 years were 94% (CI95% 87-100%) and 69% (CI95% 62-76%), respectively. No differences were found with respect to: status previous maintenance, line of therapy (first vs subsequent lines) and type of maintenance administered. From 18 pts with relapsed FL, median time to progression from the immediately previous chemotherapy was 25 months vs 36 months after maintenance R (p= 0.017). Maintenance R therapy was prematurely withdrawn

in 2 pts (recurrent infections and delayed severe neytropenia). Hipogammaglobulinemia was present in 47% and 55 % of pts before and after R maintenance, respectively. Levels of IgM were significantly lower after R maintenance (p=0.03) but levels of IgG and IgA were similar. *Conclusions*. Time to progression after rituximab maintenance is significantly increased compared with the time to progression of the immediately previous treatment in patients with relapsed FL. Rituximab maintenance is safe either in first or subsequent lines of therapy.

0272

RITUXIMAB MAINTENANCE THERAPY CAN BE SAFELY ADMINISTERED VIA A RAPID INFUSION PROTOCOL: RESULTS FROM THE MAXIMA STUDY

M. Wenger,[1] F. De Marco,[2] U. Vitolo,[3] I. Poddubnaya,[4] D. Chamone,[5] P. Warburton,[6] B. Jezersek Novakovic,[7] J.M. Rowe,[8] H. Dresler,[9] D. Thurley[10]

[1]Roche, BASEL, Switzerland; [2]Divisione Universitaria di Ematologia, Cattedra di Ematologia, TORINO, Italy; [3]Divisione Ospedaliera di Ematologia A.O. S. Giovanni Battista, TORINO, Italy; [4]Cancer Research Centre of RAMS, MOSCOW, Russian Federation; [5]Hematology Department of the Clinic Hospital of the University of São Paulo, SÃO PAULO, Brazil; [6]Wollongong Hospital, WOLLONGONG, Australia; [7]Institute of Oncology Ljubljana, LJUBLJANA, Slovenia; [8]Department of Hematology & Bone Marrow Transplatation, RAMBAM Health Care Campus, HAIFA, Israel; [9]Roche Pharmaceuticals (Israel) Ltd, PETACH TIKVA, Israel; [10]Roche Products Pty. Limited, DEE WHY, Australia

Background. The benefit of maintenance therapy in follicular lymphoma (FL) has been reported recently by various groups. Sehn *et al.* (Blood 2007) have demonstrated that rituximab (R) can be administered via a rapid-infusion protocol. *Aims*. The current study is intended to extend the safety database for R-maintenance and to examine the safety of fast infusion protocols in a real-life setting. *Methods*. 500 patients with first-line or relapsed FL having achieved CR/PR after R-containing induction therapy were eligible to receive R at the standard dose for follicular lymphoma of 375 mg/m^2 every eight weeks for a maximum of 2 years. Primary endpoint is safety, with secondary endpoints being PFS, EFS, TTNL, and OS. The study aims to detect at least one rare event with a true incidence of 0.32% with 80% power. *Results*. 349 patients with FL have been enrolled at clinical cut-off: Median age is 56, 76% were treated in first remission, and 74% were in CR. Data on a total of 809 infusions was available. Except for one patient with preexisting history of recurrent cerebrovascular incidents who received rituximab at standard infusion speed and suffered from a TIA no SAEs were recorded within 24h of the maintenance infusion. Of all patients available for analysis, 82.5% used a standard-speed infusion regimen (approx 4-6h) for the first maintenance infusion. At one year of maintenance treatment, 54% of patients were receiving R via a rapid-infusion regime (approx 1.5h). A total of 9 SAEs were recorded in the 275 patients that received at least one infusion, all but one were considered unrelated. One patient with previously known cardiac arrhythmias died 13 days after the 4th infusion of unknown causes. After clinical cut-off, three other patients died of progressive lymphoma. Hematologic toxicity occurred in 9 patients, with three grade 3/4 events (neutropenia), one resulting in febrile neutropenia. Recruitment will be completed in March 2008. *Conclusions*. R-maintenance q 8 weeks in FL after R-containing induction therapy can be safely administered. We could reproduce the finding of others that rapid-infusion regimens are no worse than standard speed infusions.

0273

A PILOT STUDY WITH RITUXIMAB, BORTEZOMIB AND HYPER-FRACTIONATED CYCLOPHOSPHAMIDE (RBC REGIMEN) FOR THE TREATMENT OF ADVANCED MANTLE CELL LYMPHOMA IN ELDERLY PATIENTS

P.M. Musto,[1] R. Guariglia,[1] G. Pietrantuono,[1] O. Villani,[1] M.C. Martorelli,[1] F. D'Auria,[1] A. Zonno,[1] A. Zupa,[1] G. Bianchino,[1] G. Improta,[1] V. Grieco,[1] L. Digiovannantonio,[1] E. Feudale,[1] R. Lerose,[1] A. Pinto,[2] F. Ferrara[3]

[1]CROB, Centro Riferimento Oncologico Basilicata, RIONERO IN VULTURE; [2]Istituto Nazionale Tumori, Fondazione Pascale, NAPOLI, Italy; [3]Cardarelli Hospital, NAPOLI, Italy

Background. Mantle-cell lymphoma (MCL) represents a distinct clinicopathologic entity which accounts for 3-10% of all non-Hodgkin lymphomas and whose median overall survival generally does not exceed 3-4 years. Patients with MCL are typically older adults with a male predominance and usually present with stage IV disease. The neoplastic phenotype is characterized by CD20 and CD5 co-expression, while CD23 surface antigen is lacking. Translocation t(11;14)(q13;q32) and cyclin D1 overexpression are the cytogenetic and molecular hallmarks of the disease. Rituximab is a key component of the current, most diffused regimens for the treatment of MCL (in particular, in R-CHOP or R-HyperC-VAD). Autologous stem cell transplantation or observation after induction therapy may be indicated, depending on patient eligibility. However, considering that MCL is frequently diagnosed in elderly subjects with relevant co-morbidities, high dose chemotherapy or the use of drugs with potential cardiotoxicity, such as anthracyclines, may result not feasible in a significant proportion of patients. In this setting, recent data suggest that the proteasome inhibitor bortezomib is well tolerated and has significant single-agent activity in patients with MCL. *Methods*. We evaluated safety and efficacy of a 21-day cycle, anthracycline-free combination of rituximab (375 mg/m^2 d 1), bortezomib (1.3 mg/m^2 d 1, 4, 8, and 11) and hyper-fractionated cyclophosphamide (600 mg/sqm given as a double, three-hour infusion d 1-3) (RBC regimen) in elderly patients with advanced MCL. Diagnosis was made according to standard histological, phenotypic and molecular criteria. So far, 14 patients (9 males, 5 females) have entered the study. Mean age was 77.2 years (range 72-84). All patients had stage IV disease, evidencing extranodal localizations (n. 4) or marrow/leukemic involvement (n. 10). IPI score was 2 in 5 patients, 3 in 7 patients and 4 in 2 patient. Four patients received RBC as first line therapy, the others were treated at relapse, generally after (R)-CHOP or (R)-CHOP-like regimens. *Results*. Two patients are currently too early, being still on therapy. Two patients who presented with very high peripheral WBC count died during the first cycles due to progressive disease, while another patient did not tolerate first cyclophosphamide infusion and was therefore removed from the study. No other extra-hematological toxicities higher than grade 1 were observed.. Hematological toxicities consisted in grade 1-2 thrombocytopenia/neutropenia (nine patients), while 3 patients experienced grade 3 neutropenia, requiring G-CSF support. One patient showed an initial response in extranodal sites but then progressed before the fourth cycle. Among the 9 patients who received at least six RBC cycles, three achieved a partial response and 6 obtained a complete response (overall response rate 75%, on intention to treat analysis excluding patients still on therapy). In 2 patients, molecular remission using PCR for t(11;14) bcl-1/IgH determination could be demonstrated. All responders maintain their remission phase 6 to 14 months after the start of RBC treatment. *Conclusions*. Although still preliminary, these results indicate that RBC regimen is feasible, well tolerated and effective (including the possibility to obtain molecular response) in elderly patients with advanced MCL.

0275

BENEFIT OF RITUXIMAB BEFORE AUTOLOGOUS STEM-CELL TRANSPLANTATION (ASCT) AT TIME OF RELAPSE IN PATIENTS WITH FOLLICULAR LYMPHOMA (FL)

K. Belhadj,[1] Y. Hicheri,[2] C. Pautas,[2] T. El Gnaoui,[2] F. Hemery,[3] C. Copie-Bergman,[4] J-L. Beaumont,[5] J. Dupuis,[2] I. Gaillard,[2] C. Haioun[2]

[1]Hôpital Henri Mondor, CRETEIL; [2]Hématologie, Hôpital Henri Mondor, CRETEIL; [3]Unité d'Information Médicale, Hôpital Henri Mondor, CRETEIL; [5]Département de Pathologie, Hôpital Henri Mondor, CRETEIL; [5]Etablissement Français du Sang, CRETEIL, France

Background. During disease's course, high dose therapy followed by ASCT can be considered as an alternative to sequential chemotherapies. *Aims*. To evaluate ASCT in the pre and post rituximab era as consolidation. *Methods*. Between 1989 and 2004, 54 patients (pts), not previously treated with rituximab, received ASCT at relapse. At initial diagnosis, median age was 47 yrs (24-61) and FLIPI score was > 3 in 18%. ASCT was performed in 2nd remission in 43 pts and in 3rd remission in 11. TBI - based conditioning regimens was used in 42 pts. Patients treated at relapse before 1998 did not receive rituximab-based salvage regimens and those treated after 1998 received rituximab before ASCT. The main characteristics of the two populations were as shown on the Table 1. *Results*. After ASCT, 49 pts achieved CR, 4 PR and one pt progressed. With a median follow-up of 8 yrs, 28 pts (52%) relapsed after a median time of 13 months (range 4-95) and 20 pts died from lymphoma progression: 9 pts, secondary malignancy: 7 pts and late infection: 4 pts. Ten-year overall survival (OS) from initial diagnosis was 68% and 10-year OS and event-free survival from ASCT were 64 and 37%, respectively. After adjusting for follow-up (median follow-up of the rituximab-treated group: 7 yrs), comparison between the 2 groups shows a significantly better 10-year OS in the rituximab-treated group (80% vs 34%, p=0.001). *Conclusion* The prognosis of pts with FL is highly improved by rituximab incorporated in salvage treatment before consolidative ASCT. after 2nd or 3rd remission in FL.

Table 1.

	rituximab-naive group	rituximab-treated group
N	29	25
FLIPI at initial diagnosis (Low/Intermediate/High) %	41/48/11	27/46/27
Median age at ASCT (yrs)	50 (range 35-60)	52 (range 30-62)
ASCT performed in 2nd remission	23	20
ASCT performed in 3rd remission	6	5

0276

RESULTS OF MAINTENANCE THERAPY WITH RITUXIMAB IN FOLLICULAR NON HODGKIN LYMPHOMA

B. Soria,[1] M.T. Olave,[2] R. Rubio-Escuin,[1] A. Rubio-Martinez,[1] L. Palomera,[2] M.A. Fuertes,[2] P. Mayayo,[1] V. Recasens,[1] P. Giraldo,[1] Group By GEHMA[3]

[1]Miguel Servet University Hospital, ZARAGOZA; [2]Lozano Blesa University Hospital, ZARAGOZA; [3]I+CS, ZARAGOZA, Spain

Follicular non-Hodgkin's lymphoma (F-NHL) is rarely curable with standard chemotherapy, the natural history of disease is characterized by successive relapses. The addition of Rituximab to different induction chemotherapy regimens increases the response rate and progression-free survival (PFS), without significantly increase of toxicity in the schedules that not including Fludarabine. The administration of Rituximab as maintenance therapy could be an useful and safe option for patients in complete remission after induction therapy with regimens based in immuno-chemotherapy, nevertheless the best maintenance schedule is not yet defined. *Objective*. To present our experience with an homogeneous maintenance schedule with Rituximab administrate every 6 months for F-NHL. *Patients and methods*. Prospective, observational study in 48 F-NHL patients in complete remission after first line therapy with immuno-chemotherapy (R-CHOP, R-CF, R-MCF, R-COP) since January 2002 to April 2007. Maintenance schedule: Rituximab 375 mg/m^2 weekly x 4 every 6 months two years in an outpatient protocol of fast infusion (24 hours before the patient received anti H1 and H2 orally and steroids 40 mg, dexclorfeniramina 5mg and paracetamol 1 g IV previously to administration every Rituximab dose in a total volume of 250 mL. The first 50 mL is administered in 30 and 200 mL in 60 minutes), patients with severe previous reactions were excluded of this schedule and received Rituximab in slow infusion. Patients were evaluated previous to start maintenance and two months after 4 courses, CT scan, PET and molecular study of t(14;18) in bone marrow were performed. Side effects according to common toxicity criteria were evaluated. Overall survival and relapsed free survival were analyzed according Kaplan-Meier and Cox regression study. *Results.* 37 patients have completed maintenance therapy (25 F/12 M); Mean age 55.3(24-77). ECOG 0(60.8%), 1(34.7%), 2(4.3%); At diagnosis: B symptoms 44.4%; FLIPI 0(8.1%), 1(43.4%), 2(30.4%), 3(17.3%); stage I(8.6%), II(17.3%), III (39.1%), IV(34.7%); grade I(43.5%), II(56.5%); Bulky 14%; extra node disease 14%; Hb<10 g/dL (14%); LDH increased 14%; B2M increased 14%. R-CHOP (69.5%), R-CHOP+local Rx (8.7%), R-FCM (17.3%), R-FC (4.3%), R-COP (2.0%). 90% of patients have received maintenance fast infusion protocol. Adverse events 2 patients (8.6%) had mild skin erythema during infusion, 2 (8.6%) previously treated with Fludarabine+Rituximab developed neutropenia grade 3-4: Overall survival mean 29.2 months (95% CI: 12-63), RFS: mean 23.3 months (95% CI:6-55). Only two patients have relapsed (4.6%), one of them with lost of CD20 expression in 70% of B-malignant cells. *Conclusions*. Rituximab maintenance therapy in an outpatient protocol of fast infusion could be a good strategy to prolong RFS in F-NHL, the tolerance is acceptable and satisfactory in most of the patients. It is necessary a longer follow-up to consider the magnitude of the effect obtained.

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Novel therapies, drug resistance and pharmacology I

0277

5-ANDROSTENE-3β,17β-DIOL ADMINISTERED TO MICE EXPOSED TO TOTAL BODY IRRADIATION RESULTS IN RAPID RECONSTITUTION OF IMMATURE BONE MARROW PROGENITOR CELLS AND SYNERGIZES WITH TPO BUT NOT WITH G-CSF

F.S.F. Aerts Kaya,[1] T.P. Visser,[1] C.L. Reading,[2] J.M. Frincke,[2] D.R. Stickney,[2] G. Wagemaker[1]

[1]Erasmus University Medical Center, ROTTERDAM, Netherlands; [2]Hollis-Eden Pharmaceuticals Inc, SAN DIEGO, USA

Background. 5-AED (5-androstene-3β,17β-diol) is a naturally occurring adrenal cortical steroid and has been shown to display radioprotective effects in both rodents and non-human primates, resulting in accelerated multilineage hematopoiesis and enhanced survival after radiation, including accelerated $CD34^+$ cell reconstitution in bone marrow of non-human primates. Where pegylated granulocyte-colony stimulating factor (Peg-G-CSF) is known to stimulate recovery of neutrophils, thrombopoietin (TPO) specifically protects short-term spleen repopulating immature cells and promotes platelet recovery. *Aims*. We studied the effects of combined treatment with 5-AED and Peg-G-CSF or TPO delivered after total body irradiation (TBI) on reconstitution of multilineage hematopoiesis and recovery of specific immature repopulating cell subsets to both further elucidate the efficacy of 5-AED and explore possible additive or synergistic effects. *Methods*. For direct measurements of the radioprotective effect of 5-AED, BALB/c mice were exposed to the midlethal dose of 6 Gy TBI. Two hours after irradiation, mice were injected with a placebo IM, 40 mg/kg 5-AED IM, 0.225 µg TPO IP or 10 µg Peg-G-CSF IP. To measure the effect of 5-AED on immature repopulating cells, BALB/c donor mice were exposed to 3x2 Gy TBI, fractions separated by 24 h, and treated after each fraction with placebo IM, 40 mg/kg/d 5-AED IM, 0.7 µg TPO IP or a single injection of 10 µg Peg-G-CSF IP and examined 24 h after the last fraction for immature cells per femur by the assay for marrow repopulating cells (MRA) and the spleen colony test (day 12 CFU-S) in 8 Gy irradiated recipient mice. *Results*. After 6 Gy TBI, BALB/c mice treated with 40 mg/kg 5-AED displayed a remarkably accelerated recovery of white blood cells ($p<0.01$), blood platelets ($p<0.01$) and red blood cells ($p<0.05$), as well as increased bone marrow cellularity ($p<0.01$) and elevated numbers of colony forming cells ($p<0.01$) at 14 days post-irradiation in comparison to placebo-treated animals. Addition of 5-AED to either Peg-G-CSF or TPO treatment did not result in an additive effect. The fractionated 3x2 Gy TBI study revealed a 5- and 7- fold increase in CFU-S in the 5-AED and TPO groups, respectively, relative to radiation controls, and a synergistic increase of 20-fold when used simultaneously. A similar effect on CFU-s was not observed for G-CSF, and neither did it synergize with 5-AED. MRA, expressed as GM-CFU per femur 13 days after transplantation in 10 mice, revealed an approximately 5- to 6-fold increased marrow repopulating ability: 1002 (range 0-5785) for 5-AED *vs* 174 (5-360) for radiation controls, contrasting TPO that promotes CFU-S reconstitution at the expense of MRA cells.[1] *Conclusions*. 5-AED as a single agent stimulates multilineage hematopoiesis and increases bone marrow cellularity following irradiation. The effect is mediated by increased survival and/or reconstitution of immature repopulating cells in a pattern distinct from that of TPO. Consistently, 5-AED strongly synergizes with TPO at the level of immature cells from which reconstitution originates, thus revealing a novel mechanism of bone marrow protection in cytoreductive therapy.

References

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0278

A POTENT SMALL MOLECULE PIM KINASE INHIBITOR WITH IN VIVO ORAL AVAILABILITY AND ACTIVITY IN CELL LINES FROM HEMATOLOGICAL MALIGNANCIES

G. Berk, E. Gourley, X.H. Liu, J. Lamb, P. Severson, J. Bearss, C. Jones, H. Vankayalapati, S. Warner, D. Bearss

SuperGen, Inc., DUBLIN, USA

Background. A small family of serine/threonine kinases known as Pim-1, Pim-2, and Pim-3 are involved in various signaling pathways in which they act as downstream effectors and potent inhibitors of apoptosis. The Pim kinases are unique in that they are expressed as active kinases and therefore gene expression levels directly correlate to their activity in cells. Pim-1 and Pim-2 are expressed in cells of hematopoietic lineage and Pim-3 appears to be more important in cells of epithelial origin. In concordance with these different patterns of expression, Pim-1 and Pim-2 are commonly overexpressed in hematological malignancies such as leukemias and lymphomas, while Pim-3 overexpression has been noted in melanoma, pancreatic adenocarcinoma, gastric, and other epithelial tumors. Thus, the Pim kinases are interesting targets for drug development, which offer promising potential in the treatment of hematological and solid malignancies. *Aims*. Develop a potent and selective, orally available Pim kinase inhibitor. *Methods*. Utilizing the published Pim-1 crystal structure and our proprietary CLIMBTM process, we identified a subset of leads from a large, virtual library from which a series of optimal analogs were synthesized to produce SGI-1776. *Results*. The IC50 of this compound in a biochemical enzyme-based assay was 6.7 nM for Pim-1, 69 nM for Pim-3, and 363 nM for Pim-2. Cell-based activity, determined by an anti-proliferative assay using HEL, K562, MV-4-11, and other leukemia, lymphoma, and solid tumor cell lines shows IC50 values as low as 54 nM. MV-4-11 cells treated with SGI-1776 shows a dramatic decrease in phospho-BAD levels (a direct substrate of the Pim kinases) as determined by western blot with an EC50 value of 7.9 nM. In conjunction with the anti-proliferative and phospho-BAD data, cell death via apoptosis was observed in cells treated with SGI-1776. As a result of these data and favorable pharmacokinetic studies, SGI-1776 was introduced into various *in vivo* studies that yielded efficacy in mouse xenograft tumor models of hematological origin. In a MOLM-13 xenograft model, only one of eight tumors remained in the group treated at 270 mg/kg SGI-1776 (PO) after fourteen days, whereas all eight tumors remained in the vehicle group. In addition to efficacy studies, pharmacodynamics studies will be presented. *Summary and Conclusions.* SuperGen's SGI-1776 exhibits potent inhibition of Pim kinase activity, translating into potent inhibition of cellular signaling pathways, cancer cell proliferation, and *in vivo* tumor progression in non-clinical models.

0279

THE PURINE NUCLEOSIDE PHOSPHORYLASE INHIBITOR FORODESINE INDUCES P53-INDEPENDENT MITOCHONDRIAL APOPTOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS THROUGH MCL-1 DOWNREGULATION AND INDUCTION OF P73 AND BIM

R. Alonso,[1] N. Villamor,[1] R. Upshaw,[2] S. Bantia,[2] C. Manz,[1] T. Mehrling,[3] E. Campo,[1] E. Montserrat,[1] D. Colomer[1]

[1]Hospital Clinic Barcelona, BARCELONA, Spain; [2]BioCryst Pharmaceuticals Inc., BIRMINGHAM, USA; [3]Mundipharma International Limited, CAMBRIDGE, UK

Background. Chronic lymphocytic leukemia (CLL) is a disease derived from the monoclonal expansion of $CD5^+$ B-lymphocytes. High ZAP-70 expression levels are associated with poorer overall survival, whereas p53 alterations convey drug resistance and short survival. Forodesine (BCX-1777) is a purine nucleoside phosphorylase inhibitor that induces an increase of plasma 2'-deoxyguanosine and intracellular accumulation of deoxyguanosine triphosphate and inhibition of DNA synthesis/repair, leading to cell death induction. Unlike other purine nucleoside analogs, Forodesine is not incorporated into DNA and represents a new selective anti-leukemic agent with a not yet fully explored mechanism of action. AIMS. To analyze the cytotoxic effect of Forodesine in CLL cells regarding their ZAP-70 levels as well their p53 status and to evaluate if the *in vitro* combination of Forodesine with Fludarabine, Bendamustine or Rituximab has a synergistic advantage. Finally, we characterized the mechanism of action of forodesine-induced apoptosis. *Methods*. The cytotoxic effect of Forodesine was analyzed by AnnexinV/PI staining in primary cells from 35 patients with CLL, 11 of them carrying p53 alterations. The synergistic or antagonic effect if combined with other drugs was analyzed using the combination index (CI) described by Chou and

Talalay. To further elucidate the mechanism of action of forodesine, several hallmarks of apoptosis were analyzed. *Results*. Pharmacologically achievable levels of Forodesine (2 μM) and dGuo (10-20 μM) induced apoptosis in CLL cells (mean cytotoxicity of 56.7±14.3 % with respect to control at 48 hours). No significant cytotoxic differences were observed between CLL cells regarding ZAP-70 expression levels (mean cytotoxicity of 57.6±10.2 % in 19 cases with ZAP-70 high *vs* 59.3±12.3 % in 16 cases with ZAP-70 low). CLL cases with 17p deletion showed a high sensitivity to forodesine (mean cytoxicity of 58.5± 20 %). A significative correlation between intracellular dGTP accumulation and forodesine-induced cytotoxicity was observed (p=0.0058). A high synergistic effect (CI<1) was observed between Forodesine and Bendamustine (mean CI=0.56) or Rituximab combination (mean CI=0.53). On the contrary, an antagonistic effect (CI>1) was observed with the combination of Fludarabine and Forodesine (mean CI=1.96). Forodesine induced an increase of the pro-apoptotic protein Bim, loss of mitochondrial membrane potential and generation of reactive-oxigen species (ROS). CLL cells express high levels of the anti-apoptotic proteins Mcl-1 and Bcl-2 that inversely correlate with *in vitro* and clinical response to chemotherapy. A decrease of the anti-apoptotic Mcl-1 protein, without affecting Bcl-2 was observed following Forodesine exposure. We demonstrated that p73 protein, which is activated upon DNA damage and ROS production and able to overcome resistance to apoptosis of CLL cells lacking p53, is induced after Forodesine incubation in both wild type- and p53 deleted CLL cases. *Conclusions*. Forodesine, as single agent or in combination with Bendamustine or Rituximab, might be highly effective in the treatment of CLL independent of the p53 and ZAP-70 status. Forodesine induced p73 and pro-apoptotic Bim, decreased the anti-apoptotic Mcl-1 protein leading to activation of the mitochondrial apoptotic pathway. No significant differences in these apoptotic markers were observed regarding p53 status, suggesting a common apoptotic pathway independent of p53-mediated cell death.

0280

SAFETY AND TOLERABILITY OF VORINOSTAT - EXPERIENCE FROM THE VORINOSTAT CLINICAL TRIAL PROGRAM

D. Siegel,[1] M. Hussein,[2] C.P. Belani,[3] F. Robert,[4] S. Rizvi,[5] J. Wigginton,[5] S. Randolph,[5] J.L. Ricker,[5] J. Lis,[5] J. Garcia-Vargas,[5] C. Sanz-Rodriguez[5]

[1]Hackensack University Medical Center, HACKENSACK; [2]H. Lee Moffitt Cancer Center, TAMPA; [3]Penn State Cancer Institute, HERSHEY; [4]University of Alabama, BIRMINGHAM; [5]Merck Research Laboratories, UPPER GWYNEDD, USA

Background. Vorinostat is an orally active histone deacetylase inhibitor with anticancer properties when used alone or in combination. *Aims*. To present an overview of safety and tolerability data from patients who received vorinostat as monotherapy or in combination with other systemic therapies for solid and hematological malignancies. *Methods*. Safety data from patients in the Vorinostat Clinical Trial Program (Merck protocols 001, 002, 003, 004, 005, 006, 008, 011, 012, 013, 015, 016, 025, 029, 030, 048, 058, 066) were collated (cut-off date December 2007). *Results*. Data from 476 patients are included. A total of 341 patients received vorinostat as monotherapy for solid tumors (mesothelioma, head and neck, renal, thyroid, laryngeal, breast, colorectal, non-small-cell lung cancer [NSCLC], and gastric cancers) or for hematological malignancies (acute myeloid leukemia, chronic lymphocytic leukemia, or chronic myeloid leukemia, non-Hodgkin's lymphomas [including cutaneous T-cell lymphoma {CTCL}, peripheral T-cell lymphoma, diffuse large B-cell lymphoma, and follicular lymphoma], Hodgkin's disease, myelodysplastic syndrome or multiple myeloma). A total of 135 patients received vorinostat in combination with other systemic therapies: pemetrexed and cisplatin in advanced cancer, bortezomib in advanced multiple myeloma, bexarotene in advanced CTCL, erlotinib in relapsed/refractory NSCLC, or carboplatin and paclitaxel in chemonaïve NSCLC. As monotherapy, the most commonly reported adverse events (AEs) were fatigue (68.3%), nausea (60.1%), diarrhea (55.4%), and anorexia (49.9%). Grade 3-4 AEs included thrombocytopenia (15.2%), fatigue (13.5%), dehydration (8.5%), and anemia (7.9%). There were three drug-related deaths (ischemic stroke, tumor hemorrhage, unspecified) observed in patients who received vorinostat monotherapy. Of the 156 patients who received vorinostat monotherapy at 400 mg q.d. (the current FDA-approved dose in CTCL patients), 13 patients (8.3%) discontinued due to AEs (predominantly anorexia [1.3%], pulmonary embolism [0.6%], and weight decrease [0.6%]), and 24 patients (15.4%) required dose modifications (commonly due to thrombocytopenia [5.8%], diarrhea [1.9%], and nausea [1.9%]). In combination therapy, the most commonly reported AEs were nausea (52.6%), diarrhea (43.0%), and fatigue (41.5%). The most common grade 3-4 events included fatigue (16.3%), diarrhea (5.9%), dehydration (5.2%), and nausea (5.2%). There was one drug-related death (hemoptysis) that occurred in a patient with NSCLC. Discontinuation due to AEs occurred in 25 (18.5%) patients, commonly due to fatigue (3.0%) dehydration and nausea (both 2.2%), and 23 patients (17%) required dose modifications (commonly due to fatigue [5.9%], diarrhea [1.5%], and hypertriglyceridemia [1.5%]). *Conclusions*. Data from the Vorinostat Clinical Trial Program demonstrate that vorinostat has an acceptable safety and tolerability profile when used either as monotherapy or in combination with other systemic therapies in cancer patients. Dose modifications are usually not required in the majority of patients who receive vorinostat as monotherapy or in combination regimens.

0281

EZN-2208, A NOVEL POLYETHYLENEGLYCOL-SN38 CONJUGATE, THERAPY RESULTS IN CURES OF ANIMALS BEARING AGGRESSIVE NON-HODGKIN'S LYMPHOMA MODELS

P. Sapra, M. Mehlig, J. Malaby, P. Kraft, H. Zhao, L. Greenberger, I. Horak

Enzon Pharmaceuticals, PISCATAWAY, USA

Background. Examination of the clinical utility of SN38 (10-hydroxy-7-ethyl-camptothecin), the highly active metabolite of CPT-11, has not been possible to date due to extremely poor solubility of SN38. We have generated, a novel polymer-drug conjugate, EZN-2208, made by linking SN38 reversibly with a multi-arm high molecular weight polyethylene glycol (PEG). *Aims*. The objectives of these studies were to evaluate the therapeutic efficacy of EZN-2208 in animals bearing non-Hodgkin's lymphomas. *Methods*. The *in vitro* cytotoxicity of EZN-2208 and CPT-11 were tested in human lymphoma (Raji, Daudi, DoHH2) cancer cell lines using the tetrazolium assay. Annexin V-FITC and propidium iodide staining followed by flow cytometry analysis were used to detect cytotoxicity at various apoptosis/necrosis stages. The therapeutic efficacy was evaluated in xenograft models of non-Hodgkin's lymphoma (Raji and Daudi and DoHH2). *Results*. *In vitro*, the IC50 of EZN-2208 ranged from 3-24 nM. EZN-2208 was about 30-45-fold more potent than CPT-11. In apoptosis assays, within 24 hours following treatment, the major mechanism of cell killing was late apoptosis and cell necrosis. When administered in a multiple dose regimen (q2d x 5), the MTDs of EZN-2208 and CPT-11 were 10- and 40 mg/kg respectively. In the Raji xenograft model, treatment with a single MTD of EZN-2208 resulted in a 500% improvement in life span (ILS) and 50% cures of animals compared with 19% ILS observed for mice treated with a single MTD of CPT-11. Multiple dose treatment of EZN-2208 resulted in 90% cures of animals compared with 63% ILS and no cures observed for the CPT-11 group. In an early disease Daudi xenograft model (treatment administered 24h postinjection of cells), a single injection of EZN-2208 treatment resulted in cures of 60% of animals and no cures were observed for CPT-11 group. Multiple injections of EZN-2208 cured 100% of animals. In the more aggressive late stage disease Daudi model (treatment administered 7 days postinjection of cells), a single dose of EZN-2208 caused 56% cures of animals, whereas CPT-11 treatment was completely ineffective. Multiple injections of EZN-2208 cured 100% of animals. Similar results were obtained in another model (DoHH2, t(14;18) chromosomal translocation), where EZN-2208 outperformed CPT-11 both in early and advanced disease models. *Conclusions*. EZN-2208 has excellent therapeutic efficacy and significantly greater antitumor activity than CPT-11 in several human NHL xenograft models. We have previously shown in solid tumor xenograft studies that EZN-2208 has prolonged circulation in the blood compared with CPT-11, resulting in high tumor exposure of free SN38. Therefore the dramatic antitumor activity seen in the NHL models may be attributed to a long circulation half-life and high exposure of the tumors to SN38. These studies merit the clinical evaluation of EZN-2208 in hematological malignancies. EZN-2208 is currently in Phase 1 clinical studies to identify optimal dose and schedule.

0282

RITUXIMAB AND ALEMTUZUMAB IN COMBINATION, BUT NOT ALONE, INDUCE COMPLETE REMISSIONS IN A PRECLINICAL ANIMAL MODEL OF PRIMARY HUMAN ALL: RATIONALE FOR COMBINATION TREATMENT.

B.A. Nijmeijer, M.L.J Van Schie, S. Stevanovic, R. Willemze, J.H. Falkenburg

Leiden University Medical Center, LEIDEN, Netherlands

Background. Acute lymphoblastic leukemia (ALL) in adults has a poor prognosis, despite intensive chemotherapy or allogeneic stem cell transplantation. Further treatment intensification is limited by toxicity. Monoclonal antibodies display a relatively favorable toxicity profile. Rituximab (RTX) recognizing CD20, and alemtuzumab (ALM) recognizing CD52, have shown clinical activity in hematological malignancies. Precursor-B ALL (preB-ALL) may express CD20 and/or CD52. *Aims*. To evaluate the activity of RTX and ALM against preB-ALL in a preclinical *in vivo* model. *Methods*. Expression of CD20 and CD52 on preB-ALL cells was determined by staining with RTX or ALM followed by secondary anti-human-Ig antibody. Mean fluorescence intensity (MFI) was analyzed by flow cytometry. To investigate *in vivo* activity of RTX and ALM, NOD/scid mice were inoculated with primary human ALL cells. Three weeks later treatment was given by daily injection of 250 µg antibody. After 4 weeks of treatment, mice were sacrificed for analysis of peripheral blood (PB) and bone marrow (BM). *Result*s. Of 18 randomly selected primary human ALL, 10 expressed CD20 (56%, median MFI 102, range 97- 951) and 12 expressed CD52 (67%, median MFI: 324, range 111-633). All CD20 positive ALL expressed CD52. In 8 CD52 positive ALL distinct CD52 negative subpopulations were observed (0.1 to 4% of leukemic cells). Two of these cases (coded COA and VBK) that expressed CD20 and CD52 were selected for *in vivo* studies. In both cases, RTX cleared blast cells from the PB but at end-point leukemic cells persisted in BM (COA: 59% of nucleated cells *vs* 76% in control animals, VBK: 23% vs 86%, respectively). Persisting cells expressed a significantly decreased amount of surface CD20, all of which was occupied by RTX. Expression of CD20 on these cells was restored after engraftment into secondary recipient mice. To determine the cause of the decreased surface CD20 expression during RTX treatment, animals were engrafted with primary cells, received a single dose of RTX or vehicle alone, and were sacrified 24 hours later. Leukemic cell numbers were strongly reduced in PB (0.1% vs 7.4%) but not in BM (5.4% vs 8.4%), indicating limited elimination of ALL cells in the BM. Expression of CD20 on these cells was however reduced 13-fold, demonstrating that CD20 (or CD20-RTX complexes) had been removed from their surface. Likewise, ALM eliminated leukemic cells from PB of engrafted animals. At end-point leukemic cells persisted in BM (COA: 20% *vs* 76%, VBK: 74% *vs* 86%, respectively) and these cells displayed complete loss of CD52. However, CD52 expression was not restored after engraftment and expansion in secondary recipient mice. Thus, although both antibodies appeared to exert anti-leukemic activity, RTX activity was limited by down modulation of CD20 in the BM and ALM activity by the emergence of pre-existent CD52 negative cells. However, RTX treatment did not affect expression of CD52, and ALM treatment did not affect expression of CD20. Therefore, we evaluated combined administration of RTX and ALM in the same experimental setting. Combined treatment (250 µgRTX+250 µg ALM daily for 4 weeks) resulted in complete disappearance of leukemic cells from the BM of 6 out of 6 COA engrafted mice and in 4 out of 5 VBK engrafted mice. *Conclusions*. A combination of rituximab and alemtuzumab may be promising as treatment for patients with preB-ALL.

0283

THE NOVEL HDAC INHIBITOR UCL67022 HAS POTENT ACTIVITY IN MULTIPLE MYELOMA CELLS AND REMAINS EFFECTIVE IN A BONE MARROW MICRO-ENVIRONMENT MODEL

R. Popat,[1] L. Maharaj,[2] H. Oakervee,[2] J. Cavenagh,[1] B. Middleton,[3] A. Rioja,[3] C. Marson,[3] S. Joel[2]

[1]St. Bartholomew's Hospital, LONDON; [2]Centre for Experimental Cancer Medicine, Barts and the London, LONDON; [3]University College London, LONDON, UK

Background. Histone acetylation and deacetylation are critical to gene expression in many cancers. Moreover, the enzymes that mediate the acetylation state of histones also alter the acetylation state of a number of other non-histone proteins. Histone deacetylase inhibitors (HDACI) represent a novel class of drugs that result in altered gene expression and the acetylation of key survival proteins such as HSP90. *Aims*. This study sought to investigate the effects of UCL67022 (a novel hydroxamic acid derived HDACI) on human multiple myeloma cell lines (HMCLs), patient derived myeloma cells and peripheral blood mononuclear cells (PBMCs). Drug activity in the presence of cytokines and stromal cells to mimic the bone marrow micro-environment was studied. Mechanisms of apoptosis resulting from HDACI exposure, and effects on ubiquitinated proteins were also examined. *Methods*. The HMCLs U266, RPMI 8226, MM1S and MM1R and the bone marrow stromal cell (BMSC) line HS-5 were used. Bone marrow aspirates from consenting patients were used to separate myeloma cells using a negative selection procedure and to generate BMSCs for co-culture studies. Cell viability and/or cell number were measured following 24 and 48hr incubations using both an ATP bioluminescence method and a fluorochrome based method. Immunofluorescence was performed by laser scanning confocal microscopy. HDACI effects on protein acetylation, caspase activation and HSPs were investigated by western blot analysis. *Results*. UCL67022 was approximately 10 fold more potent in reducing cell viability than SAHA in all HMCLs and 5 patient derived samples. IC50 values at 48 hours ranged from 45-118 nM for HMCLs and less than 360nM for patient samples. The cytotoxic effect was maintained when cells were supplemented with bone marrow derived cytokines such as IL-6 and IGF-1 and only minimally reduced when co-cultured with HS-5, BMSCs or patient derived BMSCs. In contrast, the presence of these cytokines, or BMSCs, abrogated the effects of dexamethasone. The effects of UCL67022 on BMSC cytokine production are currently being studied. UCL67022 had little effect on the viability of normal PBMCs at concentrations up to 30 µM or on BMSCs. Western blotting revealed acetylation of histone H3 and α-tubulin at 24hrs with 0.1 µM UCL67022 (compared with 1.0-10 µM SAHA) and cleavage of caspase 8 but not caspase 9. To investigate the potential HDAC6 inhibitory activity further, immunofluorescence studies were performed using acetylated α-tubulin and ubiquitin antibodies. UCL67022 caused the uniform hyperacetylation of α-tubulin which was able to disrupt bortezomib induced aggresomal formation. The combination of UCL67022 and bortezomib also altered the localisation of ubiquitinated protein to micro-aggregates. This disruption of protein homeostasis did not lead to an increase in HSP 27, 70 or 90 nor did it result in synergistic cytotoxicity by calcusyn analysis. *Conclusions*. UCL67022 is a novel hydroxamic based HDACI with potent activity in multiple myeloma. It is able to overcome the protective effects of the bone marrow microenvironment and induces apoptosis through activation of caspase 8. Finally it can disrupt bortezomib induced aggresome formation indicating HDAC6 inhibitory activity.

0284

METHYLSELENINIC ACID DEMONSTRATES ANTI-LEUKAEMIC ACTIVITY AND SENSITISES AML CELLS TO THE EFFECTS OF CYTOTOXIC AGENTS BY INITIATING THE UNFOLDED PROTEIN RESPONSE

J.M. Stevens, S. Juliger, K. Summers, J. Fitzgibbon, T.A. Lister, S.P. Joel

St Bartholomew's Hopsital, LONDONWEST SMITHFIELD, UK

Background. Evidence is accumulating that selenium modulates the activity of cytotoxic drugs and that the selenium species, methylseleninic acid (MSA) induces endoplasmic reticulum (ER) stress, initiating the unfolded protein response (UPR). *Aims*. To investigate the interaction between MSA and cytotoxic drugs in AML cell lines and evaluate the effect of MSA on markers of the UPR. *Methods*. A concentration-response curve was obtained for THP-1 and U937 cells exposed to MSA and 4 other cytotoxic agents (cytarabine, doxorubicin, etoposide and bortezomib). Subsequently cells were exposed to incremental concentrations of cytotoxic agent, combined with EC5 and EC10 concentrations of MSA. For the evaluation of chemosensitisation, results for cytotoxic drugs used as single agents were expressed relative to control viability and results for the combined effect were expressed relative to the effect of low concentration MSA alone. The concentration-response curves generated were compared using a one-way ANOVA test. Western blot (WB) and real time polymerase chain reaction (qRT-PCR) analysis were used to determine changes in the UPR (GRP78 upregulation, phosphorylation of eIF2α, splicing of XBP1 mRNA and GADD153). *Results.* MSA reduced the viability of THP-1 cell and U937 cells at 48 hours (EC50 18.2 µM and 3.9 µM respectively). Subtoxic concentrations of MSA sensitised cells to the effect of cytotoxic agents: significant supra-additive effects on viability were seen in THP-1 cells exposed to MSA in combination with cytarabine (p=<0.0001), etoposide (p=0.008), bortezomib (p=0.004) and doxorubicin p=0.03. MSA also sensitised U937 cells to etoposide (p=0.03) and bortezomib (p=0.0030), but not cytarabine or doxorubicin. Upregulation of GRP78, and phosphorylation of eIF2 were seen on exposure

of THP-1 cells to MSA. In U937 cells, GRP78 and phosphorylated eIF2α were maximally upregulated at baseline and did not change after exposure to MSA. Splicing of XBP1 mRNA and upregulation of GADD153 were demonstrated by qRT-PCR in both THP-1 and U937 cells after exposure to MSA, at both subtoxic and EC50 concentrations, in a time-dependent manner. *Conclusions*. These results demonstrate that MSA has activity against AML cells as a single agent, while at lower concentrations it sensitises cells to cytotoxic drugs and causes ER stress. However the effect is cell line specific; U937 cells were more sensitive to MSA but demonstrate less chemosensitisation by MSA than seen in THP-1 cells. This difference may be associated with the ER chaperone molecule GRP78, which exerts a cytoprotective effect against ER stress. GRP-78 was maximally upregulated at baseline in U937 cells, but was present at only low levels in THP-1 cells and could be upregulated further after exposure to MSA. This study suggests that subtoxic concentrations of selenium may be useful as a chemomodulating agent, in combination with conventional cytotoxic agents, in the treatment of AML.

0285

HIVTAT MEDIATED TRANSDUCTION OF COMPETITIVE PEPTIDES THAT DISRUPT THE BCR/ABL TETRAMERS INHIBITS THE PROLIFERATION OF PHILADELPHIA CHROMOSOME POSITIVE CELLS

A.A. Mian, M. Ruthardt, T. Beissert

Medical Clininc II/Hematology, FRANKFURT, Germany

Background. Philadelphia Chromosome (Ph) positive ALL and CML are characterized by the oncogenic BCR/ABL fusion tyrosine kinase (FTK). The BCR portion of the FTK harbors an N-terminal coiled-coil (CC) domain which induces the tetramerization of BCR/ABL. The BCR mediated tetramerization of ABL in the fusion protein leads to the constitutive activation of the ABL kinase. The subsequent permanent activation of multiple downstream signaling pathways induces the leukemic phenotype. The CC helical motif Helix-α-2 (Helix-2) represents the majority of the tetramer interface. Helix-2 is both, sufficient and necessary for BCR/ABL tetramerization. Targeted inhibition of BCR/ABL by the ABL kinase inhibitor Gleevec induces apoptosis in BCR-ABL transformed cells and leads to complete remission in Ph-positive leukemia patients. However, a large portion of ALL patients and CML patients in blast crisis relapse and acquire Gleevec resistant BCR/ABL mutations. We have previously shown that the coexpression of BCR/ABL and Helix-2 derived peptides reduces the transformation potential of Imatinib sensitive and Imatinib resistant BCR/ABL. Helix-2 peptides interfere with BCR/ABL tetramer formation and reduce the autophosphorylation of BCR/ABL. Our previous study provided a proove of concept that competitive Helix-2 peptides targeting the tetramerization domain represent a potential therapeutic approach for BCR/ABL positive leukemia. *Aims*. The aim of this study was to use the peptide transduction domain HIVTAT to deliver Helix-2 peptides (TAT-Helix-2) to Ph+ cells. We studied the proliferation of BCR/ABL-dependent Ph^+ cell lines in presence of TAT-Helix-2 as well as the autophosphorylation of BCR/ABL expressed in these cells. *Methods*. TAT-Helix-2 peptides were fused to GFP (TAT-Helix-2-GFP) and the STREP® tag. These fusion peptides were purified from bacterial lysates using the STREP® tag. The Ph-positive cell lines BV173, TOM-1 and K-562 and the Ph-negative cell line Nalm-6 were exposed to 1 μM of the TAT-Helix-2-GFP peptides. The successfull delivery to the cells was checked by FACS and western blots. The proliferation of the cells was assessed by dye exclusion and the viability by XTT assays. Anti-phospho-ABL specific immunoblotting was used to reveal the BCR/ABL autophosphorylation. All experiments were controlled using Helix-2-GFP peptides lacking a HIVTAT-domain and HIVTAT-tagged GFP devoid of Helix-2. *Results*. Here we report that i) TAT-Helix-2 peptides are efficiently delivered to Ph-positive hematopoitic cell lines; ii)) TAT-Helix-2 inhibits the growth and viability of Ph-positive cells, without affecting Ph-negative cells ; iii) the antiproliferative effects are enhanced in presence of chloroquine; iv) TAT-Helix-2 peptides decrease the autophosphorylation of BCR/ABL. *Summary and Conclusions*. Taken together these results show that cell permeable TAT-Helix-2 peptides are efficiently delivered to hematopoietic cells. Upon cellular uptake these peptides inhibit the ABL-kinase activity. TAT-Helix-2 peptides specifically inhibit the proliferation of Ph-positive patient-derived cell lines. This study provides first evidence that cell permeable Helix-2 derived peptides harbor a therapeutic potential for BCR/ABL positive leukemia.

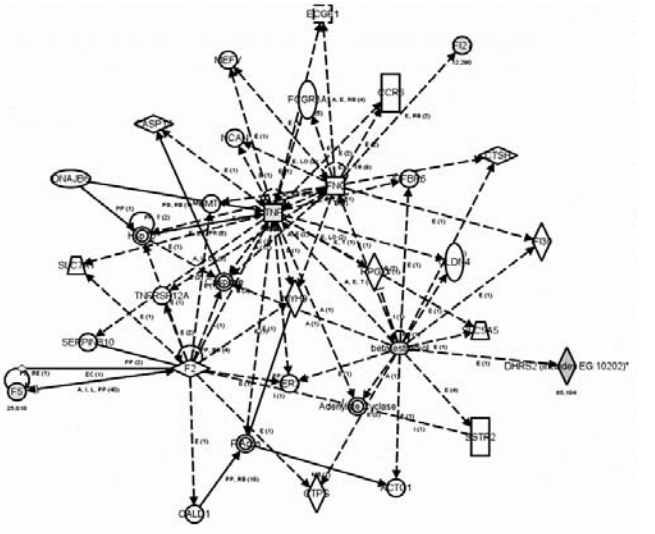
0286

HISTONE DEACETYLASE INHIBITOR ITF2357 EXERT SIGNIFICANT ANTI-PROLIFERATIVE AND PRO-APOPTOTIC EFFECTS ON THE P39 CELL LINE: A GENE EXPRESSION STUDY

S. Galimberti,[1] M. Canestraro,[1] H. Savli,[2] G. A. Palumbo,[3] B. Nagy,[4] F. Di Raimondo,[3] M. Petrini[1]

[1]Hematology, PISA, Italy; [2]Biology department, Kocaeli University, KOCAELI, Turkey; [3]Hematology Univeristy of Catania, CATANIA, Italy; [4]1st Department of Obstetrics and Gynecology, Semmelweis University, Budapest, BUDAPEST, Hungary

Background. ITF2357 is a new histone deacetylase inhibitor that has been recently reported to induce apoptosis Histone deacetylase inhibitors (HDAC inhibitors, HDI) are a class of drugs that interfere with the function of histone deacetylase.HDAC inhibitors are being studied as a treatment for cancer and neurodegenerative diseases. The exact mechanisms by which the compounds may work are unclear, but epigenetic pathways are proposed. Richon *et al.*found that HDAC inhibitors can induce p21 (WAF1) expression, a regulator of p53's tumor supressor .ITF2357, is a new histone deacetylase inhibitor, on multiple myeloma and acute myelogenous leukemia cells *in vitro* and *in vivo*. It induced histone acetylation, blocked proliferation and induced apoptosis in MM and AML cell lines. Also ITF2357 has anti-neoplastic activity *in vitro* and *in vivo* by induction of leukemic cell apoptosis.ITF2357 inhibits production of growth and angiogenic factors in IL-6 and VEGF. By this study, we assessed if ITF2357 would exert an anti-proliferative and pro-apoptotic effect on a myelo-monocytic cell line (the P39). *Methods*. Cell viability was assessed by MTT; apoptosis by the Annexin V/propidium cytofluorimetric analysis. ROS production was evaluated by dihydrorhodamine 123 (DHR) and flow-cytometry assay. Total RNA Samples were isolated using Qiamp RNA mini kit (Qiagen, Germany) and hybridized on Whole Human Genome Microarray platforms (Agilent). Obtained data imported into GeneSpring 6.1 software for analysis (GeneSpring 6.1,Silicon Genetics, Redwood City, CA, USA). The fold changes were analyzed by filtering the dataset using p-values <0.01 and a signal-to-noise ratio >2 for use in ANOVA statistical analysis. Additional filtering (minimum 2-fold change) was applied to extract the most these genes were analyzed using Ingenuity Pathways Analysis (IPA) Software (Ingenuity Systems, Redwood City, CA). Those genes with known gene symbols (HUGO) and their corresponding expression values were uploaded into the software. Each gene symbol was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base. Networks of these genes were algorithmically generated based on their connectivity. Canonical pathways analysis identified the pathways, from the IPA library of canonical pathways, which were most significant to the input data set.



The significance of the association between the data set and the canonical pathway was determined based on two parameters: (1) A ratio of the number of genes from the data set that map to the pathway divided by the total number of genes that map to the canonical pathway and (2) a P value calculated using Fischer's exact test determining the

probability that the association between the genes in the data set and the canonical pathway is due to chance alone. Results have been confirmed by TaqMan® Low Density Array Human Apoptosis Panel using Real-Time PCR (Applera). *Results*. With an IC50 of 0.8 mcM, ITF2357 was able to inhibit proliferation and induce apoptosis (measured by Annexin V and presence of apoptotic bodies) of P39 cells, in a dose- and time-dependent way. ITF2357 blocked cell cycle in the G1 phase at low dose (0.5 mcM) and in the G2 phase at higher dose (0.8 mcM). Moreover, the production of reactive oxygen species (ROS) was significantly increased after exposure to this histone deacetylase inhibitor. In order to better understand the mechanism of apoptosis, we performed gene expression assays, by using both the TaqMan® Low Density Array Human Apoptosis Panel (Applied Biosystem), and Agilent platforms. The genes identified as de-regulated by ITF2357 were then analyzed for network and gene ontology by Ingenuity Pathway Analysis software. In the untreated P39, 84 of the 93 genes involved in the apoptotic pathway and represented in the Taqman Low-Density Arrays were expressed. Already after 12h-treatment, ITF2357 down-regulated 9 genes and up-regulated 11 genes. After exposure for 24 hours, ITF2357 down-regulated 48 genes and up-regulated 3 genes only. Among down-regulated genes, *BAD, BCLXL, BCL2, BCL2L10*, whereas APAF1 was significantly up-regulated. Moreover, microarray assays showed that the IL6, IL10, inflammation, *NF-kB, PDGF, TGFβ* and apoptosis were the pathways more significantly modified after exposure to ITF2357. Interestingly, among the significantly down-regulated genes, JUN, NF-kB, TNFA, IL1β could be clinically relevant. Indeed, IL1beta has been reported to induce activation of NF-kB, with consequent cell proliferation in acute leukemia; consequently, a down-regulation of IL1beta expression, in addition to the direct down-regulation of NF-kB, could suggest an anti-proliferative effect in MDS. Moreover, TNFalpha plays a well-known pathogenetic role in MDS: it induces apoptosis in the maturing cells causing pancytopenia, but also stimulates the proliferation of the primitive progenitors, accounting for the hypercellular bone marrow frequently observed in MDS. The ability of ITF2357 of down-regulating TNFα expression would be also relevant. *Conclusions*. In summary, biological results and gene expression assays suggest the possible use of histone deacetylase inhibitors in treatment of high-risk MDS. *in vivo* trials will be useful to confirm this hypothesis coming from our *in vitro* studies.

0287

TARGETED THERAPY WITH ARSENIC TRIOXIDE AND INTERFERON ALPHA ERADICATES LEUKEMIC CELLS IN SCID MICE MODEL OF ADULT T-CELL LEUKEMIA/LYMPHOMA

A. Bazarbachi,[1] H. El Hajj,[2] H. Hasegawa,[3] M. El-Sabban,[2] G. Zaatari,[2] S. Saab,[2] R. Mahfouz,[2] R. Nasr,[2] Y. Kfoury,[2] O. Hermine,[4] H. De Thé,[5] W. Hall[6]

[1]American University of Beirut Medical Center, BEIRUT, Lebanon; [2]American University of Beirut, BEIRUT, Lebanon; [3]National Institute of Infectious Diseases, TOKYO, Japan; [4]Necker Hospital, PARIS, France; [5]Hopital Saint Louis, PARIS, France; [6]University College Dublin, DUBLIN, Ireland

Background. HTLV-I associated adult T-cell leukemia (ATL) is a severe, chemotherapy-resistant malignancy associated with poor prognosis. Recently, HTLV-I Tax transgenic mice, accurately reproducing human ATL disease were generated. To develop a more consistent and rapid model of disease development, direct transfer of Tax transgenic cells into SCID mice was assessed and showed that they die within 28 days, having developed both an extremely aggressive leukemia with characteristic flower cells, and extensive lymphomatous infiltration of the spleen, lymph nodes, bone marrow, liver, kidney and lung by malignant T lymphocytes highly expressing CD25. Furthermore, and as in ATL patients, we observed marked hypercalcemia and high level of LDH. *Aims and Methods*. We have previously identified *in vitro* several potential targeted therapies for ATL. To define the optimal schedule and drug combination to be evaluated in clinical trials, we tested the following drugs using the *in vivo* ATL SCID model: zidovudine, interferon α (IFN), arsenic trioxide, the proteasome inhibitor bortezomib, and the combinations of zidovudine and IFN, or of arsenic and IFN. *Results*. The combination of zidovudine and IFN had no effect on the survival of ATL SCID mice confirming the hypothesis that this combination targets the viral replication since these animals have Tax-transgenic leukemic cells but no entire HTLV-I retrovirus, contrary to ATL patients. Inhibition of NF-kappaB using bortezomib or arsenic alone almost doubled the mice survival but was not sufficient to eradicate ATL malignant cells. Strikingly, the most relevant effect on the mice survival was obtained when we used the combination of arsenic trioxide and interferon alpha, which targets both the HTLV-I Tax oncoprotein and the constitutive activation of the NF-kappaB pathway. Indeed, this combination cured 40% of treated mice after one cycle and 80% of treated mice after two cycles with evidence of total disease eradication. *Conclusions*. These impressive results suggest that double targeting of Tax and NF-κB by the combination of arsenic trioxide and interferon alpha may suffice to cure ATL. This combination is now tested in newly diagnosed ATL patients.

0288

IN VITRO AND IN VIVO PROPERTIES OF PT-401, A NOVEL ERYTHROPOIETIN FUSION PROTEIN

J. Sytkowski,[1] J.Y. Jeong,[1] K.L. Davis,[1] A.L. Socha,[1] H.J. Gomez[2]

[1]Beth Israel Deaconess Medical Center, Harvard Medical School, BOSTON, MA; [2]DNAPrint Pharmaceuticals, Inc., SARASOTA, FL, USA

Background and Aims. Recombinant human erythropoietin (rhEpo, epoetin) is widely used for correction of anemia due to chronic kidney disease and other conditions. Modified forms of rhEpo and other erythropoiesis stimulating agents (ESAs) with higher efficacy and longer half-life have been and continue to be developed to overcome rhEpo's short *in vivo* half-life. Previously, we described a novel Epo fusion protein, PT-401, comprising identical head-to-tail repeats and a short linker. We now report that PT-401 exhibits unique *in vitro* characteristics and enhanced *in vivo* activity in mice compared to rhEpo. *Methods*. PT-401 was expressed in Chinese hamster ovary (CHO) cells stably transfected with a mammalian expression vector containing the PT-401 cDNA. We used quantitative immunoblotting analysis with the monoclonal anti-Epo antibody AE7A5 to identify and quantify PT-401 protein in the cell culture supernatant. Highly purified PT-401 was obtained by sequential column chromatography. The interaction of PT-401 with the Epo receptor (EpoR) was characterized using radioiodinated PT-401 and BaF3 cells stably expressing the human EpoR (BaF3/huEpoR). *Results*. The amount of PT-401 secreted into protein-free cell culture medium of cloned CHO cells varied between 4 and 40 mg/L. Clones producing PT-401 with the optimal glycosylation were identified based upon mobility in SDS-PAGE and isoelectric focusing, based upon the observation that the extent of glycosylation of Epo correlates with the *in vivo* half-life. The specific biological activity of purified PT-401 measure by *in vitro* bioassay was similar to that of freshly produced PT-401 in the cell culture supernatant, demonstrating that the biological activity of PT-401 was preserved during the purification process. PT-401 exhibited specific, saturable binding to the EpoR. The equilibrium dissociation constant (Kd) of PT-401/EpoR interaction was approximately 4 nM, higher than that for rhEpo (0.14 nM, determined in the same experiment), consistent with longer-acting ESAs. The specific activity of PT-401 determined by *in vitro* bioassay was lower than that of rhEpo, reflecting the higher Kd, also consistent with other longer-acting ESAs. Importantly, PT-401 increased the mean hematocrit of mice to significantly higher levels compared to rhEpo in all injection schemes tested (three injections with 300 IU/kg or 100 IU/kg, a single injection with 100 IU/kg), demonstrating the superior *in vivo* activity of PT-401. *Conclusions*. PT-401 exhibits *in vitro* characteristics consistent with a very long-acting ESA and an *in vivo* biological activity superior to that of rhEpo, suggesting important therapeutic advantages.

0289

WOULD INACTIVATION OF GGTASE-I PREVENT THE DEVELOPMENT OF K-RAS-INDUCED MPD IN MICE?

A.-K. Sjögren, K.M.E. Andersson, B. A. Cutts, C. Karlsson, O. Khan, M. Liu, M.O. Bergö

Institute of Medicine, GÖTEBORG, Sweden

Background. We showed that inactivation of Pggt1b (the gene encoding geranylgeranyltransferase (GGTase-I)) reduces tumors and improves survival in mice with K-RAS-induced lung cancer.[1] In this model, K-RAS(G12D) is switched on not only in lung cells but also in myeloid cells, resulting in myeloproliferation. Interestingly, the inactivation of Pggt1b eliminated the myeloid phenotypes. To determine if inactivation of Pggt1b would prevent the development of a well-established myeloproliferative disase (MPD) model, we bred conditional Pggt1b knockout mice (Pggt1bfl/fl) with mice harboring a Cre-inducible K-RAS(G12D) allele (K) and a Mx1-Cre transgene (M). Injection of pI-pC in KM mice results in a lethal MPD.[2–4] Surprisingly, the inactivation of Pggt1b caused the mice to die more rapidly. One potential explanation for this finding is that mice with the K-RAS-induced MPD develop tumors in other tissues (e.g. lungs, gastrointestinal tract) due to *leaky* expression of K-RAS(G12D) and that the simultaneous inactivation of Pggt1b caused tissue damage. *Aims*. To test a tissue-specific model for K-RAS-induced

MPD and to use this model to define the impact of GGTase-I deficiency on disease phenotypes.

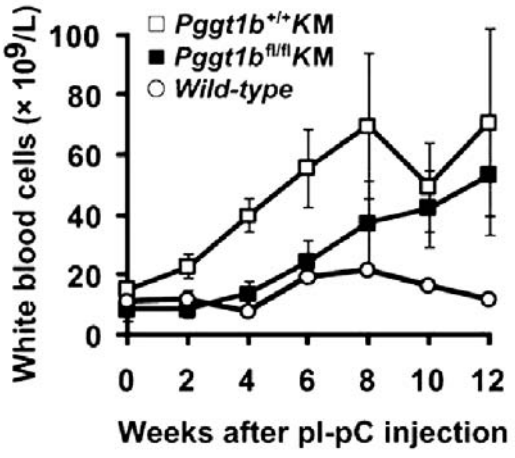


Figure 1. GGTasa-I deficiency reduces leukocytosis in mice with MPD. White blood cell counts in peripheral blood of mice receiving Pggt1b+/+ KM fetal liver cells (white rectangles, n=8-9 week 0-4; n=3-5 week 6-12), Pggt1bfl/flKM cells (black rectangles, n=8-10 week 0-4; n=4-5 week 6-12), and wild-type cells (white circles, n=3 week 0-4; n=1 week 6-12) were measured at different time points after pI-pC injection. Data are mean±SEM.

Methods. We isolate liver cells from Pggt1bfl/flKM and Pggt1b$^{+/+}$KM embryos and inject them into irradiated wild-type mice. Injection of pI-pC into mice receiving Pggt1b$^{+/+}$KM cells would turn on the expression of Cre - and K-RAS(G12D) - exclusively in the hematopoietic cells. We expected these mice to develop a K-RAS-induced MPD but not tumors in other tissues. In mice receiving Pggt1bfl/flKM cells, pI-pC should activate K-RAS(G12D) and simultaneously inactivate Pggt1b. In those mice we should be able to define the impact of inhibiting GGTase-I on the development of K-RAS-induced MPD. *Preliminary Results* Injections of pI-pC into mice receiving Pggt1b$^{+/+}$KM cells induced the development of MPD with leukocytosis, splenomegaly, and death, as predicted. K-RAS(G12D) expression was restricted to the hematopoietic cell compartment and no tumors have been detected in other tissues (n = 4 mice). Mice receiving Pggt1bfl/flKM cells showed reduced white blood cell counts (Figure 1) and splenomegaly, and improved survival. However, the mice still developed MPD, although with a prolonged latency. Genotyping of bone marrow from mice receiving Pggt1bfl/flKM (at 8 weeks) showed activation of K-RAS(G12D) but incomplete knockout of the Pggt1b allele. To overcome this incomplete Cre-inactivation of Pggt1b, we now use fetal liver cells with one conditional and one Pggt1b knockout allele (i.e, Pggt1bfl/-). In these cells, knockout of Pggt1b will be more efficient. *Conclusions.* We have tested a new approach to study mechanisms and treatment of K-RAS-induced MPD in mice. In this model, the potentially confounding effects of tumors in multiple other tissues are eliminated. We predict that we will now be able to define the impact of inactivating GGTase-I on the development of MPD in mice.

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0290

ANTILEUKEMIC ACTIVITY OF THE TYROSINE KINASE INHIBITOR BVT II +/- CYTARABINE OR DAUNORUBICIN IN ACUTE MYELOID LEUKEMIA

A Eriksson,[1] S. Bashir Hassan,[1] M. Höglund,[1] R. Larsson,[1] E. Lindhagen,[1] A. Åleskog,[1] K. Föhlenhag,[2] A. Jenmalm Jensen,[2] F. Lehmann,[2] A. Löthgren,[2] V. Parrow[2]

[1]Uppsala University, UPPSALA; [2]Discovery Research, Biovitrum AB, STOCKHOLM, Sweden

Backgroud. BVT II, a further development of the previously presented agent BVT I*, is a kinase inhibitor exhibiting activity against several receptor tyrosine kinases in enzymatic assays. *Aims*. Investigate the antileukemic activity of BVT II in hematological malignancies, as a single agent as well as in combination with two conventional antileukemic agents. *Methods*. To explore the *in vitro* activity of BVT II, the semiautomated fluorometric microculture cytotoxicity assay (FMCA) was used. The experiments were performed on a cell line panel as well as on primary tumor cells from 29 patients with hematological malignancies, (acute myeloid leukemia; AML; n=10), acute lymphocytic leukemia (n = 10) chronic lymphocytic leukemia (n = 9) and for comparison, normal peripheral blood mononuclear cells (PBMC; n=6). Combining BVT II with the AML drugs cytarabine and daunorubicin, possible synergistic effects, including sequence dependency, was studied using the FMCA In the *in vivo* experiments, semipermeable hollow fibers containing cells from 2 AML patients and the flt-3-positive AML cell line MV-4-11 were implanted subcutaneously in NMRI male mice. The animals were treated subcutaneously twice a day with 15 mg/kg of BVT II or vehicle only. The fibers were retrieved after 6 days and cell density assessed using the MTT-assay. *Results*. *In vitro*, cytotoxic activity was observed in cells from all hematological malignancies tested as well as in PBMC. AML tended to be the most sensitive cell type. Amongst the cell lines, the flt-3-positive AML cell line MV-4-11 showed the highest *in vitro* response towards BVT II in terms of EC50. *In vivo*, BVT II displayed significant antileukemic effect in both primary AML cells and MV-4-11 (Figure 1). No major toxicity, neither hematological nor non-hematological, was observed in the animals. Results from the combination studies (FMCA) suggest a sequence dependency, with better antileukemic activity when the cells were exposed to cytarabine or daunorubicin for 24 hours before adding BVT II. *Conclusions*. BVT II is a tyrosine kinase inhibitor displaying significant *in vitro* and *in vivo* effect in AML.

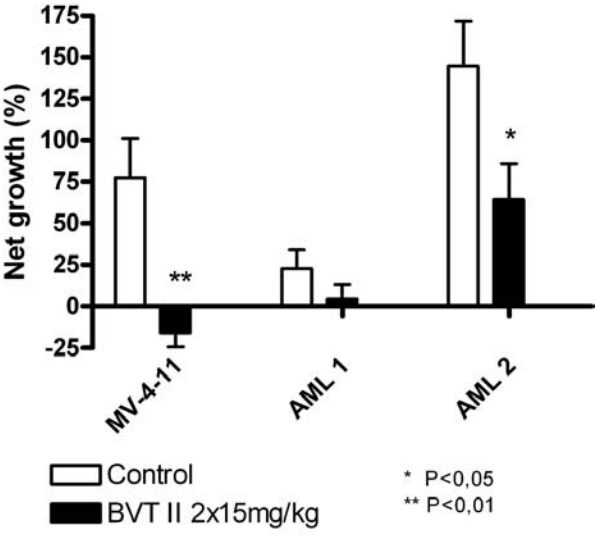

Figure 1. *In vivo* activity of BVT II against AML cells (cell line MV-4-11 and primary AML cells from two individual patients) in the mouse hollow fiber assay. Results are presented as net growth, defined as the percent change in cell density in the fibers during the 6 days of *in vivo* experiment.

0291

RITUXIMAB THERAPY FOR AUTOIMMUNE DISEASES

L. Flenghi, M. Capponi, P. Minga, L. Marcomigni, L. Stocchi, F. Falcinelli

Hematology and Clinical Immunology, PERUGIA, Italy

Background. Standard therapy for autoimmune diseases (drugs with non-specific immunosoppressive capacities) is associated with infections and toxicity. As the anti-CD20 monoclonal antibody Rituximab induces complement- or cell-mediated lysis of autoreactive lymphocyte clones, we assessed its efficacy in patients who were resistant to standard therapy for primary and secondary autoimmune hemolytic anemia (AIHA), autoimmune thrombocytopenia (AT), mixed cryoglobulinema (MC) and pemphygus (PEM). *Methods*. Intravenous Rituximab 375mg/m^2 once a week for 4 weeks for primary AIHA, AT, MC and PEM. Rituximab 375 mg/m^2 combined with cyclophosphamide 1 gr iv every fortnight for two months for secondary AIHA. *Patients*. Primary AIHA in 4 patients (2 with warm antibodies, 2 with cold); AIHA secondary to lymphoproliferative disorders in 8 patients (5 non-Hodgkins Lymphoma (NHL), 3 chronic lymphocytic leukaemia). All patients had previously received steroid therapy; 8/12 had received cyclophosphamide. All secondary AIHA had received chemotherapy; 2/8 had undergone autologous bone marrow transplantation. AT: 11 patients had idiopathic thrombocytopenic purpura (ITP), 1 had NHL, another had Sjogren's disease. All had platelet counts < 10,000/mmc, and had received steroids and high dose, intravenous immunoglobulin. Two had received Azathioprine in the previous 2 months. Chemotherapy had failed in the patient with NHL and splenectomy in 4 patients with ITP. MC was idiopathic in 1 and associated with HCV-related chronic hepatitis in 2. All presented arthralgia, asthenia, petechial lesions of the leg. Previous treatment included interferon α, plasmapheresis and steroids. PEM: paraneoplastic pemphygus was secondary to NHL in 1 patient with mucosal and cutaneous lesions; pemphygus vulgaris with cutaneous lesions was present in another. Previous therapy included steroids and azathioprine (with severe side effects). *Results*. AIHA: Haemoglobin levels increased in 11/12 patients. Steroids were suspended. Two patients achieved full response but relapsed after 18 and 8 months, respectively. After retreatment with Rituximab, 1 is in complete response after 50 months; the other relapsed after 5 months and underwent splenectomy. 1 non-responding patient died of AIHA. 9/12 patients achieved full response (12 gr/dL haemoglobin) one month after therapy and maintain it at a median follow-up of 53 months (range 4 - 106). AT: 9/13 patients reached normal platelet counts and suspended steroid treatment. 1 good responder requested a second Rituximab cycle after 16 months and obtained a second response and normal platelet count after 110 months. Median follow - up is 28 months (range 4-129). MC: Rituximab was suspended in 1 patient because of a persistent urticaria-type reaction. Cryocrit dropped from 82.7% to 44.0% with little clinical benefit. In another patient cryocrit fell from 156% to <30%. Rituximab retreatment was needed after 19 months. Cryocrit normalized (follow-up 6 months). In the third patient cryocrit fell from 60% to the present negative value after 40 months. Symptoms disappeared in the two responders. PEM: In both patients lesions improved and clinical remission was sustained in 1 patient for 52 months (without therapy) and in the other for 36 months (until death due to NHL). *Conclusions*. Rituximab seems safe and successful in patients with these autoimmune diseases who were resistant to standard immunosuppressive drugs.

Platelets and thrombocytopenia

0292

FAMILIAL THROMBOCYTOPENIA CAUSED BY A PRO-APOPTOTIC MUTATION OF CYTOCHROME C

M. Morison,[1] E.C. Ledgerwood,[1] E.M. Cramer,[2] P.L. Cheong,[1] G. Hughes,[1] A.J. Holyoake,[3] S. Fichelson[2]

[1]University of Otago, DUNEDIN, New Zealand; [2]Institut Cochin, INSERM U.567, PARIS, France; [3]Pacific Edge Biotechnology Ltd, DUNEDIN, New Zealand

Background. We have identified a large New Zealand family with mild thrombocytopenia, with normal MPV, inherited as an autosomal dominant condition. *Aims and Methods*. To determine the causative mutation and its mechanism, genetic, phenotypic, and biochemical assessments were performed. Results. To identify the causative mutation, linkage analysis and candidate gene sequencing revealed the first mutation of human cytochrome c, G41S. Recombinant G41S protein showed enhanced ability to activate caspase-3 within the intrinsic apoptotic pathway. In contrast, measures of its mitochondrial function and its structure were normal. Normal peripheral blood immature platelet fraction values indicated normal platelet survival. Electron microscopy of bone marrow showed intramedullary naked megakaryocyte nuclei and platelets indicative of dysregulated release into the marrow space rather than into sinusoids. Megakaryocytes were cultured from CD34$^+$ blood cells from two family members. Unusually numerous platelets, confirmed by EM, were produced as early as day 6. Normally CD34$^+$ cell-derived megakaryocytes produce visible platelets only after 10-12 days in culture. Flow cytometry showed a 10 fold increase in the ratio of platelet-like particles (CD41$^+$) to megakaryocytic cells at days 6 and 7 of culture. Conclusions. These results confirm the role of cytochrome c-mediated intrinsic apoptosis pathway activation in platelet formation. Affected individuals were otherwise healthy and long-lived indicating that thrombopoiesis is especially sensitive to changes in the intrinsic apoptosis pathway. Furthermore their phenotype implies that the presence of a more *active* cytochrome c has little or no effect on the apoptotic outcome in most organs during development and adult life.

0293

FIBRONECTIN FACILITATES VIABILITY EXPANSION AND MATURATION OF MEGAKARYOCYTE PROGENITORS AND CD34$^+$ HEMATOPOIETIC STEM/PROGENITOR CELLS FROM CORD BLOOD

V.R. Deutsch, E. Hubel, S. Kay, T. Ohayon, A. Many, E. Naparstek, D. Grisaru

Tel Aviv Medical Center, TEL AVIV, Israel

Background. Following cord blood (CBT) and bone marrow transplant (BMT) neutropenia and protracted thrombocytopenia remain serious clinical problems. CBT is primarily performed in children due to low numbers of hematopoietic stem/progenitor cells (HSPC) and megakaryocyte progenitors (MK-p). Platelet production following transplant depends on the availability of adequate numbers of cytokine responsive stem and megakaryocyte progenitor cells (MK-p). Thrombopoietin(TPO), had no clinical impact on thrombopoiesis in patients post BMT due to the paucity of MK-p in the grafts. If expanded, Mk-p would supply the appropriate target cells to maximize the effect of TPO and provide efficient earlier platelet engraftment. While CB CD34$^+$HSPC can be expanded, isolation of these cells from CB is not practical due to the limited number of stem and progenitor cells in the CB units. Additionally, MK expansion from purified stem cells requires long culture periods which are inappropriate for transplantation. *Aims*. To expand MK-p and HSPC, from small aliquots of whole CB, in short term cultures using progenitor enriched mononuclear cells (MNC). Growth conditions were designed to enhance viability and proliferation using fibronectin (FN), a major component of the bone marrow hematopoietic niche known to protect and stimulate HSPC, and new growth factor combinations which included the hematopoietic ARP peptide. *Methods*. Precursor enriched MNC (3) were expanded on fibronectin (FN) coated dishes in the presence of autologous plasma with various new cytokine combinations. These included r-hu-TPO, β- FGF, r-hu-SCF and ARP a peptide derived from the stress variant of acetylcholinesterase (AChE-R) recently discovered to have potent hematopoietic stem cell and MK growth factor activity (2,3). True expansion of MK, MK-p, HSPC and their subpopulations were characterized by high resolution flow cytometry on day 0 and 10 days of culture using SSC, CD45, CD41 and

CD34 and appropriate gating out of granulocyte and monocytes, which acquire CD41+ adherent platelets in culture. *Results*. High definition flow cytometry of the MK and myeloid progenitor subpopulation demonstrated that FN alone, increased the viability and expansion of MK, Mk-p (SSC^{low}/CD45-/^{dim}/$CD41^{high}$) and CD34+ HSPC. FN and ARP increased HSPC proliferation and drove Mk maturation measured by ploidy and GPIIb/IIIa expression. The combination of FN, thrombopoietin (TPO) and ARP produced a 4-6 fold increase in CD34+ HSPC, and a notable 40-70 fold increase in Mk-p, and CD34+/CD33+ HSPC. These conditions also enhanced CFU- Mk and multilineage CFU-GEMM. The MK-p expansion was calculated to potentially produce sufficient numbers of platelets to prevent bleeding in transplanted recipients. *Conclusions*. This study demonstrates for the first time that FN, maintains and expands thrombopoietic precursors and this effect is enhanced in the presence of TPO and ARP. High definition flow cytometry was used to resolve the true expansion of early MK and CD34+ progenitors and their subpopulations. We demonstrate that short term expansion of a small fraction of the CB unit is feasible, easy to perform and can comply with GTP regulations. Our novel approach may lead to improved cell therapy modalities to facilitate myelopoiesis and platelet production and shorten the neutropenia and thrombocytopenia post CBT.

0294

EFFICACY AND SAFETY OF REPEATED INTERMITTENT TREATMENT WITH ELTROMBOPAG IN PATIENTS WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

B. Psaila,[1] J. Bussel,[1] S. Vasey,[2] M. Aivado,[2] B. Mayer,[2] N. Stone,[2] M. Arning[2]

[1]*Weill-Cornell Medical College of Cornell University, NEW YORK, NY, USA;* [2]*GlaxoSmithKline, COLLEGEVILLE, PA, USA*

Background. REPEAT is an ongoing open-label phase II study evaluating the safety and consistency of response to repeated intermittent dosing of eltrombopag, a new oral non-peptide thrombopoietin receptor agonist, in adults with chronic ITP. *Aims*. This study was conducted to evaluate the safety and consistency of response to repeated intermittent administration of eltrombopag in patients with chronic ITP. *Methods*. Eltrombopag (50 mg) was administered to 66 patients with baseline platelet counts of 20K-50K/μL across 3 cycles of treatment. A cycle consisted of an on-therapy period of up to 6 weeks, followed by an off-therapy period of up to 4 weeks. Only patients responding (defined as platelets ≥50K/μL and 2x baseline) in Cycle 1 were allowed to continue with Cycles 2 and 3. The primary endpoint was consistency of response in Cycle 2 or 3, given response in Cycle 1. Written, informed consent was obtained from all study participants. *Results*. The median age of patients was 51 years; 71% were Caucasian; 68% were female; 44% and 45% had baseline platelet counts ≥20K-30K/μL and >30K-50K/μL, respectively; 30% were splenectomized; and 33% were receiving baseline ITP medication. 82% responded in Cycle 1, with 88% achieving the primary endpoint, 95% CI (72%, 97%). By Day 8 and 15 of each cycle, >60% and >75% of patients had responded, respectively. Off-therapy, median platelet counts remained ≥100K/μL 1 week after discontinuation and returned to near baseline after 2 weeks. A decrease in bleeding was observed in each on-therapy period vs baseline. Bleeding increased again during off-therapy periods, but remained lower than at baseline of Cycle 1. There were no SAEs reported. Headache was the most frequently reported AE (17%). After on-therapy periods, a transient decrease in platelet counts defined as *platelet count <10K/μL* or *<20K/μL and at least 10K/μL below baseline within 4 weeks of discontinuation of eltrombopag* was observed in 5/66 patients, and 13/66 patients, respectively. No bleeding AEs were reported in these patients during 4 weeks off-therapy. *Conclusion.* Repeated intermittent use of eltrombopag produced consistent and predictable responses in patients with chronic ITP. Eltrombopag appears well tolerated upon repeated administration with the same or greater counts with each subsequent cycle.

0295

DOSING LEPIRUDIN IN PATIENTS WITH HEPARIN-INDUCED THROMBOCYTOPENIA AND VARIOUS DEGREES OF RENAL FUNCTION IMPAIRMENT: A SINGLE-CENTRE EXPERIENCE WITH 68 PATIENTS

L. Alberio,[1] M. Tschudi,[2] B. Lämmle[2]

[1]*Department of Haematology and Central Haematology Laboratory, BERN, Switzerland;* [2]*Department of Haematology and Central Haematology Laboratory, Inselspital, BERN, Switzerland*

Introduction. Heparin-induced thrombocytopenia is a severe prothrombotic complication of heparin treatment. Lepirudin is a direct thrombin inhibitor approved for its treatment. Late in 2000 we observed that patients receiving the recommended doses of lepirudin were over-anticoagulated. *Aims.* To identify an adequate lepirudin dosing regimen for patients with various degrees of renal function impairment. *Methods*. Between 1.2001 and 2.2007 we treated 53 consecutive patients diagnosed with HIT administering lepirudin without initial bolus and reducing the recommended dose by 1/3. In addition, lepirudin dose was further adjusted according to calculated creatinine clearance (CCL). Intensity of anticoagulation was monitored with thrombin times (TT) and aPTT, its efficacy was assessed with measurements of the platelet count (PC) and D-dimers (DD). Based on this experience we defined an in-house regimen, which we prospectively validated from 3.2007 to 2.2008 treating 15 HIT patients. *Results*. Among the 53 patients of the first study phase, those with a normal renal function (CCL > 60 mL/min) which were within therapeutic range at first monitoring 4 hours after lepirudin start (17/29) received a median lepirudin dose of 0.078 mg/kg/h. The efficacy of this treatment was documented by a median aPTT-prolongation of 2.03 times at first control, by a PC increase from median 81 G/lL at d0 to 117 G/L (d1) and 272 G/L (d5), and by a decrease of DD from median 4599 mcg/L at day 0 to 1810 mcg/mL at follow-up. Patients with a moderately impaired renal function (CCL 30-60 mL/min) within therapeutic range at first monitoring (7/15) received a median lepirudin dose of 0.040 mg/kg/h. The efficacy of this treatment was documented by a median aPTT-prolongation of 2.09 times, by a PC increase from median 73 G/l (d0) to 82 G/L (d1) and 169 G/L (d5), and by a decrease of DD from median 3634 mcg/l to 1540 mcg/mL. Patients with a severely impaired renal function (CCL <30 mL/min) within therapeutic range (7/9) received a median lepirudin dose of 0.013 mg/kg/h. The efficacy of this treatment was documented by a median aPTT-prolongation of 2.11 times, by a PC increase from median 67 G/L (d0) to 82 G/L (d1) and 149 G/L (d5), and by a decrease of DD from median 2508 mcg/L to 1568 mg^3/mL. From these data we derived a dosing regimen (see conclusions), which was prospectively validated treating 15 HIT patients: all of them were within therapeutic ranges at first monitoring and experienced a resolution of the thrombocytopenia and a significant decrease of DD. *Conclusions*. The reported data confirm that the recommended dosage schedule for lepirudin is to high. We show that it is safe to avoid the first lepirudin bolus, and that administering lepirudin 0.08 mg/kg/h to patients with normal renal function, 0.04 mg/kg/h for those with moderate impairment and 0.01 - 0.02 mg/kg/h for those with severe impairment of renal function is an efficacious treatment for HIT.

0296

ORAL ELTROMBOPAG HELPS CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP) PATIENTS TO UNDERGO PROCEDURES WITHOUT BLEEDING OR ADDITIONAL TREATMENT TO RAISE PLATELET COUNTS

M. Tarantino,[1] J. Bussel,[2] M. Saleh,[3] N. Stone,[4] M. Arning[4]

[1]*Comprehensive Bleeding Disorders Center, PEORIA, ILA;* [2]*Weill-Cornell Medical College of Cornell University, NEW YORK, NY;* [3]*Georgia Cancer Specialists, ATLANTA, GA, USA;* [4]*GlaxoSmithKline, COLLEGEVILLE, PA, USA*

Background. Patients with chronic ITP may need additional treatment in preparation for planned diagnostic procedures or surgeries. Predictable, rapid platelet elevation for the duration of a hemostatic challenge would be instrumental in reducing dependence on transfusion and rescue medications. Eltrombopag is a new oral non-peptide thrombopoietin receptor agonist that has been shown to elevate platelet counts and reduce bleeding events in patients with chronic ITP. *Aims*. This analysis was conducted to determine whether eltrombopag treatment would allow patients with chronic ITP to undergo procedures without bleeding or additional treatment to raise platelet counts. Methods. Data collection on hemostatic challenges from 2 placebo (PBO)-controlled and 2 open-label eltrombopag trials (REPEAT and EXTEND) in patients with

pretreated chronic ITP. Written, informed consent was obtained from all study participants. *Results*. Data from 269 eltrombopag-treated patients and 67 PBO patients were analyzed. Three PBO patients and 22 eltrombopag patients had documented episodes of hemostatic challenges. In the PBO-controlled trials, 4 patients treated with eltrombopag had platelet responses >100K/µL and successfully managed 2 surgeries, 1 tooth extraction and 1 severe car accident without rescue medication or bleeding complications. Two PBO patients needed IVIg to elevate platelet counts prior to surgeries and 1 patient received tranexamic acid. In the open-label REPEAT study, 7 patients successfully managed hemostatic challenges without rescue therapy and unexpected bleeding (1 subject had both endoscopic sinus surgery and a cardiac catheterization, 1 patient each had TUR of the prostate and colon polypectomy, 2 patients had colonoscopies, and 2 patients had dental work). In EXTEND, 13 patients experienced ≥1 hemostatic challenge; 2 also faced a hemostatic challenge in a prior trial. The procedures included tooth repair, colonoscopies, arthroscopy, and uterine polypectomy. Two patients not responding to eltrombopag had rescue treatment (IVIg and cyclosporine) prior to the procedure; no unexpected bleeding was reported. *Conclusion*. Eltrombopag-stimulated platelet count elevation consistently allowed patients to successfully master hemostatic challenges without the use of rescue therapies, such as platelet transfusions; no excessive bleeding was reported indicating platelets produced in response to eltrombopag function normally.

0297

ELTROMBOPAG EFFECTIVELY ELEVATES PLATELETS IN PATIENTS WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP) REGARDLESS OF SPLENECTOMY STATUS

A. Newland,[1] J. Bussel,[2] N. Stone,[3] B. Mayer,[3] M. Arning[3]

[1]Barts and the London NHS Trust, LONDON, UK; [2]Weill Medical College of Cornell University, NEW YORK, NY, USA; [3]GlaxoSmithKline, COLLEGEVILLE, PA, USA

Background. Splenectomy is a standard second-line therapy for patients with chronic ITP. Patients who have refractory disease following splenectomy have a significant unmet medical need, with limited options for treatment. Eltrombopag is a new oral thrombopoietin receptor agonist that has been shown to increase platelet counts to ≥50,000/µL during short-term treatment (up to 6 weeks) in the majority of patients with chronic ITP. *Aims.* To evaluate the impact of splenectomy status on the response to eltrombopag in patients with chronic ITP. *Methods*. The importance of splenectomy status on response to eltrombopag in patients with chronic ITP was analyzed in 2 placebo-controlled trials that enrolled 164 patients to a starting dose of 50 mg eltrombopag once daily and 67 to placebo. Patients had at least 1 prior ITP therapy, which may have included splenectomy and a baseline platelet count of <30,000/µL. Prospective treatment comparisons were performed adjusting for stratification of splenectomy status at randomization. Written, informed consent was obtained from all study participants. *Results*. In the placebo-controlled trials, 28/67 placebo-treated patients (42%) and 46/106 eltrombopag-treated patients (43%) were splenectomized. Fifty-nine percent of splenectomized patients who received eltrombopag in the placebo-controlled trials achieved platelet counts ≥50,000/µL, compared with 64% of non-splenectomized eltrombopag-treated patients. Statistical significance was calculated and no significant interaction between response and splenectomy status was observed (p=0.661). *Summary and Conclusions*. This analysis demonstrated that with up to 6 weeks of treatement eltrombopag raises platelet counts to an equivalent degree in patients with chronic ITP regardless of splenectomy status. Eltrombopag may provide an effective treatment option for chronic ITP patients including those with refractory disease post splenectomy.

0298

SERUM THROMBOPOIETIN LEVELS IN PATIENTS WITH LIVER CIRRHOSIS; RELATION TO PLATELET COUNT, PLATELET FUNCTIONS SPLEEN SIZE AND SEVERITY OF THE DISEASE

M. Awad, S. EL- Sayed, M. EL-Nahas, H. EL-Askalany, T. Abdel- Hamid

Mansoura Unversity, MANSOURA, Egypt

Background. Thrombopietin (TPO) is an important regulator of megakaryocyte maturation and platelet production. The role of TPO (which is mainly produced by the liver) in thrombocytopenic cirrhotic patients is still under investigation. The aim of this study was to measure the serum TPO levels in cirrhotic patients and examine its relationship with circulating platelet count, platelet functions, splenic size and severity of disease. *Material and methods.* This study was conducted on 88 subjects, divided into 2groups, group 1 (patient group) included 72 patients with liver cirrhosis (diagnosed by combination of clinical, laboratory, ultrasound and histopathological data), they were further divided into 2 subgroups, group IA: included cirrhotic patients with thrombocytopenia (36 patients, 28 males and 8 females with age 50.3±years) , and group IB: included cirrhotic patients with normal platelet count (36 patients,26 males and 10 females, with age 50.64± 6.8 years) Group II comprised 16 healthy persons with matched age and sex used as a control group. AII included persons were subjected to: through history taking, full clinical examination, beside the following investigations: complete blood picture, kidney and liver function tests, Hepatitis Band C markers, platelet count and functions (aggregation to ADP, epinephrine and collagen) serum TPO level (Shinohara *et al.* 1996) and abdominal Doppler ultrasound. The following invasive investigation were done for group I(Patients) only: bone marrow aspiration, upper gastrointestinal endoscopy, sigmoidoscopy and liver biopsy (the latter was done for 21 patients only). Patients with pure schistosomal fibrosis were excluded from the study. Patients were classified according to the child- Pugh score into 3 classes of clinical severity: A, b and C. *Results*. Cirrhotic patients were thrombocytopenic in comparison to control (p<0.0001).Serum TPO levels were lower in cirrhotic patients (130.6±79 pg/mL) than control group (225.5±36 pg/mL) (p<0.0001) and also in patients with thrombocytopenia with normal platelet count (160.2±70.3 pg/mL) (p<0.0001). TPO had a significant positive correlation with platelet count (p=0.0001 for subgroup IA & p=0.04 for subgroup IB)TPO has significant positive correlation with platelet aggregation to ADP, epinephrine and collagen in group 1A and 1B but insignificant correlation in group 11. However serum TPO did not correlate with spleen size. Splenic size had a significant negative correlation with platelet count in cirrhotic patients (p=0.03 for subgroup IA& p=0.004 for subgroup IB). [In cirrhotic patients, serum TPO levels were found to be decreased as the disease progressed in subgroup IA, 188.25±23.28 pg/mI in class B and 51±26 pg/mL in class C ,while in group IB, 247.3±40.49 pg/mL in class A, 121.3±29.6 pg/mL in class C]. Child-Pugh score has a significant negative correlation with TPO level in both subgroups IA &IB (p=0.0001) and with platelet count (p=0.0001 for subgroup IA and 0.01 for subgroup IB) but no significant correlation with spleen size. In comparing class A, B C in both subgroups (IA & IB), spleen size was significantly larger in child class A of subgroup IA when compared to same class of subgroup IB (p=0.0001) with slight significant decrease in TPO in class A of subgroup I than class A of subgroup B (p=0.02). *Summary and Conclusion*. We concluded that low TPO production may play a role, along with hypersplenism, in the development of thrombocytopenia in patients with liver cirrhosis, In early stage of cirrhosis (Child- Pugh class A), splenomegaly and hypersplenism may be the main pathomechanism of thrombocytopenia. While advanced liver cirrhosis (Child- Pugh class B & C), causing more reduction in TPO production, plays a central role in the pathogenesis of thrombocytopenia.

0299

LESS CHRONIC ITP AFTER IVIG FOR ACUTE CHILDHOOD ITP ? A MATCHED PAIRS ANALYSIS FROM REGISTRY I OF THE INTERCONTINENTAL ITP STUDY GROUP (ICIS)

R.Y.J. Tamminga,[1] W. Berchtold,[2] M.C.A. Bruin,[3] G.R. Buchanan,[4] T. Kühne[5]

[1]University Medical Centre Groningen, GRONINGEN, Netherlands; [2]University of Applied Sciences of Aargau, BRUGG, Switzerland; [3]Wilhemina Children's Hospital, UTRECHT, Netherlands; [4]University of Texas, Southwestern Medical Center, DALLAS, TEXAS, USA; [5]University Children's Hospital, BASEL, Switzerland

Background and aims. Childhood immune thrombocytopenic purpura (ITP) is a bleeding disorder caused by destruction of platelets with a very small risk of a life threatening bleeding. ICIS Registry I was initiated in 1997 to obtain worldwide prospective data on the natural history of ITP in children. The first analyses of ICIS data showed that 25-47% of the children developed chronic ITP (defined as a platelet count < 150×10^9/L 6 months after diagnosis) depending on age. Previous studies in small numbers of patients suggested that intravenous immunoglobulin (IVIG) treatment at diagnosis might reduce the risk of chronic ITP. Therefore, the ICIS Registry I (with data on >2000 patients) was used to examine prognostic factors related to chronic ITP. *Methods and results*. Of the 2605 children with newly diagnosed ITP, between 3 months and 16 years of age, in 1984 the platelet count 6 months after diagnosis was known; 630 (32%) had a platelet count <150×10^9/L. Patient and clinical characteris-

tics associated with a significantly (univariate analysis, $p<0.001$) higher risk of chronic ITP were: age (<2 yrs: 22%; 2-10: 30%; >10:49%), platelet count at diagnosis (<10×10^9/L: 25%; 10-20: 37%; 20-50: 40%; >50:50%) and previous infection (yes: 27%; no 38%). Gender was not of prognostic significance. Investigating the effect of treatment, we performed a matched pairs analysis: two groups were composed, one with platelets >150×10^9/L (controls) and one with platelets <150×10^9/L (cases) at six months from diagnosis, but matched with regard to gender, age, previous infection, platelet count at diagnosis and country of origin. We identified 449 matched pairs. The group with chronic ITP had been more often treated at diagnosis with steroids and less often with IVIG than the controls; the difference between IVIG and steroid treatment was highly significant (p=0.003); patients who were treated with both IVIG and steroids behaved like those with steroids alone; patients without any initial drug treatment behaved in between. Odds ratio (OR) for not having chronic ITP was for IVIG: 1.8 (95% confidence interval: 1.25-2.64) and for steroids 0.60 (0.42-0.87). *Summary and conclusions*. Data from the worldwide ICIS Registry I also suggest that IVIG treatment of acute childhood ITP might reduce the risk for chronic ITP while steroids might increase that risk. However, caution is warranted because this study was not a randomized controlled trial, and a considerable number of patients could not be included in the final analyses. A prospective randomised study is needed to confirm these results.

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0300

SPLENECTOMY FOR IMMUNE THROMBOCYTOPENIC PURPURA (ITP) OR TRAUMA AND RISK OF INFECTIONS, VENOUS THROMBOEMBOLIC EVENTS AND DEATH

W.M. Schoonen,[1] R.W. Thomsen,[2] D. Körmendiné Farkas,[2] A. Riis,[2] J.P. Fryzek,[3] H.T. Sørensen[2]

[1]Amgen, UXBRIDGE, MIDDLESEX, UK; [2]Aarhus University Hospital, AARHUS, Denmark; [3]Amgen Inc., THOUSAND OAKS, CA, USA

Background. Immune thrombocytopenic purpura (ITP) is an autoimmune disorder characterised by an increased risk of bleeding due to impaired production and increased destruction of platelets. Destruction of platelets primarily takes place in the spleen; therefore, splenectomy is used in treating ITP. Splenectomy is associated with an increased risk of infections and possibly other adverse outcomes, including death, pulmonary embolism and infarction. *Aims*. The risk of adverse outcomes among splenectomised ITP patients compared to unsplenectomised population controls was estimated. Additionally, to understand how these risks may differ for ITP patients *vs* patients splenectomised for other reasons, patients splenectomised due to splenic traumatic rupture were compared to population comparisons. *Methods*. We identified patients who underwent splenectomy for either ITP or traumatic rupture between 1996 and 2005 in Danish nationwide medical databases. For each splenectomised person we identified 10 un-splenectomised population comparisons, frequency-matched on age, sex, and index date of splenectomy. We determined occurrence of death, hospitalised infection overall and pneumonia in specific, and venous thromboembolic events in splenectomised *vs* un-splenectomised persons within 90 days, 91 to 365 days, and more than 365 days, using multivariate regression for confounder adjustment. *Results*. We identified 269 splenectomised ITP patients and 765 patients with splenectomy due to trauma. Compared to the general population, relative risk of all studied adverse outcomes was highest within 90 days of splenectomy for both the ITP and trauma patient groups. In this 90 day period, death was the adverse outcome with the highest risk for trauma patients (relative risk (RR)= 125.5; 95% Confidence Interval (CI) 61.0-258.1). In later time periods the risk declined but remained 2-fold increased. Risk of death was also increased among ITP patients within 90 days of splenectomy (RR= 33.07; 95% CI 7.4-146.3) but was non-significantly increased in later time periods (Table 1). Risk of hospitalised infections remained elevated across all time periods for both ITP patients and trauma patients. More than a year after splenectomy, relative risk of infections was 3.5-fold increased for ITP patients (95% CI=2.6-4.8), and 2.5-fold increased for trauma patients (95% CI=2.0-3.2). No splenic vein thromboses were observed. However, risk of any venous thromboembolic events was strongly increased for trauma as well as ITP patients. Within 90 days of splenectomy, risk was 52-fold increased for ITP patients and 41-fold for trauma patients. Again, risk declined in later time periods but remained 3-fold increased more than a year after splenectomy. *Summary/conclusions*. Splenectomy is associated with strongly increased relative risks of hospitalised infections, death, and thromboembolic events in the 90 days following the procedure. Although risks decline in later time periods, they remain elevated compared to the general population for both ITP and trauma patients.

Table 1. Risk of adverse outcomes of splenectomy.

	ITP patients compared to general population			*Trauma patients compared to general population*		
	Splenectomised ITP patients (N=269), (%)	*Comparison patients (N=2690), (%)*	*RR (95%CI)*+*	*Patients Splenectomised after traumatic rupture of the spleen (N=765), (%)*	*Comparison patients (N=7650), (%)*	*RR (95%CI)*+*
Adverse events within 90 days after splenectomy						
Any hospitalised infection°	12 (4.5)	10 (0.4)	12.7 (5.0-32.4)	37 (4.8)	32 (0.4)	13.4 (8.0-22.3)
Hospitalised pneumonia	6 (2.2)	5 (0.2)	13.5 (3.0-59.7)	22 (2.9)	12 (0.2)	22.6 (10.7-48.1)
Other hospitalised infection	6 (2.2)	5 (0.2)	11.3 (3.1-40.8)	18 (2.4)	20 (0.3)	10.0 (5.0-19.8)
Thromboembolic events	4 (1.5)	1 (0.0)	52.4 (5.0-552.8)	8 (1.0)	2 (0.0)	41.3 (8.6-199.3)
Pulmonary embolism & infarction	2 (0.7)	1 (0.0)	27.1 (1.8-412.0)	2 (0.3)	0 (0.0)	N/A
Phlebitis & thromboplebitis	1 (0.4)	0 (0.0)	N/A	5 (0.7)	1 (0.0)	49.4 (5.6-435.4)
Portal vein thrombosis	1 (0.4)	0 (0.0)	N/A	0 (0.0)	0 (0.0)	N/A
Other venous embolism & thrombosis	0 (0.0)	0 (0.0)	N/A	1 (0.0)	1 (0.0)	10.0 (0.6-173.4)
Splenic, vein thrombosis	0 (0.0)	0 (0.0)	N/A	0 (0.0)	0 (0.0)	N/A
Death	7 (2.6)	4 (0.1)	33. (7.4-146.3)	82 (10.7)	11 (0.1)	125.5 (61.0-258.1)
Adverse events within 90 days after splenectomy						
Any hospitalised infection	11 (4.1)	20 (0.7)	6.5 (2.9-14.6)	23 (3.0)	70 (0.9)	3.6 (2.2-5.9)
Hospitalised pneumonia	2 (0.7)	10 (0.4)	1.2 (0.2-9.4)	11 (1.4)	30 (0.4)	3.7 (1.8-7.9)
Other hospitalised infection°	10 (3.7)	12 (0.4)	11.6 (4.6-29.2)	15 (2.0)	44 (0.6)	3.9 (1.8-22.6)
Thromboembolic events	2 (0.7)	7 (0.3)	4.1 (0.8-21.6)	4 (0.5)	7 (0.1)	6.3 (1.8-22.6)
Pulmonary embolism & infarction	1 (0.4)	2 (0.1)	6.4 (0.5-89.8)	0 (0.0)	1 (0.0)	N/A
Phlebitis & thromboplebitis	2 (0.7)	5 (0.2)	7.1 (1.2-43.3)	3 (0.4)	6 (0.1)	5.7 (1.3-24.4)
Portal vein thrombosis	0 (0.0)	0 (0.0)	N/A	1 (0.1)	0 (0.0)	N/A
Other venous embolism & thrombosis	0 (0.0)	0 (0.0)	N/A	1 (0.1)	1 (0.0)	N/A
Splenic, vein thrombosis	0 (0.0)	0 (0.0)	N/A	0 (0.0)	0 (0.0)	N/A
Death	3 (1.1)	16 (0.6)	1.7 (0.5-6.4)	14 (1.8)	61 (0.8)	2.6 (1.4-4.9)
Adverse events within 90 days after splenectomy						
Any hospitalised infection	56 (20.8)	176 (6.5)	3.5 (2.6-4.6)	92 (12.0)	421 (5.5)	2.5 (2.0-3.2)
Hospitalised pneumonia	29 (10.8)	84 (3.1)	3.5 (2.2-5.4)	35 (4.6)	184 (2.4)	2.2 (1.5-3.2)
Other hospitalised infection°	40 (14.9)	110 (4.1)	3.8 (2.6-5.6)	70 (9.2)	284 (3.7)	2.8 (2.1-3.6)
Thromboembolic events	7 (2.6)	24 (0.9)	2.9 (1.2-6.9)	19 (2.5)	68 (0.9)	2.9 (1.7-5.0)
Pulmonary embolism & infarction	3 (1.1)	8 (0.3)	1.8 (0.4-8.3)	4 (0.5)	21 (0.3)	1.9 (0.6-5.8)
Phlebitis & thromboplebitis	5 (1.9)	14 (0.5)	4.4 (1.5-12.5)	15 (2.0)	42 (0.5)	3.7 (2.0-6.9)
Portal vein thrombosis	0 (0.0)	0 (0.0)	N/A	1 (0.1)	0 (0.0)	N/A
Other venous embolism & thrombosis	1 (0.4)	4 (0.1)	2.9 (0.3-27.6)	3 (0.4)	8 (0.1)	4.1 (1.0-16.4)
Splenic, vein thrombosis	0 (0.0)	0 (0.0)	N/A	0 (0.0)	0 (0.0)	N/A
Death	16 (5.9)	129 (4.8)	1.4 (0.8-2.3)	57 (7.5)	359 (4.7)	1.9 (1.9-2.5)

*RR, relative risk among splenectomised JTP patients and splenectomised patients with traumatic rupture of the spleen compared to population comparisons; N/A, non applicable; N/A listed when the number of events was too small to abtain a meaningful risk measure; + Adjusted for age, sex and comorbid conditions present at the time of splenectomy; °Hospitalised infections other than pneumonia.

0301

THE BURDEN OF CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP) IN ADULTS: RESULTS OF A CHART REVIEW OF 600 PATIENTS IN EUROPE

F. Rodeghiero,[1] M. Michel,[2] J. Besalduch,[3] D. Provan,[4] M. Rummel,[5] M. Aivado,[6] K. Grotzinger,[6] N.L. Papo,[6] T. Hultsch,[7] N. Höbel[7]

[1]Ospedale S Bartolo di Vicenza, VICENZA, Italy; [2]Henri Mondor Hospital, CRETEIL, France; [3]Hospital San Dureta, PALMA DE MALLORCA, Spain; [4]Queen Mary's School of Medicine and Dentistry, LONDON, UK; [5]Universitaetsklinikum Giessen und Marburg GmbH, GIESSEN, Germany; [6]Glaxo-SmithKline Research & Development, COLLEGEVILLE, USA; [7]Kendle GmbH, MUNICH, Germany

Background. The investigation and management of patients with chronic adult ITP varies widely and there is a lack of data on current treatment strategies in Europe. *Aims.* To contribute to a better understanding of the treatment and healthcare resource utilisation of chronic ITP patients in France, Germany, Italy, Spain and the UK. *Methods.* This is part of a comprehensive project investigating the burden of chronic ITP and which involves both expert opinion as well as real patient data. A retrospective chart review for 600 patients was performed in 4 to 8 secondary and tertiary treatment centres in each participating country. Investigators were asked to include in the study the most recent 40 adults seen at their site from 31-Mar-2007 to 01-Jan-2005 with a diagnosis of chronic ITP lasting more than 12 months. Patients' demographics, medical history, current treatments and side effects, as well as medical resource utilisation were abstracted from the patient's medical charts for the 12 months prior to their most recent visit. *Results.* The mean age of the total patient sample was 54.1 years, with 67% women and 33% men. Median time from the first diagnosis of ITP to the start of the observational period was 36 months. Prior to the observational period, 23% of patients had been splenectomised and the most frequently reported treatment was corticosteroids. During the observational period, 60% of all patients were treated and received 1-12 different medical treatments (mean 2.7). The most frequent reasons given for treatment were platelet count (65%) followed by bleeding symptoms (17%). Corticosteroids represented 45% of treatments given, followed by IVIG (10%), azathioprine (7%) and rituximab (3%). Splenectomies (6% of patients) and platelet transfusions (2% of patients) were rarely performed during the observational period. A further 2% of patients refused to undergo splenectomy during the period.

In addition to regular monitoring of platelet levels, 85% of patients visited their haematologist 1 to 4 times during the year of observation. The remaining patients saw their physician 5 to 14 times (mean 2.7). Main reasons for a visit were a low platelet count (42% of visits) and bleeding (12% of visits). Overall, 11% of patients required hospitalisation; 6% of these were in an intensive care unit. 96% of hospitalisations were due to ITP (low platelet count, 65%, bleeding 35%) and 4% of hospitalisations were due to ITP treatment related side effects. Mean duration of hospitalisation was 10.7 days. *Conclusions*. This retrospective chart review of 600 patients is the largest study to date of its kind in ITP and provides the first results of actual treatment practices, outcomes and medical resource utilisation in 5 major European countries. It showed that bleeding symptoms remained quite frequent among patients with chronic ITP even though two thirds of them were actively treated. Corticosteroids were the most widely used treatment, followed by IVIG. The study results clearly highlight the burden that ITP imposes on patients and on health care systems across Europe.

0302

TREATMENT SATISFACTION IN PATIENTS DIAGNOSED WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA

F. Rodeghiero,[2] A. Matzdorff,[3] J.F. Viallard,[4] N. Chowhan,[5] S.D. Mathias,[6] Z. Cong,[7] J. Isitt[1]

[1]*Amgen, Inc, NEWBURY PARK, USA;* [2]*Department of Hematology, S. Bortolo Hospital, VICENZA, Italy;* [3]*Dept. Hematology and Oncology, Caritasklinik St. Theresia, SAARBRUECKEN, Germany;* [4]*CHU de Bordeaux Hôpital, HAUT-LÉVÊQUE, France;* [5]*Cancer Care Center, Inc, NEW ALBANY, INDIANA, USA;* [6]*Health Outcomes Solutions, WINTER PARK, FLORIDA, USA;* [7]*The Pardee RAND Graduate School, LOS ANGELES, CALIFORNIA, USA*

Background. Immune Thrombocytopenic Purpura (ITP) in adults is often a chronic disorder that requires continuous monitoring of platelet counts, bleeding events, and treatment-related side effects. Current ITP treatment options for chronic ITP include corticosteroids, intravenous immunoglobulins (IVIg), rituximab, and clinical supervision in order to prevent bleeding events by appropriate treatments. Splenectomy is reserved for those patients who do not respond or relapse after these medical therapies, or require intolerable doses to achieve safe platelet counts. Treatment-related side effects may result in patient distress and negative outcomes. *Aims*. The purpose of this study is to demonstrate that treatment satisfaction in patients with chronic ITP does not significantly improve under current standard of care. *Methods*. We present the initial results from a prospective, observational, international multi-center study in patients' previously diagnosed with chronic ITP and consecutively enrolled in this study. Patients with a primary diagnosis of ITP were enrolled from both community-based clinics and academic/referral centers. Retrospective data from patients' charts provided date of 1st ITP diagnosis, treatments received, and medical history. ITP treatments, dose, response, and duration of response were collected prospectively for 12 months. Patients completed a validated self administered treatment satisfaction questionnaire for medication (TSQM) at enrollment and monthly thereafter for 12 months. The TSQM is composed of 14 items and consists of 3 domains (effectiveness, side effects, and convenience) and an overall satisfaction score.

Table 1.

Overall Treatment Satisfaction Score				
Treatment	TSQM Prior to Treatment	TSQM End of Treatment	Change	p-Value[1]
Corticosteroid	52.2	48.4	-3.8	0.46
Rituximab	68.5	72.6	4.2	0.59
IVIG	63.0	61.7	-1.3	0.12
Anti-D	55.0	56.4	1.4	0.62
Splenectomy	44.3	60.0	15.7	0.04

[1] For TSQM score prior to treatment vs. end of treatment, t-test on residuals for change scores adjusted for age, gender, number of treatments previously received, time since 1st chronic ITP diagnosis.

Higher scores signify greater patient satisfaction. Effects were estimated using linear regression with the final TSQM score as the dependent variable, and adjusting for age, gender, TSQM score before treatment starts, number of treatments previously received and time since 1st diagnosis of chronic ITP. *Results*. The first 165 patients enrolled in the study were from 38 U.S. centers (44% male; mean age 59 years). During the study evaluation period (at baseline or beyond), 39 patients (24%) were splenectomized and 128 (78%) received ITP-related treatments including corticosteroids (n=101), IVig (n=54), Rituximab (n=46), and Anti-D Antibody (n=40). Patient scores significantly increased in effectiveness (p=0.03) and overall satisfaction (p=0.04) in patients receiving a splenectomy (Table 1). Patient side effects scores decreased significantly in patients receiving corticosteroids (p=0.02), and increased significantly in patients taking IVIG (p<0.01), and Anti-D (p=0.04). No significant changes were observed in convenience scores among all treatments. *Summary and Conclusions*. Our study indicates that while patients receiving a splenectomy reported significant improvements in effectiveness and overall satisfaction, no other medication significantly improved patients' treatment satisfaction and corticosteroids significantly worsened in side effects scores. Novel ITP interventions are desirable to improve both treatment satisfaction and reported effectiveness in patients with chronic ITP.

0303

SHOULD THE THRESHOLD FOR PROPHYLACTIC PLATELET TRANSFUSION IN NEONATES BE STANDARDISED AT 20x10⁹/L

A. Murray,[1] A.G. Roberts,[1] A. Stanworth[2]

[1]*Imperial College, LONDON, UK;* [2]*National Blood Service, OXFORD, UK*

Background. Intraventricular haemorrhage (IVH) is the commonest major haemorrhage in neonates and is a leading cause of neonatal death and disability. Most IVH occurs in preterm neonates during the first week coinciding with their highest prevalence of thrombocytopenia. As a result thrombocytopenic neonates commonly receive prophylactic platelet transfusions (Tx). However no study has defined the appropriate trigger threshold in neonates. Therefore, neonates receive prophylactic platelet Tx over a wide range of counts, commonly between 20-60×10⁹/L,[1] without evidence of efficacy. *Aims.* To document major haemorrhage in neonates and assess its relation to severity of thrombocytopenia and platelet Tx. *Methods.* Prospective observational study of neonates with platelet counts <60×10⁹/L (NT<60) in 7 neonatal intensive care units (NICUs). Major haemorrhage was defined as grade 3/4 IVH (Papile grading) plus other fresh major haemorrhage (as below). Following parental consent neonates had daily recording of platelet count, haemorrhage, and platelet Tx. *Results.* 3498 neonates were admitted to participating NICUs. 194 developed NT<60 (5.5%) and 169 were enrolled: median gestational age 27 (range 23-41) weeks, birth weight 825 (427-3733) grams. Main associated conditions: sepsis n=71; maternal pre-eclampsia/fetal growth restriction (PET/IUGR) n=36; necrotising enterocolitis (NEC) n=9. Forty-five neonates had major haemorrhage. Twenty-six entered the study with grade 3/4 IVH but no previous NT<60 or platelet Tx. Twenty-seven (19 without previous haemorrhage, 8 with previous IVH) suffered major haemorrhage on study, at median 7 (range 2-61) days: IVH n=12, pulmonary n=7, PR n=3, abdominal n=2, haematuria n=2, IVH and pulmonary n=1. However, the platelet count nadirs in these 27 neonates, and their platelet counts prior to haemorrhage, did not trend towards the lower end of the 20-60×10⁹/L range (Figure 1).

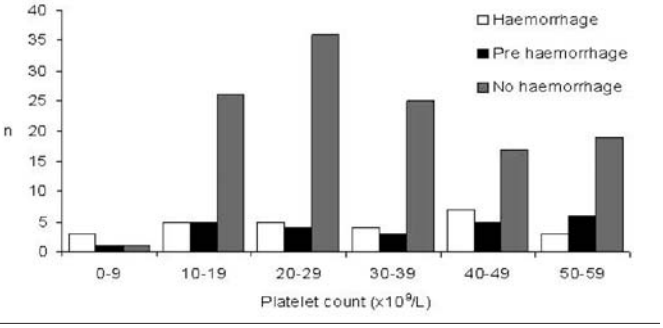

Figure 1. Lowest platelet counts in study neonates

Four hundred and fourteen platelet Tx were administered on study raising the median pre-transfusion count form 27 (range 2-75) to 79 (4-513)×10⁹/L. Neonates without major haemorrhage received 223 prophylactic Tx, those with major haemorrhage 115, 78 post-haemorrhage. Only 13 Tx were given to treat haemorrhage and 12 hours post transfusion manifestations of haemorrhage remained the same in 5 and reduced

in 8. The remaining 63 Tx were given for clinical reasons. Outcome differed by the cause of thrombocytopenia. Despite similar platelet nadirs, neonates with maternal PET/IUGR had on study bleeding and mortality rates of 4 and 7%, respectively, whereas those with sepsis/NEC had rates of 15 and 33%. *Conclusions.* These data suggest that in thrombocytopenic neonates without active bleeding the risk of major haemorrhage does not alter within the platelet count range 20-60×10⁹/L. Consequently prophylactic platelet Tx at thresholds above 20×10⁹/L may not alter outcome and Tx at these levels could be reserved for treatment of haemorrhage. Progress likely depends on reducing prophylactic Tx of doubtful benefit, and tailoring platelet Tx practice to the outcomes of different patient groups. Both objectives will require well-designed randomised controlled trials.

Reference

1. Del Vecchio et. al. Transfusion 2001;41:803-8.

0304

HEPATITIS C VIRUS INFECTION AND LOW PLATELET COUNT: A COMMUNITY-BASED STUDY IN A HEPATITIS B AND C ENDEMIC AREA

C.Y. Dai, C.K Ho, W.L Chuang, J.F Huang, M.Y. Hsieh, W.Y. Chang, M.-L. Yu

Kaohsiung Medical University, KAOHSIUNG, Taiwan

Background. Hepatitis C virus (HCV) infection has been considered as associated with abnormal blood count. *Aims.* To evaluate the association between virologic status and platelet count in individuals with HCV infection in a hepatitis B virus and HCV endemic area. *Methods.* A large-scale survey, enrolling 11,239 residents aged 40 to 65 years, was conducted in the Kaohsiung area of Taiwan. Platelet count, alanine aminotransferase (ALT) level, hepatitis B surface antigen (HBsAg) and anti-HCV were checked. For anti-HCV-positive subjects the serum HCV RNA was tested by using a standardized automated qualitative polymerase chain reaction assay. Non-invasive markers of fibrosis (FibroTest; FT) derived from five biochemical markers including α2-macroglobulin, apolipoprotein A1, haptoglobin, total bilirubin and γ-glutamyltranspeptidase by a quantitative assessment was computed on the website (http:\\www.biopredictive.com). *Results.* Overall, 703 (6.3%), 1536 (13.7%), 84 (0.7%) and 9,084 (80.8%) were positive for anti-HCV, HBsAg, and both anti-HCV and HBsAg, and negative for anti-HCV and HBsAg, respectively. Subjects with anti-HCV had significantly higher mean age and mean ALT value, lower mean platelet count, higher frequency of male sex, abnormal ALT and low platelet count than those who were anti-HCV-negative (all $p<0.001$). In multivariate logistic regression (MLR) analysis, the independent factors significantly associated with positive anti-HCV were lower age, female sex, abnormal ALT value and low platelet count. Subjects with positive HCV RNA had significantly higher mean ALT value, lower mean platelet count, lower frequency of positive HBsAg, and higher frequency of abnormal ALT value and low platelet count than those who were HCV RNA-negative (all $p<0.001$). In MLR analysis, the independent factors significantly associated with positive HCV RNA in anti-HCV subjects were abnormal ALT value, positive HBsAg and low platelet count. Subjects with a low platelet count had a significantly higher mean ALT value, higher frequency of abnormal ALT value and higher frequency of positive serum HCV RNA (all $p<0.001$). In MLR analysis, the independent factors significantly associated with low platelet count were abnormal ALT value and positive HCV RNA. A significant trend for higher prevalence of HCV RNA in patient groups with lower platelet count was demonstrated ($p=0.019$, chi-square for trend). The FT score was checked among 137 patients with positive anti-HCV (17 were HCV RNA-negative). When compared to patients with no or mild fibrosis, those with advance fibrosis had significantly lower platelet counts ($p=0.002$) and higher prevalence of positive HCV RNA ($p<0.001$). The mean platelet count was significantly lower and mean FT score and mean fibrosis stage were significantly higher among 120 HCV RNA-positive individuals than 17 HCV RNA-negative ones by univariate analyses (all $p<0.001$). In MLR analyses, lower platelet count and higher fibrosis stage were independent factors associated with positive HCV RNA. *Conclusions.* Based on this large-scale community study, our results implicate that platelets may be affected directly by HCV. Decreased platelet counts in anti-HCV-positive individuals strongly implies positive HCV RNA and is associated with elevated ALT levels, which indicates active HCV-related liver disease.

0305

ORAL ELTROMBOPAG INCREASES PLATELET COUNTS AND REDUCES BLEEDING IN CHRONIC ITP PATIENTS: POOLED RESULTS OF PHASE II AND PHASE III TRIALS

M.N. Saleh,[1] J.B. Bussel,[2] N. Stone,[3] B. Mayer,[3] J. Jenkins[3]

[1]Georgia Cancer Specialists, ATLANTA, GA; [2]Weill-Cornell Medical College of Cornell University, NEW YORK, NY; [3]GlaxoSmithKline, COLLEGEVILLE, PA, USA

Background. Current treatments for thrombocytopenia have a poor risk-benefit profile and often have no level 1 evidence of their tolerability or efficacy profile. Eltrombopag is the first-in-class oral non-peptide thrombopoietin receptor agonist, being developed as treatment for various thrombocytopenias, including chronic idiopathic thrombocytopenic purpura (ITP). *Aims.*This pooled analysis assessed the efficacy and safety of eltrombopag treatment in adult patients with chronic ITP. *Methods.* Efficacy and safety data from two independent double-blind, placebo-controlled studies in adult patients with chronic ITP have been pooled [Bussel *et al.* New Engl J Med. 2007; Bussel *et al.* Haematologica. 2007]. In both studies, patients were required to have a baseline platelet count <30K/μL. The primary endpoint was the proportion of patients with a platelet count ≥50K/μL after up to 6 weeks of treatment. Secondary endpoints included platelet count pharmacodynamics (PD), incidence and severity of bleeding (WHO bleeding scale), and safety parameters. Written, informed consent was obtained from all study participants. The first study was a Phase II dose finding study. 117 patients were randomized to one of 4 arms to receive once daily oral doses of placebo (n=29), 30 mg (n=30), 50 mg (n=30) or 75 mg (n=28) eltrombopag for up to 6 weeks. The second study was a Phase III study in which 114 patients were randomized (2:1) to one of 2 arms to receive once daily oral doses of placebo (n=38) or 50 mg (n=76) eltrombopag for up to 6 weeks. Randomization was stratified by concomitant ITP medication, splenectomy status, and baseline platelet count (>/≤ 15K/μL). *Results.* (Table 1). *Conclusions*. Eltrombopag, administered as a once daily tablet for up to 6 weeks, is an effective therapy for the treatment of ITP, increasing platelet counts to safe levels in more than 60% of the patients, while reducing the incidence and severity of bleeding symptoms. There were no clinically meaningful differences in incidence or severity of AEs between patients treated with eltrombopag 50 mg compared to placebo.

Table 1. Pooled Safety and Efficacy Data

Baseline Characteristics	Placebo (n=67)	50 mg (n=106)
Age, median (range)	46 (18-85)	47 (19-84)
Female, n (%)	43 (64)	64 (60)
Concomitant ITP medication, n (%)	23 (34)	44(42)
Splenectomy status, n (%)	28 (42)	46 (43)
Baseline platelet count ≤15K/μL, n (%)	31 (46)	50 (47)
At least 3 prior ITP therapies, n (%)	30 (45)	60 (57)
Median platelet count at Day 1 (range), K/μL*	17 (3-29) (n=50)	18 (0-30) (n=86)
Patients with any bleeding at Day 1*	38 (59) (n=64)	63 (62) (n=102)
Efficacy	**Placebo (n=65)**	**50mg (n=101)**
Patients with platelet count ≥50K/μL), n (%)	9 (14)	62 (62)
Median platelet count at Week 6 (range), K/μL	18 (2-419)	84 (1-1,312)
Patients with any bleeding at Week 6, n (%)	34 (56)	44 (43)
Safety	**Placebo (n=67)**	**50mg (n=106)**
Number of patients with an adverse event (AE)	35 (52)	70 (66)
Number of patients with a drug-related AE	16 (24)	31 (29)
Number of patients with a serious adverse event (SAE)	8 (12)	12 (11)
Number of patients with a SAE leading to withdrawal	4 (6)	3 (3)

* Based on efficacy population.

0306

ORAL ELTROMBOPAG SPARES CORTICOSTEROIDS AND REDUCES BLEEDING IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

J.B. Bussel,[1] G. Cheng,[2] M.N. Saleh,[3] B. Meddeb,[4] B. Mayer,[5] N. Stone,[5] M. Arning,[5] M. Aivado[5]

[1]Weill-Cornell Medical College of Cornell University, NEW YORK, NY, USA; [2]Prince of Wales Hospital, SHATIN, NT, Hong Kong; [3]Georgia Cancer Specialists, ATLANTA, GA, USA; [4]Hopital La Rabta, TUNIS, Tunisia; [5]GlaxoSmithKline, STOCKLEY PARK, UK

Background. Eltrombopag is a new oral non-peptide thrombopoietin receptor agonist shown to increase platelets and reduce bleeding symptoms during short-term placebo-controlled trials in chronic ITP patients. EXTEND is an ongoing, open-label study designed to assess the long-term clinical benefit of eltrombopag, including the potential for patients to reduce/eliminate concomitant ITP therapies. *Aims.* This analysis was conducted to determine the long-term clinical benefit of eltrombopag treatment, including the potential for patients with chronic ITP to reduce/eliminate the use of concomitant ITP therapies. *Methods*. In EXTEND, patients receive a starting dose of 50 mg eltrombopag/day that can be adjusted in response to platelet counts. Bleeding symptoms using the WHO Bleeding Scale were assessed at each visit. Written, informed consent was obtained from all study participants. *Results*. To date, 109 patients have received eltrombopag; at baseline, 37% were receiving concomitant ITP medication, 44% were splenectomized, 70% had baseline platelet counts <30K/μL, 17% between 30K-≤50K/μL, and 14% >50K/μL. 14 out of 40 patients (35%) taking concomitant ITP medications at baseline were able to stop ≥1 ITP med(s) (prednisone [n=10], danazol [n=4], and one subject each with dexamethasone, mycophenolic acid, and oxymetholone). The proportion of patients with any bleeding and any clinically significant bleeding (WHO Grade 2-4) decreased from baseline as platelet counts increased. At baseline, >60% of patients had any bleeding and >20% of patients reported clinically significant bleeding. After 12 weeks, <30% of patients had any bleeding and <5% had clinically significant bleeding. For the majority of weeks, the proportion of patients with any bleeding or clinically significant bleeding was reduced by approximately half from baseline. *Conclusion*. The findings from this ongoing study demonstrate the clinical benefit of eltrombopag during long-term treatment in patients with chronic ITP, as measured by reduction of both concomitant ITP medications and bleeding symptoms.

0307

RISK OF THROMBOEMBOLIC EVENTS AMONG PATIENTS WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

I. Bennett,[1] U. Forssen,[1] C. Enger,[2] J. Nelson[1]

[1]GlaxoSmithKline, PHILADELPHIA, USA; [2]I3 Drug Safety, ANN ARBOR, USA

Background. ITP is a disease caused by inadequate platelet production as well as increased platelet destruction. There is very little published data on the risk of TE in ITP patients. *Aims*. To examine the risk of TE among patients with chronic ITP compared to a non ITP population. *Methods*. This was a retrospective database analysis using eligibility and medical claims data from a large U.S. health plan affiliated with i3 Drug Safety. Chronic ITP patients were defined using the following criteria: a) at least two physician claims separated by at least six months with ICD-9 CM diagnosis code 287.3x for primary thrombocytopenia, b) at least 12 months of continuous enrollment prior to the date of the diagnosis code eligibility, and c) at least 18 years of age between January 1, 2000 and September 30, 2006 with follow-up through December 31, 2006. The non ITP reference group was selected from the same time period. TE were defined as deep vein thrombosis (DVT), pulmonary embolism (PE), myocardial infarction (MI), unstable angina (UA), ischemic stroke (IS), transient ischemic attack (TIA), portal vein thrombosis (PVT), and other TE using ICD-9 CM diagnosis codes. Patients in both groups with a history of TE during the 12-month baseline period were excluded from the analysis. The incidence rate ratio (IRR) and 95% Confidence Interval (CI) of occurrence of TE comparing the ITP to non ITP populations was estimated using Poisson regression. *Results*. All chronic ITP patients (N=2,873) who met the above mentioned criteria formed the ITP cohort; N=116,933 non ITP patients created the reference group. Among the ITP patients, 197 (6.9%) patients experienced at least one TE during a median follow up of 15 months. Among the non ITP patients, 4,027 (3.4%) developed at least one TE during follow up (median 17 months). After adjusting for age, gender, history of hypertension, and past and recent history of steroid use, the cumulative IRR comparing the ITP to non-ITP patients for any TE was 1.65 (95% CI: 1.42-1.91). The adjusted cumulative IRR was significantly greater than one for the individual TE such as unstable angina (IRR 1.45; 95% CI: 1.11-1.89), ischemic stroke (IRR 2.21; 95% CI: 1.48 - 3.32), transient ischemic attack (IRR 1.70; 95% CI: 1.30 - 2.23), portal vein thrombosis (IRR 37.82; 95% CI: 12.39-115.41), and other TE (IRR 2.01; 95% CI: 1.60-2.53). There was increased risk for DVT (IRR 1.69; 95% CI: 0.88-3.25) and PE (IRR 1.52; 95% CI: 0.64-3.62) in chronic ITP patients *vs* the non ITP population that did not reach statistical significance. There was no increase in risk for MI (IRR 0.98; 95% CI: 0.66-1.45). *Summary*. The study found an association of increased risk for thromboembolic events in patients with chronic ITP compared to the non ITP population.

0308

RETROSPECTIVE SAFETY REVIEW OF IV RHO (D) IMMUNE GLOBULIN

C.J. Sinclair, C.J. Sinclair, C. Muller, H. Li, M.G. Genereux

Cangene Corporation, WINNIPEG, Canada

Background. Cangene IV Rho (D) Immune Globulin (IV RhIG), a purified human Anti-D immunoglobulin, has been licensed to treat immune thrombocytopenic purpura (ITP) for over 12 years in North America and recently in numerous European countires. In pivotal studies, IV RhIG demonstrated strong efficacy characteristics and duration of response, with common side effects being headache, chills and fever. Since licensure, rare haemolytic reactions have been reported through post-marketing surveillance, ranging from mild haemoglobin decrease to intravascular haemolysis (IVH) and its potential complications. *Aims*. Cangene evaluated the reporting rate of haemolytic reactions and assessed if there was any contribution of gender, age, dosing, or previous medical history to the severity of haemolytic reactions. *Methods*. Data from the Cangene's IV RhIG pharmacovigilance database was analyzed. Haemolytic reactions were characterized as definite, probable or possible IVH using an internal algorithm. Possible and probable cases are regarded as other haemolytic reactions. *Results*. Since January 1994 to December 2007, 57 Definite and 184 Probable or Possible IVH events have been reported. Using the estimated number of doses administered in this period (2,500,000), reporting frequency of definite IVH is 0.0023, and other haemolytic reactions are 0.0074, with an overall total of 0.0097. Gender was analyzed for 238 cases (3 unknown), of which females contributed 55%. Age group was analyzed for 216 cases (25 unknown), with 0-20, 20-50 and >50 years of age representing 31%, 20% and 49%, respectively. Dosing in (mcg/kg) was analyzed for 175 cases (66 cases were unknown), with 6%, 55% and 39% of patients receiving <40, 40-60 and >60 mcg/kg, respectively. The overall dose used in the IVH group was 60 mcg/kg and for patients with other haemolytic reactions was 56 mcg/kg. A review of medical history was conducted in 179 cases (62 cases did not have complete medical history) to evaluate whether specific conditions may increase the severity of haemolytic reactions. Pre-existing haematologic malignancies were reported in 15% of definite IVH cases and in 10% of other haemolytic reactions; hepatic dysfunction was reported in 19% of definite IVH cases and in 8% of other haemolytic reactions; pre-existing viral infection was reported in 19% of definite IVH cases and in 8% of other haemolytic reactions; autoimmune disorders were reported in 7% of definite IVH cases and in 5% of other haemolytic reactions. *Conclusions*. Based on a 12 year period of Cangene IV RhIG safety data, the reporting rate of overall haemolytic reactions such as definite IVH is rare. No significant reporting rate differences by gender or dose are apparent, while the relationship with age needs to be further analyzed. As reported in recent literature, pre-existing medical conditions including hepatic dysfunction, recent viral illness or haematologic malignancy may contribute to haemolytic events; however, the low number of cases and incomplete data reported in the passive system make conclusions difficult.

0309

ALTERATIONS OF MORPHO-FUNCTIONAL STATUS IN CIRCULATING PLATELETS ASSOCIATED WITH THROMBOCYTOPENIAS

I. Vasilenko,[1] I. Kastrikina,[1] E. Pustovaya[2]

[1]Institute of Rheumatology RAMS, MOSCOW, [2]Hematological Research Center, MOSCOW, Russian Federation

Quantitative platelet disorders are always associated with qualitative platelet alterations. The circulating platelet multiplicity reflects cell distinctions in size, density, metabolic, functional, biochemical features and the level of megakaryocyte polymorphism. The relation between this platelet attributes is extensively discussed but the some details are sufficiently unclear. The purpose of this study was to evaluate the various aspects of platelet heterogeneity and activity in patients with idiopathic thrombocytopenic purpura (ITP) and secondary thrombocytopenias. Platelet-rich plasma was separated from blood samples of healthy volunteers (20), patients with immune thrombocytopenic purpura (15) and secondary thrombocytopenias associated with antiphospholipid syndrome (APS) (15) and lupus erythematosus (LE)(12) by centrifugation at 1000 g for 10 min at room temperature. Morpho-functional status of peripheral blood platelet we determined by express-method of vital computer morphometry using computer phase-inteference microscope Cytoscan: height accuracy 0,5nm, coordinate accuracy 10nm, image area 256×256 pixels, optical magnification 1000, acquisition time 4-12 sec. The complex algorithm of morphometry included definition of optic and geometrical characteristics of unfixed and unstained living platelets, statistical analysis of data and creation of medical documents. We have analyzed the optic-geometrical parameters of each isolated living platelet and the distribution of platelets by sizes to detect the heterogeneity of cell population. It allowed to identify four platelet forms that have different morphological features and different parameters of size distribution. In the cell population we distinguished 4 morphologic forms of platelet with according to various activation levels. So 64% of resting platelets (discoid forms), 21% of platelets with low activation level, 12% of platelets with high activation level and 3% of degenerate functionally incomplete platelets were registered in healthy volunteers. In ITP, APS and LE patients had the high level of platelet activating state: 48, 50, 45%-the resting platelets; 41, 42, 45%-activating forms; 11, 8, 10%-degenerating forms, respectively). The average metric platelet parameters (diameter, perimeter, height, area and volume) in ITP were constituted 3,2±0,9 mkm; 9,4+/2,7 mkm; 1,1±0,4 mkm; 6,0±3,0 mkm^2; 2,9±2,0 mkm^3 (M±σ) contrary to 2,6±0,8 mkm; 8,2+/3,4mkm; 1,3±0,5mkm; 4,6±2,1 mkm^2; 1,8±1,3mkm^3 in norm (p<0,05). The average metric platelet parameters in APS and LE were constituted 3,2±1,2 mkm; 10,4+/5,1 mkm; 1,5±1,0 mkm; 7,5±3,7mkm^2; 3,3±2,9mkm^3 (M±σ) and 3,7±1,5 mkm; 12,0+/3,3 mkm; 0,9±0,4 mkm; 8,7±2,7 mkm^2; 2,5±1,7 mkm^3 (M±σ), respectively. It is important the values of phase height objectively reflected platelet granule contents in platelets. The subpopulation of giant platelets (with diameter>5 mkm) were revealed and described in patient with thrombocytopenias: 10, 18, 27% in ITP, APS and LE, respectively. Furthermore we discriminated giant platelets with low and high density corresponding cells with reduced or enlarged significances of phase high. The relation between the activating platelet status, platelet count and percentage of giant platelet is well established. Computer phase interference morphometry of living platelets provides the sufficient degree of objectivity and information for the real evaluation of the haemostatic state of patients and allowed to prognosticate the possible disturbances. Advances in the understanding of the platelet structure features in ITP and secondary thrombocytopenias will promote to improving our knowledge of pathogenesis for this diseases.

0310

RAPID INCREASE OF PLATELET COUNT WITH IGPRO10 IN CHRONIC IMMUNE THROMBCYTOPENIC PURPURA

A. Salama,[1] T. Robak,[2] L. Kovaleva,[3] Y. Vyhovska,[4] S.V. Davies,[5] M.G. Mazzucconi,[6] O. Zenker[7]

[1]Charité University Medicine Berlin, BERLIN, Germany; [2]Medical University of Lodz, LODZ, Poland; [3]Russian Academy of Medical Sciences, MOSCOW, Russian Federation; [4]Institute of Blood Pathology and Transfusion Medicine AMS, LVIV, Ukraine; [5]Taunton and Somerset Hospital, TAUNTON SOMERSET, UK; [5]University La Sapienza, ROMA, Italy; 7CSL Behring, BERN, Switzerland

Background. Immune thrombocytopenic purpura (ITP) is an autoimmune bleeding disorder with an estimated annual incidence of 55-66 new cases per 1,000,000 individuals. The condition is characterized with decreased counts of circulating platelets due to antibody-mediated platelet destruction. Intravenous immunoglobulin (IVIG) is a widely recommended treatment for both acute and chronic ITP. IgPro10 is a new 10% liquid IVIG product formulated with L-proline, which combines a ready-to-use formulation with the convenience of room temperature storage for its entire shelf life; both are considered advantages in IVIG therapy. *Aims*. The primary objective of this prospective, open-label, phase III trial was to evaluate the efficacy, safety and tolerability of IgPro10 in patients with chronic ITP. *Methods*. Fifty-seven patients aged 12-65 years with a platelet count of ≤20×10^9/L at screening were treated with 1 g/kg body weight of IgPro10 intravenously on each of two consecutive days. The primary efficacy parameter was the number of patients who respond to IgPro10 treatment with an increase of platelet count to ≥50×10^9/L within 7 days after the first infusion. Secondary efficacy endpoints included time to platelet response, duration of platelet response, platelet counts at specified time points, and regression of hemorrhages at defined bleeding sites. Regression of hemorrhages was defined as a decrease of severity of bleeding status from baseline. *Results*. IgPro10 therapy resulted in an increase of platelet count to ≥50×10^9/L in 46 (80.7%) of the 57 patients (95% CI: 69.2%, 89.3%) within 7 days after the first infusion. An important feature of the treatment was the rapid response: 43% of the patients achieved response with only one infusion. The median time for response was 2.5 days after start of IgPro10 administration (Figure 1).

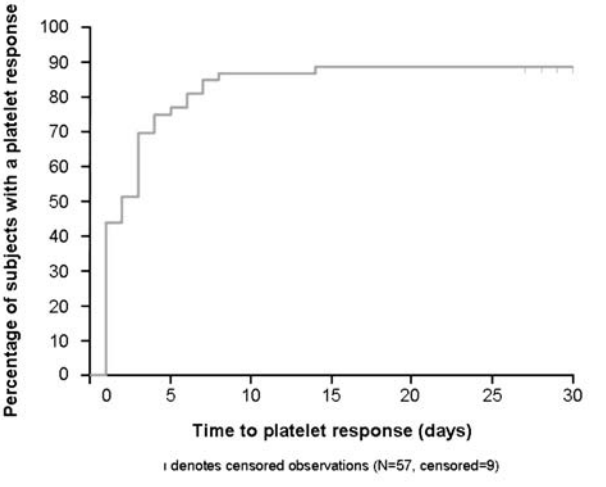

Figure 1.

Platelet response was sustained for a median time of 15.4 days (range, 1 to >82 days). In line with the almost instant increase of platelet counts, the bleeding status of patients improved rapidly. A significant reduction in the number and severity of hemorrhages was observed 2 days after treatment. By Day 8, the proportion of patients with bleeding events had dropped from 70.2% at baseline to 21.8% and most of the remaining hemorrhages were mild to moderate in severity. IgPro10 administration was well tolerated and 104 of the 114 infusions could be performed at the maximum allowed infusion rate of 4 mg/kg/min. Adverse events (AEs) were generally mild or moderate and typical for the disease and treatment. The most frequent AE was headache. *Summary/conclusions*. IgPro10 treatment produces a rapid increase in platelet counts to a safe level of ≥50×10^9/L and has a good tolerability.

Stem cell biology and microenvironment

0311

COMPARISON OF TWO BONE MARROW (BM) CELL TYPES AND ADMINISTRATION ROUTES FOR THE TREATMENT OF HINDLIMB ISCHEMIA USING THE GFP-TIE2 TRANSGENIC MODEL: ANY OPTION WORKS BUT WITHOUT TRANSDIFFERENTIATION

F.M. Sanchez-Guijo,[1] E. Oterino,[1] M.V. Barbado,[1] S. Carrancio,[1] N. Lopez-Holgado,[1] S. Muntion,[1] P. Hernandez-Campo,[1] L.I. Sanchez-Abarca,[1] J.A. Perez-Simon,[1] J.G. Briñón,[2] J.F. San Miguel,[1] M.C. Del Cañizo[1]

[1]Hospital Universitario de Salamanca, SALAMANCA, Spain; [2]Departamento de Biología Celular. Universidad de Salamanca, SALAMANCA, Spain

Background. There is growing evidence suggesting that cells from BM contribute to revascularization in experimental models of ischemia and in phase I and II clinical trials. Nevertheless, there is no definitive clue regarding the best cellular type to be used, since some groups have employed selected stem cell populations (e.g. CD133$^+$ cells) whereas others have used BM mononuclear cells, all with encouraging results, but with no direct comparison between them. The same applies to the route of cell delivery, where intravenous (IV) and direct intramuscular (IM) administration in the ischemic area have proven their value. The mechanism of action of the transplanted stem cells is also controversial. *Aims.* To compare the neovascularization capacity of CD133$^+$ selected BM cells, which have been claimed as the primitive endothelial progenitor cell subset, and the whole myeloid (CD11b$^+$) population in a murine hindlimb ischemia model assessing, in addition, IV *vs* IM administration. Finally, we wanted to gain insight into the mechanism of action of the transplanted cells by using Tie2-GFP (Green Fluorescent Protein) transgenic mice as donors: any cell from these donor mice expresses GFP only if it activates the Tie2 promoter, a specific endothelial marker. *Methods.* Transgenic FVB-Tie2-GFP mice were used as donors. BM mononuclear cells from these mice underwent magnetic cell sorting to obtain CD133^1 cells and CD11b$^+$ cells independently. Wild-type FVB mice were used as recipients and underwent left iliac artery ligation and section. 24 hours later, 1×10^6 CD133$^+$ cells or 1×10^6 CD11b$^+$ selected cells were either intravenously administered or intramuscularly injected in the left quadriceps. Each one of the four experimental groups (CD133 IV, CD133 IM, CD11b IV, CD11b IM) contained 6 wild-type FVB ischemic mice, that were maintained for 7, 14 and 21 days after the transplant (72 recipient mice in total), and then sacrificed for tissue processing. A non-ischemic sham operated group was included as control, as well as a group with limb ischemia but treated with no cells. Peripheral blood flow (μL/sec) in the ischemic limb was assessed at days 7, 14 and 21 by laser-Doppler, and tissue analysis performed in the ischemic muscles (abductor and quadriceps) consisted on capillary density quantification and immunofluorescence analysis to find donor cells with endothelial phenotype, that would appear as GFP positive. *Results and Conclusions*. In all four experimental groups, blood flow significantly recovered compared to the ischemic mice that received no cells. There were no differences between groups, with the exception that flow recovery was significantly faster (at day +14) for the mice treated with CD133$^+$ cells either IV or IM. Capillary density showed again a significant improvement in all cell-treated mice compared with the control, but also without differences at day +21 between groups. Finally, we did not find significant numbers of GFP cells in the ischemic muscles analyzed. This fact indicates that the beneficial effects observed with either cell type or delivery route were not related to Tie2 expression, and therefore transdifferentiation of BM cells into endothelial cells is lacking in our experimental model.

0312

ALDEHYDE DEHYDROGENASE ACTIVITY IDENTIFIES DISTINCT SUBCLASSES OF PRIMITIVE HEMATOPOIETIC CELLS IN MYELOID MALIGNANCIES

A. Bogen,[1] M. Pietschner,[1] C. Eaves,[2] W. Hiddemann,[1] C. Buske,[1] O. Christ[1]

[1]University of Munich, 3rd Dept. of Medicine, MUNICH, Germany; [2]Terry Fox Laboratory, British Columbia Cancer Agency, VANCOUVER, Canada

Background. The selection of cells with high aldehyde dehydrogenase (ALDH) activity in samples of normal human bone marrow, peripheral blood or umbilical cord blood has been shown to enrich for cells with colony-forming and *in vivo* repopulating potential. In human cord blood, usually 0.5-1 percent of all low-side scatter mononuclear cells show high ALDH activity. These rare cells have a predominantly primitive (CD34$^+$) phenotype and contain most of the short-term and the entirety of long-term repopulating cells present in these samples. However, little is known about the usefulness of ALDH activity as a marker of primitive cells in samples of leukemic hematopoiesis. *Aims*. In the present study, we aimed to functionally and phenotypically characterize subsets of acute (AML) and chronic (CML) myeloid leukemia cells with distinct levels of ALDH activity. *Methods*. Light-density (LD) cells were isolated from AML and CML samples and stained with the ALDEFLUOR fluorescent substrate. Cells were then sorted and analyzed for their CFC content, immunophenotype, and the presence of karyotype abnormalities using fluorescence *in situ* hybridization (FISH). *Results*. In all CML samples analyzed, ALDH$^+$ cells were detectable at higher percentages and with higher mean fluorescence intensities than in samples of normal hematopoietic cells. As in normal hematopoiesis, these cells were highly enriched for cells with a primitive phenotype and showed markedly increased colony forming activity. Preliminary studies using FISH suggest that in primary CML samples, selection for high ALDH activity may allow the isolation of the leukemic (Ph$^+$) subfraction within the most primitive (CD34$^+$CD38$^-$) population. Conversely, AML samples less consistently contained ALDH$^+$ cells, and the phenotype of these showed a considerable inter-sample variability. *Conclusions*. We and others have previously shown that selection of cells with high ALDH activity enriches for a variety of primitive cells with clonogenic and repopulating potential in populations of normal human hematopoietic cells. The results presented here show that high ALDH activity can be reproducibly found in samples of myeloid neoplasias, and that these cells contain the majority of phenotypically primitive and functionally immature cells. Assessment of ALDH activity appears to be a valuable tool for the selection of primitive cells in myeloid malignancies. Transplantation studies are underway to determine whether high ALDH activity is a specific feature of transplantable leukemic cells.

0313

OPTIMAL CONDITIONS FOR MESENCHYMAL STEM CELLS EXPANSION IN A FBS-FREE MEDIUM

K. Chieregato, M. Maddalena, S. Castegnaro, M. Marchesi, D. Madeo, F. Rodeghiero

San Bortolo Hospital, VICENZA, Italy

Background. Mesenchymal stem cells (MSCs) are multipotent cells isolated from several tissues capable to differentiate into several cell lineages. They interact with hematopoietic stem cells and exert immunoregulatory function. These characteristics make MSCs excellent candidates for regenerative medicine and gene therapy. Due to their scarce quantity, MSCs require *ex vivo* expansion. To this aim there is a need to identify growth factors that might be used in serum-free formulations to avoid transmissible diseases. *Aims.* In this study we evaluated the effects of a pool of human recombinant growth factors (hr-GFs including Epidermal Growth Factor [EGF], basic-Fibroblast Growth Factor [bFGF], Granulocyte Colony-Stimulating Factor [G-CSF], Hepatocyte Growth Factor [HGF], Insulin-like Growth Factor I [IGF I], Platelet-Derived Growth Factor-bb [PDGFbb] and Transforming Growth Factor β1 [TGFβ1]) on MSCs obtained from human adipose tissue (AT) and umbilical cord (UC) cultured in standard conditions. *Methods.* MSCs at the end of fist passage were plated in a 96-well plate at 1500 cells/cm^2 in DMEM supplemented with human Platelet Poor Plasma (hPPP, 3%). On subsequent day cells were treated for 72 hours with different media containing respectively: Fetal Bovine Serum (FBS, 10%); Platelet Lysate (PL, 10%); hPPP; hPPP with hr-GFs at 5 or 10 or 30 ng/mL; hPPP with EGF at 10 ng/mL and other hr-GFs at 5 or 10 or 30 ng/mL. Cell proliferation was measured with BrdU incorporation. *Results.* In UC-MSCs cultures (fig. 1A-B) EGF, bFGF and PDGFbb increased fluorescence intensity respectively by 2,5 , 2,6 and 2,9 fold compare to control (hPPP at 3%), showing a similar effect of FBS, but lower than PL. Only when EGF and PDGF-bb were added together we obtained a proliferation rate near to PL; whereas the combination of EGF and bFGF had a moderate additive effect. HGF and G-CSF did not induce proliferative response when added alone; furthermore they reduced EGF-induced proliferation.The same effect was detected for IGF I. TGFβ1 induced a robust inhibition alone or in combination with EGF or EGF-bFGF-PDGFbb. In AT-MSCs cultures (Figure 1 C-D) EGF and PDGFbb had an effect similar to that of UC cells. b-FGF alone had a more remarkable proliferative effect than on UC-MSCs, and higher than PL. With EGF we did not appreciated a further increase of proliferation rate. G-CSF and HGF, alone and with EGF, had no effect. IGF I alone induced an increased cell proliferation, whereas

EGF did not. TGFβ1 caused inhibition in AT-MSCs too, but only when added alone, in fact with EGF it potentiated its proliferative effect.The addition of EGF, bFGF and PDGFbb represented the best condition for AT-MSCs growth, more than PL. *Conclusions.* AT and UC-MSCs showed a good expansion in a FBS-free medium containing hPPP and a mixture of hr-GFs. In particular a combination of EGF-PDGFbb (10 ng/mL) for UC-MSCs and EGF-bFGF-PDGFbb (10 ng/mL) for AT-MSCs has shown a proliferation rate higher than with FBS and similar to that obtained with PL addition. Further studies are required to investigate the uptake potential *in vivo* of expanded MSCs and their immunoregulatory capacity.

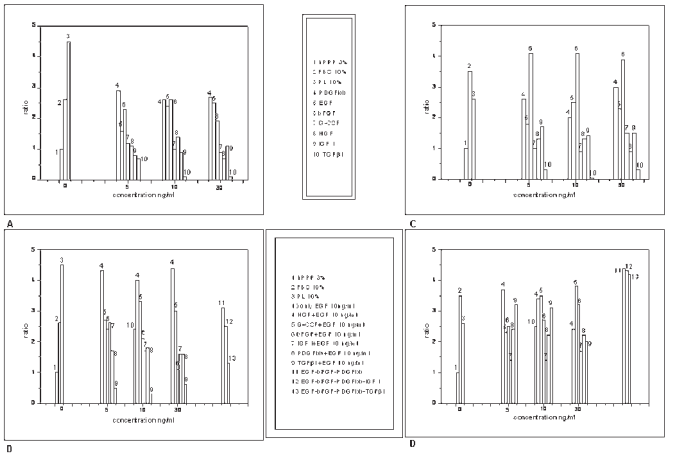

Figure 1 A-C Ratio between fluorescence of UC or AT MSCs, respectively, expamded in hPPP, or FBS, or PL, or with hr-GFs alone and control (hPPP 3%). B-D Ratio between fluorescence of UC or AT MSCs cultures in FBS, or PL or EGF (10 ng/mL) plus GFs and vehicle control.

0314

OPTIMIZATION OF MESENCHYMAL STEM CELL EXPANSION PROCEDURES BY CELL SEPARATION AND CULTURE CONDITIONS MODIFICATION

S. Carrancio,[1] N. Lopez-Holgado,[2] F.M. Sánchez-Guijo,[2] E. Villarón,[2] V. Barbado,[3] S. Tabera,[2] M. Díez-Campelo,[2] J. Blanco,[4] J.F. San Miguel,[2] M.C. Del Cañizo[2]

[1]University Hospital of Salamanca, SALAMANCA; [2]Department of Hematology. University Hospital of Salamanca, SALAMANCA; [3]Cell Biology and Pathology Anatomy Deparment. University of Salamanca, SALAMANCA; [4]Department of Traumatology. University Hospital of Salamanca, SALAMANCA, Spain

Background. The use of mesenchymal stem cells (MSC) in gene and cell therapy holds promise as a potential therapeutic tool for several human diseases, and a number of clinical trials with MSC transplantation have been initiated in the last few years. The great numbers of these cells required for clinical approaches, together with the low percentage of them in adult bone marrow, make the *in vitro* expansion a mandatory process. Well-defined isolation, expansion and characterization protocols are required to facilitate comparison of MSC clinical results. Several methods have been proposed in order to improve the number and quality of MSC including the use of platelet lysate (PL) and low O2 tension but none of them has included a different isolation method. Some years ago, it was reported that a greater number of fibroblast progenitors was obtained when gravity sedimentation was used. We have hypothesized that within these progenitors MSC would be included and we propose a new approach for cell separation. *Aims.* The present study was designed to optimize the culture of MSC by combining and comparing several cell isolation methods and culture conditions. *Methods.* Human MSC were isolated from BM cells of eleven healthy volunteer donors after informed consent was obtained. Mononuclear cells (MNC) from bone marrow aspirates were obtained by both density gradient centrifugation (standard method) and gravity sedimentation. Cells were cultured with or without platelet lysate (PL) preparations and under different O2 concentrations until fourth passage. Time of expansion, number of cells obtained, morphology, cell surface markers and differentiation potential were evaluated. *Results.* The number of cells obtained by using gravity sedimentation was significantly higher (11.7×10^6, range from 5.52 to 20.4×10^6 cells/mL) than that obtained with density centrifugation (4.4×10^6, range from 2.0×10^6 to 6.4×10^6 cells/mL) ($p<0.05$). Morphologically, MSC expanded in the presence of PL were smaller and exhibited faster proliferation rates that those expanded in conventional culture medium. As shown in Figure 1, the number of MSC obtained by gravity sedimentation and cultured with PL (with or without hypoxia) were significantly higher than those obtained in the other groups ($p<0.05$) from P1 until P4. When the oxygen tension was studied, we could observe that reduced oxygen tension lead to a faster proliferation, particularly during the first passages and this fact led to a significantly shorter time of expansion when compared with other groups ($p<0.05$). MSC obtained by any of the different culture conditions expressed comparable inmunophenotype and were able to differentiate into osteoblasts, adipocytes and chondrocytes.*Conclusions.* MSC isolation by MNC gravity sedimentation together with culture medium supplementation with 5% of PL in a hypoxic atmosphere (5% O2) significantly improved MSC yield and reduced expansion time compared to the standard accepted protocols.

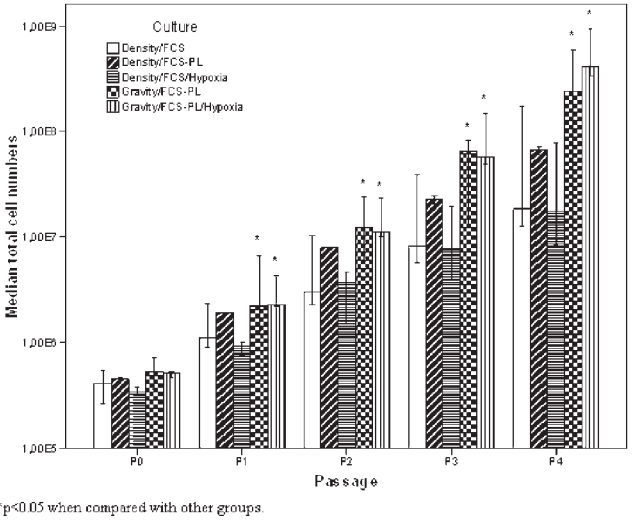

Figure 1. Effect of culture conditions on isolation and proliferation of BM MSC. Total number of adherent cells isolated at passages 0-4 from 10' BM mononuclear cells.

0315

IMMUNOPHENOTYPIC AND GENE EXPRESSION PROFILE CHANGES OF EX VIVO EXPANDED UMBILICAL CORD BLOOD CD34+ HEMATOPOIETIC STEM CELLS

D.L. Zanette

FMRP-USP-CTC, RIBEIRAO PRETO, Brazil

Background. The number of hematopoietic stem cells (HSC) in UCB samples is a limiting factor for its use in transplants and *in vitro* expansion of these cells seem promising. Nevertheless, the use of animal derived culture components and the adverse effects on cells viability and differentiation potential of commonly used expansion protocols hamper their clinical application. *Aims*. To allow future improvements, this study was carried in order to gain knowledge on molecular mechanisms acting on HSC upon culture conditions allowing the expansion of primitive sets of UCB HSC. Material and Methods. 13 samples of immune-magnetically purified UCB $CD34^+$ cells (mean purity 93.5%) were expanded in serum and stroma-free conditions with FLT3-lig, TPO, SCF and IL-6. Following purification (T0) and after 3 days of culture (T3), cells were evaluated by flow cytometry for the percentage of apoptotic cells (anexin V-expressing cells), of primitive cell subsets ($CD34^+/CD38^-$ and $CD34^+/CD90^+$) and of $CD34^+/CXCR4^+$ cells. Differentiation and clonogenic potential was accessed by methylcellulose assays. RNA extracted from samples were pooled to generate two independent gene expression profiles from cells at T0 and at T3 (total of four) using oligonucleotide microarrays encompassing over 10,000 genes. Differentially expressed genes were selected for validation based on multiple comparisons between both time points. Individual RNA samples were used to evaluate the expression level of IL9R, SMAD6, BIRC5 and CDC25C genes by real time quantitative PCR (dual-labeled probes, 2-ddCt comparative method). *Results*. A mean fold increase in

total cell counts of 2.8 was seen on day 3, with at least 80% of these cells expressing CD34. Absolute numbers of $CD34^+/CD38^-$ and $CD34^+/CD90^+$ subsets were increased two and six-fold, respectively. At T3 cultures had a greater percentage of viable cells, compared to T0 (mean 75% *vs* 63%). The percentage of $CD34^+/CXCR4^+$ cells was significantly lower at T3 (p=0.005). The absolute and relative number of colonies, as demonstrated by clonogenic progenitor assays, was not altered between T0 and T3, indicating that the differentiation capacity of cultured cells was maintained. Cell cycle related genes involved in mitosis were the most upregulated, such as CDC25C (fold=8), whereas the expression levels of known intrinsic regulators of HSC self-renewal such as HOXB4, Bmi-1 and STAT5B, did not change. In addition, the downregulation of CXCR4 (fold= -9), involved in homing, and the upregulation of genes such as IL9R (fold=56), associated with proliferation through JAK/STAT pathway, SMAD6 (fold=12), a negative regulator of TGF-β1 pathway and BIRC5 (fold=7), a negative regulator of apoptosis, were all confirmed by qPCR on the samples evaluated (p<0.05, Mann Whitney T test). *Conclusions*. Using a protocol free of animal derived components, we were able to expand, in a short period of time, the absolute numbers of cell subsets typically associated with HSCs, without affecting cell viability and clonogenic potential. Functional evaluation of the significant gene expression changes that accompany expansion the may help to improve protocols to expand HSC while maintaining desired characteristics.

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0316

IMPAIRED MOBILIZATION OF THALASSEMIC MICE DUE TO INCREASED SEQUESTRATION OF HEMATOPOIETIC STEM CELLS IN THE THALASSEMIC SPLEEN

N. Psatha, E. Yannaki, A. Tasouli, E. Athanasiou, G. Karponi, V. Constantinou, D. Bougiouklis, A. Papadopoulou, A. Xagorari, A. Anagnostopoulos, A. Fassas

Gene and Cell Therapy Center, Hematology-BMT Unit, George Papanicolaou Hospital, THESSALONIKI, Greece

Background. Gene therapy has recently been considered a therapeutic potential for thalassemia, therefore, there is a necessity to determine the most preferable source of autologous $CD34^+$ cells. It is well known that Granulocyte-Colony Stimulating Factor (G-CSF)-mobilized peripheral blood stem cells (PBSCs) provide a higher yield of stem cells than conventional bone marrow harvest. However, the administration of G-CSF in conditions of splenomegaly may not prove safe, as G-CSF is related to splenic enlargement rarely resulting in rupture. On the other hand, an enlarged spleen may potentially pool stem cells from the periphery and negatively affect mobilization. *Aims.* We decided to investigate the G-CSF effect in a thalassemic mouse model (HBBth-3) as compared to a normal strain (C57Bl6), in terms of safety, mobilization efficacy and distribution of stem cells among hematopoietic compartments. *Methods*. G-CSF was administered intraperitoneally at 200 μg/kg once a day for 7 days. Control mice received no G-CSF-treatment. Hematopoietic stem/progenitor cell (HSPCs) analyses were carried out by flow cytometry (FCM) and clonogenic assays (CFU-GM) on three hematopoietic tissues: peripheral blood, bone marrow, and spleen. HSPCs were defined as Lin-sca-1+c-kit+ cells by FCM. Histological and immunohistochemical analyses (MPO, TER-119, c-kit, Ki-67, Gomori) were performed into harvested tissues. Spleen and liver weight was determined as a ratio (x1000) to total body weight. *Results*. G-CSF caused dramatic alterations in the histology of thal spleens represented by excessive hypercellularity and intense trilineage hemopoiesis in a greatly expanded red pulp dominating over the white one. No death or splenic rupture in G-CSF-treated animals of either strain was observed (0/68), however, hemorrhagic infarcts could be detected in the spleens of G-CSF-treated HBBth-3 mice at the time of sacrifice (5/40). The size difference was significant in favor of thal spleens over normal ones, both at steady state (p<0,0001) and post G-CSF(p<0,0001), however, the G-CSF effect was stronger in normal than in thalassemic strain finally resulting in 133% increase as compared to 65% increase in the thal spleen size. Calculation of LSK+cells/μL blood and CFU-GM/mL blood demonstrated that the HBBth-3mice mobilized less effectively than C57Bl6 mice (p=0,01 and p=0.01, respectively) due to the sequestration of LSK+cells and CFU-GM progenitors in the spleen (p=0,007 and p=0.06 respectively). Splenectomy restored the mobilization efficacy in thal mice at comparable levels to the normal strain by increasing LSK+ cells in the blood by 2.5 fold and CFU-GM by 4.3 fold. The liver post splenectomy developed a compensatory increase of extramedullary hematopoiesis which became intense after G-CSF. *Conclusions*. Our data imply that in view of human gene therapy for thalassemia, multiple cycles of mobilization or bone marrow harvest may be required for a sufficient yield of transplantable HSCs in non-splenectomized patients and strategies to reduce the risk of G-CSF-induced infarcts in the spleen should be explored in a clinical setting.

0317

CHOLESTEROL-ENRICHED DIET PROMOTES LEUKEMIA ENGRAFTMENT AND PROGRESSION

R. Fragoso,[1] T. Carvalho,[1] A. Gomes,[1] L. Martins,[2] J. Barata,[2] S. Dias[1]

[1]Portuguese Institute of Oncology, LISBON; [2]UBCA, Institute of Molecular Medicine, LISBON, Portugal

Subsets of acute leukemia patients have low cholesterol levels (hypocholesterolemia) associated with poor clinical outcome and evidence of extramedullary disease. In the present study, to address *in vivo* the contribution of cholesterol metabolism on leukemia development, we xenotransplanted T and B lymphoblastic leukemias into nod-scid mice subjected to a high fat diet (that results in increased cholesterol levels in the peripheral blood and in the bone marrow). We observed that the high fat diet condition worsened the disease, and significantly decreased the survival of leukemic mice. The increased mortality was due to an increased BM engraftment and, at the latter stages of the disease, to the early onset of extramedullary disease in organs, such as the spleen and the lungs. The increased BM engraftment in high fat diet mice was diminished by treating the mice with cholesterol lowering drugs (statins). According to these results we hypothesized that cholesterol levels may modulate the BM microenvironment, making it more permissive to leukemia cells engraftment (and, at the latter stages of the disease, to their exit). Mechanistically, we first focused on the effects of high cholesterol in endothelial cells. We started by performing trans-endothelial leukemia cell migration assays using endothelial cell monolayers pretreated or not with high levels of cholesterol. Importantly, we observed that cholesterol enrichment significantly increased leukemia cells trans-endothelial migration (~3 folds over control). We observed that some of the key molecules involved in leucocytes adhesion to endothelium (such as P-selectin, VCAM and I-CAM) were up-regulated by this cholesterol treatment, as well as the complement component factor C3a and C5 receptors, known to regulate endothelial monolayer permeability and adhesion. On the other hand, we observed that cholesterol treatment induces complement component factors C3a and C5 expression on leukemia cells, suggesting that cholesterol metabolism may modulate leukemia cells/BM microenvironment interaction through the complement pathway activation involving leukemia:endothelial cell interactions. Accordantly, blocking the complement pathway activation (with a C3a receptor antagonist) impaired cholesterol-induced leukemia trans-endothelial cell migration. Next, we sough to analyze the importance of the complement pathway in regulating leukemia engraftment and expansion *in vivo*, by inoculating different leukemia cells on nod-scid mice fed or not with a fat diet and treating or not with a complement inhibitor before/during leukemia BM engraftment and after, during leukemia progression. According to our results, complement pathway inhibition decreased BM engraftment and also prevented/delayed the onset of extramedullary disease on fat diet mice, by impairing endothelial cell permeability/survival in the BM and in the peripheral organs. Our results demonstrate for the first time the mechanisms by which a high-cholesterol diet may modulate the interaction between subsets of leukemia and BM endothelial cells, contributing towards increased leukemia onset and progression.

0318

DIFFERENTIAL BCR EXPRESSION IN SEQUENTIAL STAGES OF MURINE HEMATOPOIETIC HIERARCHY

H.K. Khoury

Princess Margaret Hospital, TORONTO, Canada

Background. The BCR gene was originally cloned due to its involvement in the t(9;22) translocation found in CML and B-ALL. In this translocation the chimeric gene BCR-ABL is under the control of the promoter of BCR. Although BCR-null mice do not exhibit major defects in the development of definitive hematopoietic cells, they show abnormal stress responses to infective stimuli. It has been shown that BCR transcriptional activation is tissue and lineage specific. *Aims.* The goal of this study was to analyze BCR expression in sequential stages oh hematopoietic hierarchy in order to add insight into the importance of the potential role of BCR in normal hematopoiesis and the contribution

of its dysregulation to leukemic hematopoiesis. *Materials and Methods.* We quantified by real-time RT-PCR, the transcript level of this gene in different stages of maturation of the murine hematpoietic hierarchy that include long-term (LTR-HSCs), short-term repopulating hematopoietic stem cells (STR-HSCs), Multipotent B+, pentapotent, tetrapotent, the bipotent (pE/pMeg) and neutrophilic/macrophage (pNeut/pMac), unipotent (BFU-E, CFU-E, pMeg, pMac and pNeut) progenitors and, finally, the terminally differentiated cells (E, Meg, Mac, Neut, Mast , B and T). The LTR- and STR-HSCs are able to reconstitute the entire murine hematopoietic system for a period of 1 year or more and 3-12 months and have the phenotypes (Rho-Kit+Sca1+Lin-CD49b-) (Rho-Kit+Sca1+Lin-CD49b+), repectively. Complementary DNA was obtained from sorted cells (HSCs, mature B and mature T) or pooled at each stage of maturation from single cell precursors whose differentiation potential was determined by the outcome of cultured sibling cells (Multipotent B+ and myeloid progenitors and mature cells). *Results.* BCR transcript expression was biphasic. The first phase was restricted to the LTR-HSC and STR-HSC with BCR/GAPDH levels of 0.0027 and 0.0017, respectively. The second phase included mainly the bipotent progenitors (pE/Meg and pNeutro/Mac) with BCR/GAPDH ratios of 0.009 and 0.002, respectively. BCR Expression was not detected in the Multipotent B+, pentapotent, tetrapotent, unipotent or terminally-differentiated cells. Then we analyzed the BCR gene transcript levels in HSCs that are arrested at G0 phase and after stimulating them to undergo mitosis. Interestingly, LTR-HSCs and STR- HSCs in G1 phase showed 10-fold increase in BCR transcripts when compared to HSCs in G0. Moreover, this augmentation persisted during all stages of mitosis. *Conclusions.* Our findings suggest that BCR transcript levels are tightly regulated during murine hematopoietic differentiation; whether this regulation is achieved at the pre-transcriptional (through methylation of DNA regulatory regions or histone modification) or post-transcriptional (by microRNA) level is still to be determined. The increase in BCR levels during mitosis may indicate a potential role of BCR in mitosis or cell cycle, such a role is still to be fully deciphered. Moreover, our results may suggest that the abnormal stress response of $BCR^{-/-}$ mice is likely due to abnormal compensatory function of the pNeut/Mac progenitors rather than to defective function of the terminally maturing cells. It remains to be determined if the murine expression pattern is recapitulated in the human hematopoietic precursor hierarchy, and in particular whether the expression of the BCR-ABL fusion transcript and protein follow the same pattern in CML.

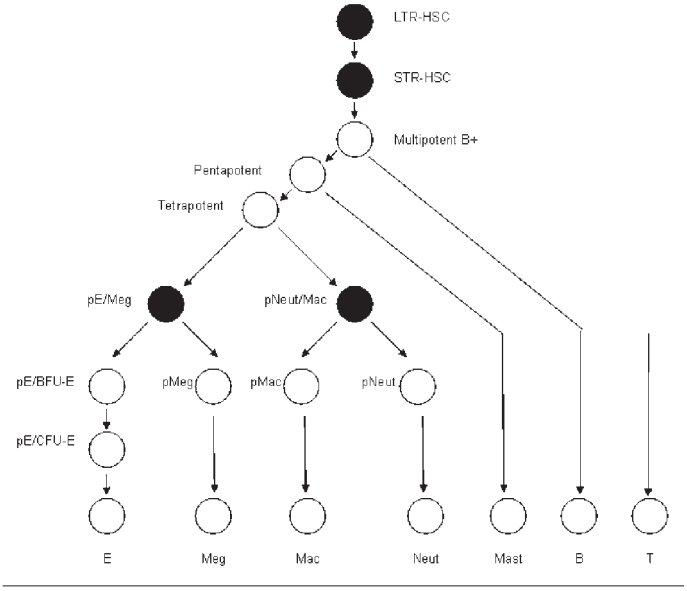

Figure 1. BCR expression in murine hematopoietic Hierarchy.

0319

A QUALITATIVE AND QUANTITATIVE ANALYSIS OF THE CD68+ CELL MICROENVIRONMENT COMPONENT IN REACTIVE LYMPH NODES AND FOLLICULAR LYMPHOMA AT PRESENTATION AND RELAPSE

T. Todd,[1] S. Barrans,[2] K. Turner,[2] R. Tooze,[2] A.S. Jack[2]

[1]*Cambridge University Hospitals NHS Foundation Trust, CAMBRIDGE;*

[2]*HMDS, Leeds Teaching Hospitals NHS Trust, LEEDS, UK*

Background. The importance of the tumour microenvironment in follicular lymphoma (FL) has been highlighted by both gene expression profiling (GEP) and quantitative analyses of the $CD68^+$ cell component. However, whole tissue GEP does not distinguish the contribution of different cell types; and quantitation of a single, non-lineage specific marker, may be over simplistic. *Aims.* To determine phenotypic and quantitative differences in the CD68+ cell population between reactive lymph nodes, and FL presentation and relapse. *Methods.* Quantitative analysis of the $CD68^+$ cell population was performed by staining sections from 43 matched pairs of formalin fixed paraffin embedded lymph nodes of presentation and relapsed FL with anti-CD68 (PGM1) antibody using standard immunohistochemistry. Cells were counted at x1000 magnification in both the 5 most densely populated fields (as previously published) and 40 random fields per sample. The composite phenotype of $CD68^+$ cells was further investigated in 10 reactive, 10 presentation and 10 relapse FL lymph nodes using 2 or 3 colour immunofluorescence (MCIF). CD68 was used to identify the cells of interest in combination with antibodies against inflammatory factors (PU.1, CEBP/beta, COX2), markers of M1 (iNOS, IL23) and M2 (the novel markers Gas-3 and Cadherin-1) macrophage differentiation, transcription factors (IRF1, IRF2, IRF4, IRF8 , BCL6) and genes from prognostically significant groups previously determined by GEP and attributed to the microenvironmental component (LGMN, ACTN1, C1q, STAT1). S100 was used to identify non-macrophage antigen presenting cells (APC). *Results.*No quantitative difference in $CD68^+$ cells was identified between FL presentation and relapse. The composite phenotype of $CD68^+$ cells was unchanged between FL presentation and relapse but differed markedly between reactive and FL lymph nodes. In FL fewer cells expressed iNOS than in reactive lymph nodes (45% *vs* 85%), IL23 was unchanged though weaker, COX2 was virtually absent, IRF2 was expressed in a higher proportion of cells (25% *vs* 10%) and IRF1 in a lower proportion (63% *vs* 82%). Gas3 and Cadherin-1 were more frequently expressed in FL (84% vs 18% and 87% vs 10% respectively) as was LGMN. ACTN1 and LGMN were also expressed in tumour and normal lymphocytes. 5% of $CD68^+$ cells co-expressed S100. *Summary and Conclusions*. This study has demonstrated the potential utility of MCIF for evaluating the tumour microenvironment. We have shown that a proportion of $CD68^+$ cells are non-macrophage APCs and that some genes highlighted in prognostically significant gene expression profiles and attributed to the microenvironment are also expressed in tumour cells. No quantitative or phenotypic difference in the $CD68^+$ component of the microenvironment was demonstrable between presentation and relapse samples suggesting that the microenvironmental response to tumour remains constant. Clear phenotypic differences in $CD68^+$ cells between FL and reactive samples were demonstrated. FL showed diminished M1 and inflammatory marker expression but enhanced M2 marker and differing transcription factor expression. We have shown for the first time that Gas3 and cadherin-1 are expressed in markedly more $CD68^+$ cells in human FL compared to reactive lymph nodes. These findings may be of value in future evaluation of the microenvironment in this and other diseases.

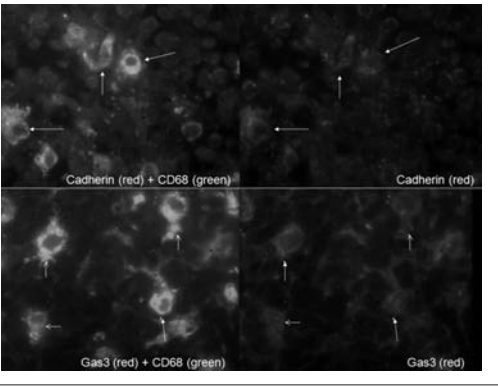

Figure 1. $CD68^+$ cells expressing Cadherin-1 and Gas3 in FL.

0320

NOTCH1, GATA3 AND NF-KB PARTICIPATE IN A TRANSCRIPTION NETWORK THAT REGULATES PRIMITIVENESS OF HEMATOPOIETIC STEM CELLS

R.A. Panepucci,[1] L.H.B. Oliveira,[2] D.L. Zanette,[1] G.A. Molfetta,[1] R.C.V. Carrara,[1] V.C. Oliveira,[1] A.G. Araujo,[1] M.D. Orellana,[1] P.V.B. Palma,[1] C.C.B.O. Menezes,[1] D.T. Covas,[1] M.A. Zago[1]

[1]Fundacao Hemocentro de Ribeirao Preto (FMRP-USP), RIBEIRAO PRETO; [2]FCF-UNESP, ARARAQUARA, Brazil

Background. NFKB2 and RELB are sub-units of a transcription factor (TF) that mediates constitutive signaling of the nuclear factor kappa B (NF-kB) pathway. We have previously shown that the increased signaling of this pathway in umbilical cord blood (UCB) CD34$^+$ hematopoietic stem cells (HSC) as compared with bone marrow (BM) CD34$^+$ cells is probably related to their more primitive state, to which higher levels of NOTCH1 could also contribute. However, differences of the proportion of more primitive CD34$^+$ cells among BM and UCB CD34$^+$ HSC could also account for this finding. CD133 is a surface marker expressed in a more primitive sub-population of CD34$^+$ cells that are highly enriched in long-term culture-initiating and NOD/SCID-repopulating cells with a proposed hemangioblast potential. Aims. Identify molecular mechanisms related to the more primitive characteristics of UCB and CD133$^+$ HSC. Material and Methods. Human CD34$^+$ and CD133$^+$ cells were immunomagnetically purified from BM and UCB obtained from donors and from the placenta in term births. Oligonucleotide microarray gene expression profiles were carried out followed by in silico promoter analysis using the TELIS on-line tool. Real-time quantitative PCR for selected genes were carried on a total of 65 immunomagnetically purified samples. Interference RNA (iRNA) experiments were carried on BM CD34$^+$ HSC. Results. UCB CD34$^+$ cells contained a significantly higher proportion of CD133$^+$ than BM (70% and 30%, respectively). Cluster analysis of the expression profiles, encompassing around 10.000 genes, showed that UCB CD133$^+$ are more similar to UCB CD34$^+$ than to BM CD133$^+$ cells, whereas BM CD34$^+$ showed the lesser similarity to these cells, a result probably related to the cell sub-populations composition. Compared with CD34$^+$ cells, CD133$^+$ cells had a higher expression of many well-known factors related to the hemangioblast potential of primitive HSC. In fact, TFs such as RUNX1/AML1, GATA3, USF1, TAL1/SCL, HOXA9 and HOXB4 were all present at higher levels in CD133$^+$ HSC. Promoter analysis of these differentially expressed TF revealed a frequency of NF-kB TF binding sites on these genes significantly higher than the expected frequency for random selected genes, and included potentially novel NF-kB targets such as RUNX1, GATA3 and USF1. NOTCH1, GATA3, NFKB2 and RELB levels evaluated by real-time PCR were significantly higher (Mann-Whitney) for UCB (CD34$^+$ or CD133$^+$) samples than for BM CD34$^+$ samples. NOTCH1, GATA3 and NFKB2 levels were also higher on BM CD133$^+$ samples compared to BM CD34$^+$ samples. Significant positive correlations (Sperman's correlation coefficient) were demonstrated for the levels of all the transcripts evaluated, thus indicating that GATA3 levels in HSC can be regulated by NOTCH1 and NF-kB members. To further test this hypothesis, we used iRNA against NKFB2 in HSC. As a result, levels of NFKB2, GATA3 and RELB (a known target of NFKB2/RELB dimmers) were down-modulated, in comparison with cells transfected with control iRNA. Conclusions. Our results indicate that constitutive NF-kB signaling may act together with NOTCH1 to upregulate transcription factors related to a more primitive state of HSC. Supported by FAPESP, CNPq and FINEP.

0321

MAINTENANCE OF PRIMITIVE STEM CELL ACTIVITY DURING MASSIVE AMPLIFICATION OF COMMITTED PROGENITORS IN CLINICAL SCALE SERUM-FREE *EX VIVO* CULTURES OF THAWED CORD BLOOD CD34$^+$ CELLS.

Z. Ivanovic,[1] P. Duchez,[1] J. Chevaleyre,[1] M. Vlaski,[1] B. Dazey,[1] X. Lafarge,[1] E. Robert-Richard,[2] F. Mazurier,[2] J.M. Boiron[3]

[1]Etablissement Français du Sang Aquitaine - Limousin, BORDEAUX; [2]INSERM E0217, BORDEAUX; [3]Université V Segalen Bordeaux 2, BORDEAUX, France

Background. The *ex vivo* expansion of cord blood (CB) cells could potentially resolve the problem of the low cell content in the graft and of the long period of post-transplantation neutropenia, as was demonstrated for *ex vivo* expanded autologous peripheral blood CD34$^+$ cells (Boiron *et al.*, Transfusion 46:1934). We upscaled (Duchez *et al.*, J Hematother Stem Cell Res 12: 587, 2003) the experimental procedure (Kobari *et al.*, Exp Hematol 28: 1470, 2000) of CD34$^+$ cell expansion in clinical-scale cultures in presence of SCF, FLT3 ligand, MGDF (100 ng/mL each) and G-CSF (10 ng/mL). *Aims.* Since the long-term engraftment capacity is crucial after allogeneic transplantation, the activity of primitive stem cells was investigated before and after expansion of thawed cord blood CD34$^+$ cells in our culture system. *Methods.* Expansion of CD34$^+$ cells selected [(Isolex (Duchez *et al.*, J Hematother Stem Cell Res 12: 587, 2003) or Miltenyi, (Ivanovic *et al.*, Stem Cells. 22: 716, 2004)] from previously cryopreserved and thawed volume-reduced (Dazey *et al.*, Stem Cells Dev 14: 6, 2005) or non-reduced cord blood units was performed in two-step cultures (diluted 1:4 after 6 days ; VueLife culture bags) in the presence of SCF, FLT3 ligand, MGDF and G-CSF in Macopharma HP01 serum-free medium (Ivanovic et al, Transfusion 46: 126, 2006). The activity of stem cells was evaluated on the basis of detection of human cells in the bone marrow of NOD/Scid mice (primary recipients) 6-8 weeks after transplantation (i.v.). The maintenance of very primitive stem cells was studied by a serial engraftment: the secondary recipients were engrafted by an intramedullary injection of primary recipient bone marrow cells. Six weeks later, injected femurs were analyzed as in primary recipients : human CD45, CD33, CD19 as well as the number of clonogenic progenitors, (CFC), of human-origin. *Results.* The number of total cells, of CD34$^+$ cells as well as of committed progenitors were expanded ~500 fold, ~100 fold and ~150 fold, respectively. The activity of primitive stem cells (SRC : SCID Repopulating Cells) at the end of culture, was maintained at the Day-0 level as judged by the phenotypical markers (corresponding to relatively less primitive subpopulation of SRC), and doubled as judged by human CFC content in primary recipient bone marrow (SRC - CFC, representing relatively more primitive subpopulation of stem cells). The engraftment of secondary recipients (considered to be the measure of very primitive stem cells) was not impaired by culture whether judged by human phenotypical markers or by human CFC numbers. *Conclusions.* In the course of a massive expansion of hematopoietic progenitors from CD34$^+$ cord blood cells in this clinical-scale serum-free cultures, the activity of the primitive stem cells is not impaired. Furthermore, the activity a stem cell subpopulation (SRC-CFC) was enhanced. These data actualize the *ex vivo* expansion as an approach that could significantly improve the transplantation of cord blood stem and progenitor cells.

Stem cell transplantation - disease related

0322

UNRELATED CORD BLOOD TRANSPLANTATION AFTER MYELOABLATIVE CONDITIONING IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA

J. Ooi, S. Takahashi, A. Tomonari, N. Tsukada, T. Konuma, S. Kato, S. Kasahara, A. Tojo, S. Asano

Institute of Medical Science, University of Tokyo, TOKYO, Japan

Background. Recently, unrelated cord blood has been increasingly used for an alternative stem cell source in adult patients. We previously reported promising results of a pilot study of unrelated cord blood transplantation (CBT) in adult patients with *de novo* acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS)-related AML. *Aims.* The main purpose of this study was to confirm the safety and efficacy of unrelated CBT for adult patients with AML after myeloablative conditioning regimen as well as to identify factors related to the transplant outcome. *Methods.* Between August 1998 and September 2007, 75 adult patients with AML were treated with unrelated CBT at The Institute of Medical Science, University of Tokyo. Written informed consent was obtained from all patients. Diagnoses at transplantation included *de novo* AML (n=56) and MDS-related secondary AML (n=19). 44 (59%) patients were transplanted in an advanced status of the disease (defined as AML in third or subsequent complete remission, relapse or refractory disease). All patients received four fractionated 12 Gy total body irradiation and chemotherapy as myeloablative conditioning. 72 patients received standard cyclosporine (CyA) and methotrexate, and 3 patients received CyA only as a graft-versus-host disease (GVHD) prophylaxis. Among the patients the median age was 45 years (range, 18-55 years), the median weight was 55 kg (range, 36-76 kg), the median number of cryopreserved nucleated cells was 2.44×10^7/kg (range, $1.16\text{-}5.29\times10^7$/kg) and the median number of cryopreserved CD34 positive cells was 1.00×10^5/kg (range, $0.15\text{-}8.97\times10^5$/kg). All patients received a single and HLA mismatched cord blood unit. *Results.* 71 patients had myeloid reconstitution and the median time to more than 0.5×10^9/L absolute neutrophil count was 21 days. A higher CD34 positive cell count was independently associated with faster neutrophil recovery (p=0.0001). A self-sustained platelet count more than 50×10^9/L was achieved in 67 patients at a median time of 37 days. Acute GVHD greater than or equal to grade III occurred in 7 of 71 evaluable patients and chronic GVHD occurred in 61 of 66 evaluable patients. Among 61 chronic GVHD patients, 19 patients were extensive type. 52 patients are alive and free of disease at between 7 and 115 months after CBT. With a median follow-up of 71 months, the probability of event-free survival (EFS) at 5 years was 67%. The 5-year cumulative incidence of transplant related-mortality and relapse was 11%, 25%, respectively. In multivariate analyses, advanced disease status was an adverse factor for EFS (p=0.01) and relapse (p=0.02). HLA compatibility had no impact on any transplant outcomes. *Conclusions.* These results suggest that adult AML patients without suitable related or unrelated bone marrow donors should be considered as candidates for CBT.

0323

MYELOABLATIVE HEMATOPOIETIC ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA IN FIRST OR SECOND COMPLETE REMISSION OR REFRACTORY DISEASE: A RETROSPECTIVE STUDY OF 168 PATIENTS ENROLLED IN TWO CONSECUTIVE FRENCH TRIALS

E. Bachy,[1] C. Pautas,[2] C. Cordonnier,[2] X. Thomas,[1] M. Michallet[1]

[1]Edouard Herriot Hospital, LYON; [2]Henri Mondor Hospital and Paris 12 University, CRETEIL, France

Background. Prognostic factors of acute myeloid leukemia (AML) at diagnosis are well identified in the literature. However, whether type of induction may impact on the outcome of myeloablative (MA) allogeneic stem cell transplantation (alloHSCT) is debated. Aims. The aim of this study was to confirm the role of pretransplant prognostic factors and of the induction regimen on overall survival (OS), disease free survival (DFS) and relapse rate (RR). Methods. We retrospectively analysed 168 patients (median age 36 (15-52) years old) enrolled in two consecutive prospective trials (ALFA 9000 trial and ALFA 9802 trial; database for transplantation obtained from Societe Francaise de Greffe de Moelle et de Therapie Cellulaire (SFGM-TC)) who underwent MA alloHSCT in first or second complete remission (CR1 or CR2) or in refractory disease (RD) stage between June 1996 and December 2004. Thirty-four patients received standard 3+7 induction with standard doses of cytarabine (Ara-C) and 134 patients received intensified (double or time-sequential) induction with intermediate doses of Ara-C. Statistical analyses were performed to evaluate the correlation between OS, RR, or DFS, and age at time of transplantation (before 40, n=108; after 41, n=60), cytogenetic status (available for 134 of 168 patients; favorable, n=11; intermediate, n=92; unfavorable, n=31), status at transplant (CR1, n=98; CR2 , n=29; RD, n=41), conditioning regimen (endoxan plus total body irradiation (EDX-TBI), n=96; endoxan plus busulfan (EDX-BUS), n=37; other, n=35), source of stem cells (peripheral blood, n=11; bone marrow, n=156; unknown, n=1), type of donor (matched related, n=133; matched unrelated, n=35). Results. Median OS and DFS were 1.10 and 0.86 years respectively. OS, RR, and DFS at 5 years were respectively 45.7%, 35.4% and 41.5%. By Kaplan-Meier univariate analysis, OS was significantly improved for patients receiving related matched alloHSCT (p=0.004) and bone marrow stem cells (p=0.012). While no significant correlation was found between OS and cytogenetic status or type of induction treatment or age at time of transplant, status at transplantation markedly correlated with OS (CR1 vs CR2 vs RD, p<0.0001), as well as type of conditioning regimen (EDX-TBI or EDX-BUS vs other, p=0.0004). By Cox multivariate analysis, only related (vs unrelated) matched donor (p=0.009) and status at transplantation (CR1 vs RD, p=0.041) were associated with a significant better OS. In a subgroup analysis of the 98 patients transplanted in CR1, OS was significantly improved for patients receiving standard vs intensified induction (p=0.031). However, no further association was found when adjusted for cytogenetic status in a Cox model mainly because patients who underwent HSCT in CR1 after 3+7 standard induction showed all more favorable cytogenetic features than those who received an intensified induction. Conclusions. We confirmed in this study that status at transplantation as well as matched related donor type remain strong prognostic factors. Furthermore, in univariate analysis, the outcome was better after CR1 if patients received standard induction. However, in multivariate analysis, after adjusting for potentially confounding factors, this trend was not confirmed. Further studies are needed to determine the role of intensified induction regimens before alloHSCT especially for patients with adverse cytogenetic characteristics at diagnosis.

0324

CLOFARABINE/ARA-C (CLARA) TREATMENT FOLLOWED BY REDUCED-INTENSITY CONDITIONING AND ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH-RISK, RELAPSED, OR REFRACTORY ACUTE LEUKEMIA

S. Buchholz, J. Krauter, E. Dammann, S. Ehrlich, M. Port, E. Weissinger, M. Stadler, M. Eder, A. Ganser

Hannover Medical School, HANNOVER, Germany

Background. Allogeneic stem cell transplantation (SCT) is the most effective treatment for high-risk, refractory or relapsed AML and MDS. However, most AML and MDS patients are older than 50 years and not eligible for conventional myeloablative conditioning. Combined cytoreductive therapy with reduced-intensity conditioning regimen (RIC) is increasingly used for these patients, but limited anti-leukemic activity still results in significant relapse rates. Clofarabine, a nucleoside analogue with properties of both fludarabine and cladribine, has shown remarkable activity in relapsed AML and ALL. *Aims.* The aim of this pilot study is to evaluate the antileukemic and immunosuppressive effects of clofarabine/ara-C in the context of RIC allogeneic SCT for patients with high-risk AML, ALL, and MDS. *Methods.* Up to February 2008 fourteen patients (median age: 62, range 39 - 69, male/female: 10/4) with high-risk, relapsed or refractory acute leukemia or MDS (8 AML, 2 ALL, 4 MDS; high-risk cytogenetics n=4) were treated. At SCT, patients were in CR (n=3), in relapse (n= 5), refractory (n=3), and untreated (n=3). Cytoreductive therapy consisted of clofarabine 30 mg/m^2 and ara-C 1000 mg/m^2 for 5 days. After 3 to 5 days of rest, RIC was given with 4 Gy of total-body irradiation, 80 and 120 mg cyclophosphamide /kg for related and unrelated donors, respectively, and ATG. All patients were transplanted with unmanipulated G-CSF mobilised peripheral blood stem cells from matched (n = 7) and mismatched (n=3) unrelated or matched related (n = 4) donors. GvHD prophylaxis consisted of cyclosporine A and mycophenolate mofetil. *Results.* Overall, treatment was well tolerated and all but two patients are alive and in haematological remission (follow-up median day + 120, range day + 8 to 286). All but one (early death) patient engrafted (median day + 19 for ANC > 0.5 G/L, range: day +12 to 31). So far no acute GvHD > I° was observed with follow-up > 100 days for 7 patients. Non-hematological toxicity included reversible hepatotoxicity (increase in ALT and AST CTC II - III°) in 11 patients, CTC IV° in one patient, hem-

orrhagic cystitis in one patient, and mild to severe reversible hand-foot-syndrome in 9 cases. One patient suffered from veno-occlusive disease and infectious complications and died due to multiorgan-failure on day + 33, and one patient died due to candida septicemia on day +8. *Conclusions.* Antileukemic therapy with clofarabine and Ara-C followed by RIC allogeneic HSCT is feasible in elderly patients with high-risk acute leukemia or MDS with normal engraftment and no increase in GvHD. Non-hematological toxicity of this regimen mainly liver and skin is acceptable. Updated results of this ongoing pilot study will be presented.

0325

UNRELATED TRANSPLANTS FOR POOR PROGNOSIS ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: LONG-TERM COMPARATIVE ANALYSIS BASED ON THE HEMATOPOIETIC PROGENITOR SOURCE

C. Ferra,[1] J. Sanz,[2] R. Cámara,[2] G.F. Sanz,[2] A. Bermúdez,[2] D. Valcárcel,[2] M. Rovira,[2] M. Morgades,[2] I. Espigado,[2] I. Heras,[2] D. Serrano,[2] C. Barrenetxea,[2] C. Solano,[2] R. Duarte,[2] A. García-Noblejas,[2] A. Iriondo,[2] J.L. Díez,[2] E. Carreras,[2] J. Sierra,[2] M.A. Sanz,[2] J.M. Ribera[2]

[1]H. Germans Trias i Pujol - Institut Català d'Oncologia, BADALONA; [2]GETH and PETHEMA groups, VALENCIA, Spain

Background. Adults with high risk ALL features at diagnosis, slow responders or with recurrent disease have a poor outcome with standard chemotherapy and are considered for unrelated transplants in most centers if a matched sibling donor is not available. Unrelated cord blood (UCB) has emerged as an option for unrelated transplant in adult patients. Aim of the study: to compare the outcome of adult patients with unrelated transplant for poor prognosis ALL based on the hematopoietic source used for transplant. *Patients and Methods.* One hundred and thirty-seven adult patients (median 29 years [15-59], 82M/55F) with poor prognosis ALL received an unrelated transplant in 12 Spanish institutions from 2000 to 2007. ALL was of precursor B lineage in 73 (53%), T-cell lineage in 54 (39%) and undetermined lineage in 10 (7%) patients. ALL was in 1st CR in 76 (56%) patients, in 2nd CR in 33 (24%), in 3rd CR in 8 (6%) and with overt disease in 20 (14 %) patients. Both groups were comparable for the main clinical and biologic ALL features. Conditioning therapy consisted on TBI-CY in 63 (46%), BU-CY in 8 (6%), Thiotepa-BU-CY/FLU in 57 (42%) patients (all of them UCB transplants) and other regimens in 9 (7%) patients (6 of them were non-myeloablative conditioning). The source of hematopoietic progenitors was UCB in 61 (45%), mobilized peripheral blood in 37 (27%) and bone marrow in 39 (28%) patients. HLA compatibility requirements for selecting unrelated donors (bone marrow and mobilized peripheral blood) were 7-8 out of 8 allelic identities, and for UCB were 4-6 out of 6 A / B antigenic and DR allelic identities. *Results.* The median follow-up was 20 months (0.3-96). DFS estimated at 5 years for patients transplanted with any source were significantly better in transplants in 1st CR *vs* 2nd CR or more advanced disease (median 10 [2-19], 6 [0-13] and 3 [0-7] months, p=0.023). There was no statistical difference in OS or DFS at 5 years between UCB and conventional unrelated transplant (peripheral blood stem cell transplant [PBSCT] and bone marrow transplant [BMT]). TRM was significantly lower in UCB transplants.

Table 1.

	OS (5 y, IC 95%:)	Median OS (month, IC 95%:)	DFS (5y, IC 95%:)	Median DFS (month, IC 95%:)	TRM (5y, IC 95%:)
Whole series	23% (13-33)	10 (4-16)	20% (11-29)	7 (3-11)	57% (45-69)
PBSCT + BMT	18% (6-30)	7 (1-14)	18% (7-29)	5 (3-8)	67% (52-82)*
UCB transplant	31% (15-47)	11 (3-19)	23% (8-38)	9 (2-16)	40% (25-55)*
Patients in 1st CR	29% (14-44)	38 (28-49)	25% (11-39)	10 (2-19)	58% (44-72)
PBSCT + BMT	23% (4-42)	27 (3-50)	22% (4-40)	12 (2-21)	65% (44-86)
UCB transplant	35% (14-56)	10 (0-23)	28% (6-50)	10 (0-26)	50% (31-69)

*p=0.029

The relapse probability was 23% for the entire group without differences between both groups of unrelated transplants. *Conclusions.* UCB transplant, unrelated PBSCT and unrelated BMT are equivalent options for poor prognosis adult ALL patients without a sibling donor. However, all unrelated transplant modalities are associated with high transplant related mortality.

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0326

TREATMENT OF ACUTE LEUKAEMIA WITH UNMANIPULATED HLA-MISMATCHED/ HAPLOIDENTICAL BLOOD AND BONE MARROW TRANSPLANTATION

X.-J. Huang, D.-H. Liu, L.-P. Xu, K.-Y. Liu, H. Chen, W. Han, Y.-H. Chen, X.-H. Zhang, D.-P. Lu

Peking University Institue of Hematology, BEIJING, China

Background and aims. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains one of the best therapeutic options to cure acute leukaemia (AL). However, many patients lack a human leukocyte antigen (HLA) matched donor. HLA mismatched family donors are associated with insufficient long-term survival because of infection and GVHD if T cells are depleted. Recently, we developed a new method for HLA-mismatched/haploidentical transplantation without *in vitro* T-cell depletion (TCD). *Methods*. We analyzed the outcome of 250 consecutive patients with AL who underwent HLA-mismatched/haploidentical transplantation with 1-3 mismatched loci from family donor via our new transplant protocol. The factors that might influence the outcome of transplantation were discussed. *Results*. 249 patients achieved sustained, full donor chimerism. The incidence of grade 2-4 acute graft-versus-host disease (GVHD) was 45.8%, and that of grades 3 and 4 was 13.4% which was not associated with the extent of HLA disparity. The cumulative incidence of total chronic GVHD was 53.9% and that of extensive chronic GVHD was 22.6% in 217 evaluable patients. 150 of the250 patients survived free of disease recurrence throughout the follow-up of 862 days (range: 212-2207 days). 8 patients received DLI as a treatment for relapse after transplantation and all achieved leukemia free survival (LFS). The 2-yr probability of LFS for AML was 69.1% and 55.4% and for ALL was 68.9% and 17.5% in standard-risk and high-risk groups, respectively. Lower overall survival and LFS were associated with diagnosis of acute leukaemia in high-risk group (p< 0.005) and the occurrence of acute GVHD of grades 3 and 4 (p<0.02). *Conclusions*. Treatment of acute leukaemia with unmanipulated HLA-mismatched/haploidentical blood and bone marrow transplantation was feasible.

0327

STUDY OF WT-1 EXPRESSION AND ANALYSIS OF JH REARRANGEMENT MAY PREDICT RISK OF RELAPSE IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA SUBMITTED TO ALLOGENEIC BMT

M. Miglino, R. Varaldo, N. Colombo, R. Grasso, M. Clavio, L. Vignolo, C. Ghiggi, F. Ballerini, L. Canepa, I. Pierri, M. Gobbi

Chair of Haematology, GENOVA, Italy

The study of the monoclonal JH rearrangement is widely applied in the minimal residual disease (MRD) analysis of chronic and acute B-lymphoproliferative diseases, when a specific intrinsic marker is lacking. Patients who are JH positive at the end of therapy do not necessarily have an increased risk for relapse. Wilms' tumor gene (WT1) is a tumor suppressor gene involved in the regulation of cell growth and differentiation. WT1 transcripts and nuclear protein have been detected in the majority of human acute leukemias. The level of WT1 expression has been associated with the presence, persistence or reappearance of a leukemic clone. A significant association between WT1 level and prognosis has been shown in patients with acute myeloid leukaemia and acute lymphoblastic leukaemia (ALL) receiving chemotherapy. We analyzed both WT1 expression and JH rearrangement in 32 adult ALL patients submitted to allogeneic BMT to investigate whether the sequential monitoring of both markers is able to predict the outcome and improve the disease management. JH assay was performed by fingerprint method and WT1 expression by Real-time PCR , normalized on Abl expression . In this scenario WT1 expression could reflect a functional state of leukemic clone and predict relapse.14 males and 18 females, with a median age of 35 years (range, 19 to 51) have been studied . Ten patients showed t(9;22), 2 t(4;11), 1 a complex karyotype; 17 patients had a normal karyotype. All patient had received a conventional induction and consolidation therapy . Molecular study was performed before BMT, at day +100, and then every 3 months. In all the patients one or more JH monoclonal bands had been identified on the marrow sample studied at diagnosis .In the pre-BMT samples JH rearrangements have been detected in all the 32 patients, whereas WT1 expression was shown in 15 patients. In 3 patients in complete hematologic remission the high level of WT1 expression was predictive of an early relapse. At day +100 post BMT 9 patients were JH negative: 7 of these patients are alive and disease free, 2 have died, due to extensive GVHD, in complete remission. All these patients had normal levels of WT 1 expression . Twentythree

patients were JH positive : 7 had high level (≥100) of WT1 and experienced an early relapse, 2 had intermediate level (50-100) and both relapsed in extramedullary site, 14 patients had normal level of WT1 (< 50) and maintain CR with a median follow up of 24 months. Nine of these 13 received donor lymphocyte infusions for persisting JH positive molecular status: 4 of them developed cGVHD and one patient died of extensive cGVHD. In conclusion, our data suggest that elevated WT 1 level post BMT (day +100) may have an unfavorable prognostic value . JH negative status post BMT is always associated with a low level of WT1 expression and is associated with a very low risk of disease recurrence.

0328

ALLOGENEIC TRANSPLANTATION FOR MYELOFIBROSIS IN MYELOPROLIFERATIVE DISEASE -A SINGLE CENTER EXPERIENCE

M. Markova, J. Schwarz, A. Vitek, V. Valkova, D. Pohlreich, P. Cetkovsky

Institute of Haematology, PRAHA 2, Czech Republic

Background. Although new discoveries in gene mutations bring a new hope for future targeted therapy of myelofibrosis in the setting of myeloproliferative disease (MF-MPD), allogeneic hematopoietic stem cell transplantation (HSCT) still remains the only curative procedure. However, it is not devoid of causing quite high transplant-related mortality, especially when myeloablative conditioning regimens are used. *Aims*. To evaluate retrospectively the results of HSCT performed in a single institution for MF-MPD and to analyze the prognostic parameters at diagnosis and at time of transplant. *Patients and Methods.* 19 patients with MF-MPD underwent HSCT. 17 of them had primary myelofibrosis, 2 had postpolycythemic MF. The male/female ratio was 8:11, the median age was 49.3 years (38-63). The conditioning regimen used was myeloablative in 12 patients and 7 patients received a reduced intensity conditioning regimen (RIC); fludarabine plus busulphan was used in all but one patient. A matched related donor was used in 21.0% (4 pts), matched and mismatched unrelated and mismatched related donors were used in 78.9% (15 pts). The median post-transplant follow-up was 8 months (2-61 mos). Survival curves were constructed and evaluated by the Cox regression method (Mantel-Haenschel test). *Results.* Transplant-related mortality was 26.3% (5 patients), the projected overall survival (OS) was 57% at 2 years following HSCT. The regimen intensity did not translate to any difference in OS (p=0.38). The same was true for comparisons of related or unrelated (p=0.61) and HLA-matched and mismatched donors (p=0.76). Engraftment failure or graft rejection was seen in 21.0% (4 patients), all but one following RIC. OS seemed to be better in patients with a spleen of <10 cm below costal margin at the time of transplant (Fig. 1), albeit this has not reached statistical significance (p=0.35). The spleen size was also not significant for time to engraftment of leukocytes and thrombocytes (p=0.28 and p=0.23, respectively). Surprisingly, myeloablative conditioning led to earlier engraftment of leukocytes (median 16 days) than RIC (23 days; p=0.01, Mann-Whitney test). Venoocclusive disease (VOD) was present in 52.6% (10 patients), without any impact of the regimen intensity. However, the presence of VOD grade 2-3 significantly deteriorated the projected OS at 2 years (22.2% *vs* 75.0%; p=0.01). The presence of acute graft-vs-host disease (aGvHD) in 47% (9 patients) also tends to impair OS (23.8% vs 90.0%; p=0.07). The incidence of aGvHD significantly correlated with the incidence of of VOD (p=0.04). Among pretransplant characteristics, notably both patients with Lille score 2 died. Patients with erythroblasts in peripheral blood at diagnosis tended to have poorer OS (p=0.12). Alike, immature granulocytes at diagnosis seemed to predict worse OS (p=0.27). *Discussions and conclusions*. Allogeneic transplantation offers a chance for long-term OS for high-risk patients with primary myelofibrosis. Many of the weaker associations described above may become significant if a larger number of patients is studied. We believe that proper selection of patients for HSCT (without significant numbers of immature granulocytes and normoblasts, without gross splenomegaly, transfusion-independent) will substantially improve the long-term results.

0329

REDUCED INTENSITY CONDITIONING STEM CELL TRANSPLANTATION FOR HIGH RISK AML AND MDS - OUTCOME OF PATIENTS IN A SINGLE CENTER

M. Uzunov,[1] S. Wittnebel,[2] C. Boccaccio,[2] J.H. Bourhis[2]

[1]*Hopital Pitié-Salpetriere, PARIS;* [2]*Institut Gustave Roussy, VILLEJUIF, France*

Background. Reduced intensity conditioning (RIC) hematopoietic stem cells transplantation (HSCT) was developed in order to obtain long term disease control using graft-versus-disease (GVD) effect in a variety of patients unfit for a standard transplantation procedure. *Aims.* We aimed to assess the long term disease control after a RIC HSCT. *Methods.* We retrospectively assessed the medical records of 43 consecutive AML and MDS high risk patients transplanted in a single center between 2000 and 2006. *Results.* The majority of patients (97%) were older than fifty, median age of 56. RIC was proposed to the four younger patients because of invasive fungal infection (2patients), heart failure (1patient) and relapse after a previous standard HSCT (1patient). RIC was considered in older patients because of the high risk MDS or leukaemia: failure of first line chemotherapy (8 patients - 18.6% in 2nd complete remission), partial remission (6 patients -14%), poor risk cytogenetics (14patients - 32.5%) or molecular biology features(Flt3+ - 3patients - 6.9%), secondary leukaemia (8patients - 18.6%), detectable minimal residual disease (1patient - 2.3%). Thirty nine patients received a Fludarabine based conditioning regimen. Fludarabine was associated to TBI2Gy, Busulfan, ATG, Endoxan, Ida, Ara-C and 4 patients received 6 Gy TBI associated to 60mg/kg cyclophosphamide. 29 patients - 67% received SCT from an identical sibling, 11 patients- 26% from an MUD and 3patients- 7% from un unrelated cord blood. Post transplant immunosuppression consisted in CSA and/or MMF or short course MTX. Engraftement was achieved in 41 patients (96%) - 1 patient died on D10 and was not assessed for engraftement and 1 patient rejected his graft. Cumulative incidence of acute and chronic GVHD was 30% (13 patients). With a median follow up of 43 months (range 12-94) 22 patients (52%) are alive and 18 in complete remission (CR) and 21 died. Death was due to disease progression - 16 patients (37%), due to sepsis in the context of severe GVHD - 2 patients, to respiratory failure - 2 patients, fulminant hepatitis - 1 patient and due to complications of severe psychiatric disease - 1patient. The estimated OS for the whole group was 52% at 3 years and 48% at 4 years. For the AML patients the 3 years OS was 48%. The estimated 3 years OS was 49,6% for patients receiving a graft from an identical sibling and 64,3% from a MUD. Estimated 3 years DFS was 75% in patients experiencing either acute or chronic GVHD and 29% in the absence of GVHD. Durable remission was achieved in 57,9% of the pts in CR1 compared to 28.6% of pts beyond CR1. *Conclusions*. Less than 25% of patients older than 50 treated with chemotherapy alone are long term survivors, failure of first induction and bad risk blasts karyotype carries usually a dismal prognostic with a very high incidence of relapse. Our results support the use of RIC HSCT for patients having high risk myeloid malignancies which are not candidate for a conventional allogeneic HSCT. Transplant in CR1, GVHD and graft from a MUD seem to be associated with a better outcome

0330

SINGLE UK CENTRE EXPERIENCE OF ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION FOR CHRONIC MYELOMONOCYTIC LEUKEMIA

Z.Y. Lim, P.K. Krishnamurthy, W. Nagi, M. Kenyon, A.Y.L. Ho, G.J. Mufti, A.P. Pagliuca

Kings College London and Kings College Hospital, LONDON, UK

Background. Chronic myelomonocytic leukemia (CMML) carries a relatively poor prognosis with a median survival of 20-40 months with transformation to acute myeloid leukaemia in up to a third of cases. Allogeneic stem cell transplantation is a potentially curative treatment for CMML but data regarding outcomes is limited. *Aims*. We performed a single centre retrospective analysis on the outcomes of 18 patients who have received allogeneic transplantation at King's College Hospital, London since 1988. *Patients and Methods*. The median recipient age was 54 years and the median Dupriez score was 2. 41 patients had CMML and 39% had Acute Myeloid Leukaemia (AML) transformed from CMML. 25% of the cohort had complex cytogenetic abnormalities. The median interval from diagnosis to transplant was 20.9 months. All but one patient received induction chemotherapy prior to transplant, with most patients receiving either one (53%) or two (12%) courses of a FLAG or FLAG-Ida containing regimen. Two patients had previously undergone autologous stem cell transplantation. Conditioning was with a T-cell depleted reduced intensity conditioning regimen, Fludarabine/Busulphan/Campath (FBC, 83% of patients) or Fludarabine/Busulphan/ATG (11%). 1 patient (6%) was treated with a myeloablative Bu/CY regimen. 56% of the transplants used male donors; 39% of the donors were HLA-matched siblings, 44% were HLA-matched unrelated donors, with 17% HLA-mismatched unrelated donors. The source of progenitor stem cells was peripheral blood in 67% of cases.

Results. The median time to neutrophil and platelet engraftment was d+17 and d+19 respectively. No patients experienced primary graft failure. The median follow-up of the cohort was 895 days. The 2-year survival of the cohort was 31%, with a relapse incidence of 43%. The median time to relapse was 202 days post-transplant. Transplant-related mortality at 2-years was 33% with the primary cause of death secondary to GvHD and infection. CMV reactivation occurred in 6 patients at a median of 45 days post-transplantation. Acute GvHD occurred in 39% of patients at a median of 65 days post-tramsplanation: skin GvHD (60%), gut GvHD (14%) or both. A total of 6 patients received DLI post-transplant (median: d+232 post transplant). The indication for DLI was loss of chimerism in 4 cases and for morphological relapse in the other 2 cases. 3 of the patients who received DLI subsequently developed GVHD. However, only 2 patients had a response to DLI (both with initial loss of chimerism), with restoration of chimerism. *Conclusions*. In a cohort of patients with high risk CMML, our data suggests that allogeneic transplantation is a feasible therapeutic option in patients with CMML, with durable responses achievable in a proportion of patients.

0331

ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION FROM UNRELATED DONORS FOR CHRONIC LYMPHOCYTIC LEUKAEMIA: A POPULATION-MATCHED ANALYSIS FROM THE EBMT REGISTRY

M. Michallet,[1] P. Dreger,[2] A. Van Biezen,[2] M. Sobh,[3] D. Milligan,[2] D. Niederwieser,[2] L. Sutton,[2] A. Buzyn,[2] R. Tapani,[2] A. Nagler,[2] J. Schetelig,[2] V. Koza,[2] T. De Witte[2]

[1]Hôpital Edouard Herriot, LYON, France; [2]EBMT CLL Sub-committee CLWP, LEIDEN, Netherlands; [3]Edouard Herriot Hospital, LYON, France

We performed from the EBMT registry a population-matched analysis of allogeneic for CLL after either myelo-ablative or non myelo-ablative conditioning. Two hundred ninety four patients were studied, 161 from HLA identical siblings and 133 from unrelated donors. We matched the centre, the type of conditioning (standard or reduced intensity), recipient gender and age, HSC source, year of transplantation and we defined almost 3 common factors. We then obtained 120 pairs matched allotransplantations, 60 in each group (Table 1). In group 1, on 59 evaluable patients, 28(47%) developed acute GVHD with 15(25%) ≥grade II [12(20%) grade II and 3(5%) grade IV], on 58 evaluable patients, 21(36%) patients developed cGVHD [10limited and 11(19%) extensive]. At the last follow-up, 21(35%) patients relapsed and, 23 patients died and 37 are alive. In group 2, on 57 evaluable patients, 37(65%) patients developed acute GVHD with 24(42%) ≥grade II [15(26%) grade II, 7(12%) grade III and 2(4%) grade IV], on 50 evaluable patients 30(60%) developed cGVHD (15 limited and 15(30%) extensive). At the last follow-up 10 patients (17%) relapsed, 27died and 33 are alive. We found a very significant difference concerning the non relapse mortality with 12% (2-19) for group1 and 39% for group2 (p<0.001). In addition, we observed no significant difference concerning the probability of overall survival and event-free survival at 3 years: for group 1, 66% (54-81) and 56%(44-72) respectively with a median follow-up of 45 months (3-109) and for group2, 52% (39-68) and 46% (34-62) respectively with a median follow-up of 35 months (6-76) (p=0.11 and p=0.24). In conclusion, this study showed no difference between group 1 and group 2 due to higher non relapse mortality after unrelated transplantation and higher relapse rate after related transplantation for CLL.

Table 1.

Variables	Related (Group 1)	Unrelated (Group 2)
Gender : Male/Female	49 (81.6%) /11(18.4%)	48 (80%) / 12 (20%)
HSC Source BM/PBSC	5 (8.3%) / 55 (91.7%)	6 (10%) / 54 (90%)
Conditioning Std/RIC	6 (10%) / 54 (90%)	6 (10%) / 54 (90%)
ABO Compatibility Comp/Minor/Major	39(65%)/4(6.6%)/14 (23.4%)	26(43.3%)/11(18.3%)/19(31.6%)
Patients median age (Min-Max)	54 (37-66.5)	51.5 (35-66)

0332

DOUBLE UMBILICAL CORD BLOOD TRANSPLANTATION IN PATIENTS WITH HIGH RISK HEMATOLOGICAL DISEASES: A SINGLE-CENTRE EXPERIENCE

A. Ruggeri,[1] R. Peffault de Latour,[2] V. Rocha,[2] M. Carmagnat,[3] J. Larghero,[4] A. Madureira,[2] M. Robin,[2] C.A. Rodrigues,[2] D. Rea,[4] R. Traineau,[5] P. Ribaud,[2] C. Ferry,[2] A. Devergie,[2] C. Rabian,[3] A. Toubert,[3] E. Gluckman,[2] G. Socié[2]

[1]Bone Marrow Transplantation unit, PARIS; [2]Service d'Hématologie Greffe, PARIS; [3]Service d'Immunologie, PARIS; [4]Unité de Thérapie cellulaire, PARIS; [5]Service d'Hémobiologie, PARIS, France

Background. Double cord blood transplantation (dUCBT) has extended cord blood use to adults with hematological disease. *Aims.*We report a phase II study on 35 dUCBT from 2004 to 2007. *Methods.* Twentythree patients had high risk hematological malignancies (ALL=6, AML+MDS=8, secondary AL=5, CML=2, Hodgkin disease= 2) and 12 high risk of rejection (SAA=5, PNH=1, Fanconi Anemia=6). Among all patients, 9 (28%) (3 AML, 3 SAA, 1 FA, 1 MDS and 1 CML) had undergone previous non-engrafted transplants. Previous transplant source was UCB and BM in 5 and 4 cases respectively. Analyses of T, B and NK cells phenotype were done once a month during first 3 months and ever 3 months until 12 months. Patient data were collected after obtaining informed consent.Median age was 35 years (6-55) median weight was 59kg (17-90). Median follow-up was 17 months (4-39). Half of patients (n=17) received myeloablative conditioning, reduced intensity regimen was performed mostly for second transplant and 22 patients received ATG. Most of cord blood units were 4/6 or 5/6 HLA A, B and DR match with the patient. GVHD prophylaxis consisted in cyclosporine+steroids in 21 patients (60%) and associated with mycophenolate in 14 (40%). Median infused cell doses were 4×10^7 NC/Kg (1.8-9.7) and 3×10^5 $CD34^+$cells/Kg (0.5-7.46). *Results.* Twentyfive patients (71%) engrafted at median time of 25 days (11-42). Before day 100, chimerism data were evaluable in 29 patients. A single UCB unit predominated in 18 patients showing full donor chimerism. Mixed chimerism was detected in 6 patients and 5 patients had autologous recovery. After day 100, 16 out of 19 evaluable patients mantained complete donor chimerism and 3 had evidence of both UCB units engraftment. Acute GVHD was observed in 18 patients (43%) (grade III-IV, n=6) and chronic GVHD in 15 out of 26 patients at risk. During the first 100 days 19 CMV reactivations were detected; 4 HSV (resistant to acyclovir); 1 HHV6-meningoencephalitis; 4 EBV reactivations; 3 adenovirus diseases, 4 VRS infections, 3 septicemias, 6 fungal infections, 2 toxoplasmosis. After day 100 we observed 5 CMV reactivations, 1 CMV disease, 1 HSV, 1 EBV-PTLD and 3 fungal infections. Delayed immune reconstitution was observed in all patients with important lymphopenia. Median numbers of lymphocytes were 352 mm^3 at 3 months (n=15); 460 at 6 months (n=13) and 703 at 9 months (n=13). Median numbers of CD3/CD4 at 3, 6 and 9 months were: 34, 40, and 49 mm^3, respectively; of NK cells 223, 294 and 368 mm^3 and of B cells were 1, 5 and 65 mm^3, respectively. At 12 months overall survival was 56 (±8%). Relapse occurred in 5/35 patients (18%) and was major cause of death in 3 of them. TRM at 6 months was 39%±9. Infection was a major cause of death in 7 subjects. Five out of 9 patients have been rescued of previous non-engraftment and are alive and well (5-37 months). Conclusion:dUCBT is an option to treat patients with high risk diseases lacking a suitable HLA matched donor despite a long lasting immune deficiency.

0333

HIGH DOSE IMMUNOSUPPRESSIVE THERAPY (HDIT) WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT) IN MULTIPLE SCLEROSIS (MS): CLINICAL AND PATIENT-REPORTED OUTCOMES

Y.L. Shevchenko,[1] A. Novik,[1] A. Kuznetsov,[1] B. Afanasyev,[2] I. Lisukov,[3] O. Rykavicin,[4] T. Ionova,[5] V. Melnichenko,[1] D. Fedorenko,[1] S. Shamanski,[4] A. Kishtovich,[5] R. Kruglina,[1] A. Kondrashov,[1] R. Ivanov,[1] G. Gorodokin[6]

[1]Pirogov National Medical Surgical Center, MOSCOW, Russian Federation; [2]Department of Bone Marrow Transplantation, Pavlov State Medical University, ST. PETERSBURG, Russian Federation; [3]Institute of Clinical Immunology, Siberian Branch of Russian Academy of Science, NOVOSIBIRSK, Russian Federation; [4]Department of Haematology, Burdenko Central Military Hospital, MOSCOW, Russian Federation; [5]Unit of QoL Research in BMT, Multinational Center of Quality of Life Research, ST. PETERSBURG, Russian Federation; [6]New Jersey Center for Quality of Life and Health Outcome Research, NEW JERSEY, USA

During the last decade HDIT+AHSCT has been used with increasing frequency as a therapeutic option for MS patients. The goal of our research was to study long-term treatment outcomes in patients with different types of MS after early, conventional and salvage/late HDIT+AHSCT. Fifty-six patients with MS (secondary progressive - 27 patients, primary progressive -10, progressive-relapsing - 1, and relapsing-remitting - 18) from 6 medical centers were included in this study (mean age - 32.0, range: 17-51; male/female - 22/34). Fourteen patients underwent early AHSCT (EDSS 1.0-3.0), 38 patients - conventional (EDSS 3.5-6.5) and 4 patients - salvage/late AHSCT (EDSS 7.0-8.5). Median EDSS at base-line was 6.0 (range 1.5-8.0). The median follow-up duration was 18 months (range 6-84 months). Neurological and quality of life (QoL) evaluation was performed at baseline, at discharge, at 3, 6, 9, 12 months, and every 6 months thereafter following HDIT+AHSCT; MRI examinations - at baseline, at 6, 12 months, and at the end of follow-up. FACT-BMT and FAMS were used for QoL evaluation. Notably, no transplant-related deaths or unpredictable severe adverse events were observed. All of 45 patients included in the efficacy analysis experienced improvement (n=28) or clinical stabilization (n=17). Among the patients with improvement there were 15 SPMS, 4 PPMS, 8 RRMS and 1 PRMS; 9 SPMS, 4 PPMS and 4 RRMS stabilized. Among the patients with improvement there were 20 patients after conventional, 6 - after early and 2 - after salvage AHSCT. In the group with stabilization there were 15 patients after conventional AHSCT and 2 - after salvage AHSCT. Two patients (SPMS and PPMS; conventional AHSCT) deteriorated to a worse score after 18 months of stabilization; 2 others progressed after 12 and 30 months of improvement (RRMS, early AHSCT and SPMM, conventional AHSCT), respectively. Results of MRI scans were available in 37 patients. Sixteen patients (43.3%) had active lesions at baseline and all turned to inactive status except two cases. Of the 21 patients without active lesions pretransplant 20 remained inactive, whereas one patient showed disease activity after transplantation. No active, new or enlarging lesions were registered in patients without disease progression. Out of 24 patients included in QoL analysis 22 exhibited improved QoL 6 months post-transplantation. Further QoL improvement was observed at longer follow-up. All the patients with out disease progression were off therapy throughout the post-transplant period. In conclusion, HDIT+AHSCT appears to be an effective treatment for MS both in terms of clinical and patient-reported outcomes. The data obtained point to feasibility of early, conventional and salvage transplantation in MS patients. Further studies should be done to establish the best timing for transplantation and to validate regimens for early, conventional and salvage/late HDIT+AHSCT.

0334

IMPACT OF DONOR AND RECIPIENT BIRTH ORDER ON OUTCOME OF HLA-IDENTICAL SIBLING STEM CELL TRANSPLANTATION (SCT) IN ACUTE MYELOID LEUKEMIA (AML)

C. Dobbelstein, H. Kamal, E. Dammann, E. Weissinger, M. Stadler, J. Krauter, A. Ganser, M. Eder

Hannover Medical School, HANNOVER, Germany

Background. Among well established risk factors for outcome of SCT such as patient´s age, HLA-identity between donor (D) and recipient (R), stage of disease, the impact of primacy of birth in HLA-identical sibling transplantation has recently been described in a retrospective analysis (Bucher *et al.* Blood 2007). In this cohort, first-born patients had the best survival, the lowest incidence of acute graft-versus host disease (aGvHD), and a reduced relapse mortality rate. The underlying mechanism may include pre-existing microchimerism due to fetomaternal and transmaternal sibling cell trafficking in younger siblings. *Aims.* The aim of this study was to analyze the impact of relative birth order of recipients and donors on outcome of HLA-identical sibling SCT in AML. *Methods.* We retrospectively analyzed HLA-identical sibling transplantation for all consecutive evaluable patients with *de novo* AML from 1986 to 2007 at our centre. 123 patients transplanted from HLA-identical siblings were assigned to one of two groups: 61 recipients had an older sibling donor (D>R group), and 62 patients were older than their donors (R>D group). Data for age, sex, stage of disease, cytogenetics, transplantation date, conditioning regimen, stem cell source, T-cell depletion, incidence and severity of acute GvHD, relapse rate, relapse mortality (RM), overall survival (OS), and treatment-related mortality (TRM) were analysed. *Results.* Both groups were comparable for sex, transplantation date, high-risk cytogenetics, conditioning regimen, stem cell source, and T-cell depletion. In contrast, the R>D group encompasses slightly more patients with primary induction failure prior to SCT (10% *vs* 2%, p=0.05). Median age and follow-up (FU) were 38 years (range: 17-65 years) and 32 months (range: 0.3-187 months) for the D>R group, and 41 years (range: 21-66 years) and 34 months (range: 0.6-220 months) for the R>D group, respectively. In the D>R group, there was a trend for higher incidence of aGvHD ≥II° as compared to the R>D group: 33% *vs* 19% (p=0.09). In addition, relapse incidence in the D>R group seems to be higher than in the R>D group (30% *vs* 18%, p=0.12) with a higher RM (25% *vs* 8%, p=0.01) as compared to the D>R group. In contrast, TRM was not different among both groups (26% *vs* 27%). Finally, OS at 180 months was 60% ±7.1% and 39% ±7.9% for the R>D group and the D>R group, respectively. *Conclusions.* In AML, a trend to superior OS, lower incidence of aGvHD ≥II°, and lower relapse incidence and a reduction in RM were observed in recipients transplanted from younger sibling donors. These data on a homogeneous group of AML patients are in line with a previous report on a cohort of patients with heterogeneous diseases. Multi-centre studies for specific diseases are required to establish the impact of donor and recipients birth order on outcome of HLA-identical sibling transplantation.

0335

INFLUENCE OF GRAFT COMPOSITION ON OUTCOME OF ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION. LOW CD34+ CELLS PREDICTED LOW TRM IN PATIENTS WITH STANDARD-RISK HAEMATOLOGICAL MALIGNANCIES

F. Bijou,[1] R. Tabrizi,[2] X. Lafarge,[1] M. Sauvezie,[2] J.M. Boiron,[1] K. Bouabdallah,[2] T. Leguay,[2] D. Fizet,[1] A. Pigneux,[2] G. Marit,[2] C. Foucaud,[2] S. Vigouroux,[2] M. Dilhuydy,[2] R. Bouzgarou,[1] B. Dazey,[1] C. Melot,[2] N. Milpied[2]

[1]Etablissement Français du Sang, BORDEAUX; [2]CHU de Bordeaux, Service Maladies du Sang, PESSAC, France

Many reports have studied factors associated with the donor influencing outcome of patients (pts) after allogeneic transplantation. We have reviewed the influence of cell subsets [CD34, B, T, NK] of granulocyte-colony-stimulating factor mobilized peripheral blood stem cells (G-PBSC) on clinical outcome in 78 allogeneic transplantations from January 2002 to December 2006 for pts with standard- risk myeloid and lymphoid malignancies in a single center. All grafts were monitored for CD34 median 6,8×10^6/kg (1,7-28.1), CD19: 45.2×10^6/kg (6-152), CD3: 180×10^6/kg (24-567), CD56$^+$cells: 25.3×10^6/kg(2-115)). Diagnoses included standard-risk diseases N= 78 (Chronic lymphocytic leukaemia, low grade lymphoma, high grade lymphoma in first CR, AML/ALL first CR, Refractory anaemia with ringed sideroblasts of myelodysplastic syndrome, chronic myeloid leukaemia first CP, multiple myeloma in CR or partial remission). G-PBSCs have been harvested from 54 matched related and 24 unrelated donors (MRD and MUD). The pts and donors median age was 53 yrs old (23-65) and 46 (24-67) respectively. Eleven myeloablative and 67 reduced intensity conditioning regimens were administered and followed by cyclosporine A alone or±mycophenolate mofetil or±methotrexate. The median follow-up was 19 months. 71 pts engrafted. Cumulative incidence (CI) of day 100 grade 2-4 aGVH and 3 years cGVH were 33 % and 61 % respectively. CI of TRM, Relapse, EFS and OS at 3 yrs were 27 %, 33 %, 53% and 59 % respectively. In univariate analysis, low TRM was correlated with low CD34 (p=0,02), low B cells (p=0,047) and MRD (p=0,005). Low relapse incidence (RI) was strongly associated with low NK cells number (p=0,007), donor chimerism

(p<0,001) and cGVH (p=0,05). Type of donor and cGVH predicted better EFS (p=0,001 and p=0,03) respectively. NK cells, cGVH and type of donor offered better OS respectively (p=0,03; p=0,005 and p=0,01). In multivariate analysis, low NK cells number is the only factor affecting independently RI [95% confidence interval, 1-7, RR=2,6 (p=0,05)]. cGVH is affecting independently OS (p=0,04). These results show a role of NK cells in relapse to be futher explored.

0336

OUTCOMES OF PATIENTS RELAPSING FOLLOWING T-CELL DEPLETED REDUCED INTENSITY CONDITIONING ALLOGENEIC TRANSPLANTATION FOR MDS OR AML

Z.Y. Lim, T. Munir, S.G. Gandhi, M. Kenyon, A.Y.L. Ho, A.P. Pagliuca, G.J. Mufti

Kings College London and Kings College Hospital, LONDON, UK

Background. Disease relapse following reduced intensity conditioning allogeneic stem cell transplantation remains one of the major causes of post-transplant mortality, particularly in patients with AML or MDS. Management of these patients poses a significant challenge and at present, it remains unclear as to the optimal form of treatment for this subgroup of patients. *Aims*. We retrospectively reviewed the outcomes of 162 consecutive patients treated with a uniform fludarabine/busulphan/alemtuzumab RIC allograft regimen at Kings College Hospital, London from 2003-2006. We identified 40 patients with high risk MDS and AML who had relapsed following allograft and proceeded to review the management and outcomes of these patients. *Methods.* The median recipient age of time of transplantation was 54.5 years. The diagnoses were MDS=18 (45%), AML=18 (45%), CMML=4 (10%). The stem cell source for the initial transplant was PBSC in 34 cases (85%), and bone marrow in 6 (15%). 16 patients (40%) received stem cells from a sibling donor with 24 from an unrelated donor (60%). The median number of courses of intensive chemotherapy received prior to transplant was 2 courses (range: 1-7). The median time to disease relapse post-HSCT was 259 days (range: 34-1034). 13 patients had cytogenetic relapse and 27 had frank morphological relapse of disease (>5% blasts in bone marrow). Patients were analysed according to 4 groups: 12 patients (30%) did not receive further treatment and only received supportive care. 6 patients (15%) were treated with escalating doses of DLI alone. 8 patients (20%) received chemotherapy alone. 14 patients (35%) received a combination of re-induction chemotherapy followed by DLI. *Results*. The median survival post-relapse was 162 days (95%CI: 57-266). There was no significant difference in recipient age, disease type, stem cell source, type of relapse (cytogenetic/morphological) or stem cell dose between the subgroups. The median survival of the 4 groups was: supportive care (35 days), DLI only (147 days), chemotherapy alone (207 days), intensive chemotherapy + DLI (1033 days). There was no significant difference in the survival outcomes between patients receiving chemotherapy or chemotherapy + DLI (p=0.57), but patients who received chemotherapy + DLI and a significantly improved outcome when compared with those receiving DLI alone (1 year actuarial survival: 62% *vs* 0%, p=0.02). *Conclusions*. Our findings are consistent with published data indicating that the use of DLI alone is ineffective for the treatment of AML or MDS patients with morphological relapse post-transplantation. However, the use of re-induction chemotherapy followed by DLI can potentially induce durable long-term remissions in a significant proportion of patients.

0337

RESPONSE TO COMBINED USE OF DONOR LYMPHOCYTE INFUSIONS AND IMATINIB MESYLATE IN PATIENTS RELAPSED AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR CHRONIC MYELOID LEUKEMIA

P. Bavaro,[1] F. Pompetti,[2] P. Olioso,[1] G. Papalinetti,[1] S. Santarone,[1] P. Di Carlo,[1] E. Di Bartolomeo,[1] P. Di Bartolomeo[1]

[1]Centro Trapianti Midollo Osseo, PESCARA; [2]Dip. Medicina Trasfusionale, Laboratorio Biologia Molecolare, PESCARA, Italy

Imatinib Mesylate (IM) is an effective drug against chronic myeloid leukemia (CML) and actually it has become the standard therapy for patients with newly diagnosed CML-chronic phase. IM is able to induce remission also in patients with CML who relapse following allogeneic stem cell transplantation (SCT). Nevertheless, IM should be continued indefinitely in responding patients because usually leukemia recurs when the drug is stopped. Donor lymphocyte infusions (DLI) are very successful in restoring complete remission for patients who relapse in chronic phase after SCT. Unfortunately, DLI can be complicated by potentially life-threatening graft-versus-host disease (GvHD) and myelosuppression. To study the possible synergy between DLI and IM, we assigned the CML patients with relapse after SCT to receive IM 400 mg/die and DLI in escalating doses of 1, 5 and 10×10^7 CD3+cells/kg given every 6 weeks provided there were persistence of relapse and absence of clinical signs of GvHD. It was scheduled to stop IM therapy after 3 months of continuous molecular remission (MR), defined as undetectable BCR/ABL transcripts PCR on bone marrow and peripheral blood samples. Four CML patients allografted from HLA-identical sibling between January 1998 and January 2001, who showed recurrent disease at a median of 62 months (range, 28-101) following SCT, entered this study. One patient was in molecular relapse, 2 in cytogenetic relapse, and 1 in hematologycal relapse. The median duration of IM therapy was 180 days (range, 170-220). The median number of DLI was 2 per patient (range, 2-3). The total $CD3^+$ cells dose infused per patient was $1,5\times10^7$/kg, $5,1\times10^7$/kg, 6×10^7/kg, and 15×10^7/kg, respectively. The first dose of DLI was reduced to 0.5×10^7 $CD3^+$ cells/kg because of limited chronic GvHD in the patient who was in molecular relapse. The subsequent dose was delayed by 12 weeks. The patient with haematological relapse underwent IM therapy for 4 years. He obtained the MR and 6 months later a reduction of IM to 200 mg/die was attempted. Following 12 weeks of treatment at this dosage, a molecular relapse was diagnosed. He received DLI and achieved a new MR which is maintained at 7 months after IM discontinuation. The other three patients started combined therapy with IM and DLI at a median of 2 months (range, 2-3) following diagnosis of relapse. They reached the MR at median of 3 months (range, 2-4) of treatment. All 4 patients are currently off-therapy. A continuous MR is maintained at a median of 7 months (range, 2-14). No signs of acute or chronic GvHD were observed and haematological values remained constantly in normal range. The combined therapy was generally well tolerated. In our small series, the fast achievement of a complete MR, the persistence of response after the IM discontinuation and the absence of GVHD are of interest. These observations suggest a potential synergy between cytoreductive ability of IM and immune-mediated cures of CML by DLI, despite the concern of reported immunosuppressive effect of the IM. These preliminary results are particularly encouraging and deserve further studies with more patients and longer follow-up.

0338

THE COMPARISON BETWEEN MATCHED RELATED DONOR AND ALTERNATIVE DONOR FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACQUIRED APLASTIC ANEMIA: KSBMT2007-02 STUDY

H. Kim,[1] H.H. Koo,[2] H. Kook,[3] B.S. Kim,[4] S.H. Kim,[5] H.K Kim,[6] H.J. Kim,[3] S.H. Bae,[7] J.J. Seo,[8] S.K Sohn,[9] H.J Shin,[10] H.S. Ahn,[11] J.H. Won,[12] S.S. Yoon,[11] Y.H. Lee,[13] D.C. Jeong,[14] D.Y. Jo,[15] Y.D. Joo,[16] M.S Hyun,[17] K.H. Lee[8]

[1]Ulsan University Hospital, University of Ulsan College of Medicine, ULSAN; [2]Samsung Medical Center, SEOUL; [3]Chunnam National University Hwasun Hospital, HWASUN; [4]Korea University Hospital, SEOUL; [5]Dong-A University Medical Center, PUSAN; [6]The Catholic University of Korea St. Marry's Hospital, SEOUL; [7]Daegu Catholic University Hospital, DAEGU; [8]Asan Medical Center, SEOUL; [9]Kyungpook National University Hospital, DAEGU; [10]Pusan National University Hospital, PUSAN; [11]Seoul National University Hospital, SEOUL; [12]Soon Chun Hyang University Hospital, SEOUL; [13]Hanyang University Hospital, SEOUL; [14]The Catholic University of Korea Our Lady of Marry Hospital, INCHEON[15]Chungnam National University Hospital, DAEJEON; [16]Inje University Pusan Baik Hospital, PUSAN; [17]Yeungnam University Medical Center, DAEGU, South-Korea

Backgound. Although allogeneic hematopoietic stem cell transplantation (alloSCT) from matched related donor (MRD) is a standard therapy for severe acquired aplastic anemia (AA), alternative donor (AD) alloSCT is increasing and the survival of alloSCT from AD improves nowadays. *Aims.* We planned this study to review the recent result of alloSCT survival and to compare AD with MRD. *Methods.* A retrospective study comparing MRD and AD for alloSCT in patients with AA was conducted by Korean Society of Blood and Marrow Transplantation (KSBMT2007-02 study). *Results.* The patient population was AA, pure red cell aplasia, paroxysmal nocturnal hemoglobinuria, and they underwent alloHSCT from 1997 and 2007. Total 336 patients were enrolled in 24 Korean alloSCT centers. Survival analysis was done in 331 patients because data were not available in 5 patients. Two hundred five adult patients with AA were also analyzed to define the characteristics of adult AA patients. AA was 97.3% and median age at alloSCT was 20.4

(1-62) years old. Median time from diagnosis to alloSCT was 6.2 (0.2-248.4) months. Male was 48.9%. Seventy nine percent patients received bone marrow (BM) as a stem cell source. AD had longer time from diagnosis to alloSCT (p=0.049) and received more peripheral blood (PB) or cord blood (CB) as a stem cell source (16.3% *vs* 31.7%, p=0.004). Univeriate analysis showed MSD (p<0.001), age less than 15 years (p=0.010), BM as stem cell source (p=0.036) and disease duration less than 12 months (p=0.007) were significant predictor for better survival. However, multivariate analysis revealed that donor type was not significant factor (p=0.087) whereas age and BM was predictors for better survival. When 205 adult AA patients were analyzed, AD had more graft failure (p=0.005), delayed neutrophil engraftment (p=0.035), more acute graft versus host disease (GvHD; p=0.009). However, there were no different between AD and MRD in terms of platelet engraftment (p=0.618), incidence of SOS (p=0.735) and relapse rate (p=0.360). MRD (p=0.011), age less than 30Y (p=0.001), disease duration less than 12M (p=0.003), no prior immune suppression therapy, (p=0.007) and platelet transfusion less than 90U (p=0.010) significantly affected survival. Only age and platelet transfusion were significant factor for better survival in multivariate analysis. *Summary.* In conclusion, our study showed that donor type was not a significant factor for survival. Therefore, AD should be considered earlier when there is no MRD in patients with AA.

0339

CURRENT STATUS OF THE KOREA MARROW DONOR PROGRAM (KMDP) AND ITS INTERNATIONAL COOPERATIVE ACTIVITIES

J. Seo,[1] E. Choi,[1] H. Im,[1] B. Kim[2]

[1]Asan Medical Center, SEOUL; [2]Korea Marrow Donor Program, SEOUL, South-Korea

Background. The KMDP was established in March 1994 to facilitate the unrelated hematopoietic cell transplantation (HCT) in Korea. After the first case of unrelated HCT in July 1996, 1,000th case of unrelated HCT facilitated by the KMDP was achieved in October 2006. The need for HCT is steadily increasing, but the possibility of finding a matched related donor is becoming more difficult in Korea. *Aims*. We tried to evaluate the domestic and international coordinating activities of KMDP to enhance the feasibility of unrelated HCT in patients who do not have matched related donor. *Methods*. Data on unrelated HCT using unrelated adult donor and unrelated umbilical cord blood collected by KMDP until Dec. 2007 was analyzed retrospectively. *Results*. The cumulative number of patients who underwent unrelated HCT through the coordination of the KMDP doubled during recent 3 years. The cumulative number of volunteer donors registered at the Korea Network of Organ Sharing (KONOS) reached 144,970 by Dec. 2007. The match rate for a Korean patient who requested donor search through KMDP to find HLA-matched volunteer donor registered at KONOS was 72% in 2007. The most common cause of UBMT was AML followed by ALL, CML, SAA, MDS and other miscellaneous disorders in the descending order. The KMDP established the Korea Network for Cord Blood (KoreaCord) in June 2001, and 14,307 cord blood units are preserved at the KoreaCord by Dec. 2007. The number of cord blood transplantations in Korea reached 364 cases by the end of 2007, and it includes the 223 cases facilitated by KMDP using the units of the KoreaCord (197 pediatric patients, and 26 adult patients). The levels of HLA tests for volunteer donors registered at KONOS were serologic for HLA-A, -B, and DNA level for HLA-DR for those registered until early 2001. Since the latter half of 2001, all the volunteer donors are tested at DNA level for HLA-A, -B, -C, and -DR. The major international cooperations of KMDP with foreign registries has been with the Japan Marrow Donor Program (JMDP) and Buddist Tsu Chi Stem Cell Center (BTCSCC) of Taiwan. The international cooperation between KMDP and JMDP was started in March 1999, and 136 Korean patients underwent unrelated HCT using JMDP donors by 2007. Also the KMDP facilitated unrelated HCT in 14 Japanese patients using KONOS donors. One hundred thirty Korean patients received unrelated HCT using donors from BTCSCC by 2007, which includes 23 cases facilitated by KMDP. The KMDP signed contract to cooperate donor exchange activities with NMDP in 2005, and with Chinese Marrow Donor Program (CMDP) in 2007. *Summary*. The KMDP is expanding the number and quality of volunteer donor registry, and the match rate for a Korean patient to find a matched unrelated donor is increasing. The international cooperative activities with foreign registries together with continuous development of the KoreaCord will further enhance the feasibility of unrelated HCT for patients who can not find matched related donor for HCT.

Stem cell transplantation - miscellaneous

0340

HOMING OF LIN- / CD117+ HEMATOPOIETIC STEM CELLS IN LETHALY IRRADIATED MICE

J. Vavrova,[1] M. Rezacova,[2] S. Filip,[2] J. Mokry,[2] Z. Sinkorova[1]

[1]Faculty of Military Health Sciences, University of Defence, HRADEC KRALOVE; [2]Charles University, Faculty of Medicine in Hradec Kralove, HRADEC KRALOVE, Czech Republic

Background. High doses of ionizing radiation damage not only hematopoietic system, but also other tissues, such as intestinal epithelium or thymus. Very little is known about effect of transplantation of primitive hematopoietic cells on regeneration of these tissues after lethal irradiation. *Aims.* In this report, we describe the homing of hematopoietic stem cells (HSCs) to non-hematopoietic tissues in lethally irradiated (9 Gy) hybrid mice transplanted intravenously with lineage-/ CD117$^+$ bone marrow cells from ROSA-26 mice (lacZ$^+$). Methods and *Results.* On day 8 post irradiation, we were able to demonstrate recovery of hematopoiesis as the the number of CD117$^+$/B220$^-$ cells in the bone marrow reached the level of non-irradiated controls. Bone marrow CFU-GM numbers did not reach levels found in non-irradiated controls even 33 days after transplantation. Regeneration of differentiated cells in peripheral blood was significantly slower. On 12th day regeneration of lymphocytes was observed, increase in granulocytes was detected as late as on 33rd day. Transplanted cells containing lacZ gene were detected in reciepient hybrid mice by histochemistry and their location in the thymus, liver, stomach and ileum was followed during 33 days post transplant. On day 8 and 33 after transplantation, we found massive presence of donor (lacZ$^+$) cells in the thymus cortex. In the stroma of small intestinal villi, the lacZ$^+$ cells could be demonstrated sporadically on day 8 post transplant; higher positivity was seen in the stromal villi and lower positivity in lamina propria mucosae were observed on day 33 post transplant. By day 33 individual lacZ$^+$ cells were dispersed in relatively great numbers throughout the liver parenchyme. *Conclusions*. Transplantation of sorted lin-/ CD117$^+$ effectively resulted in hematopoietic recovery ensuring survival of lethally irradiated animal. Hematopoietic stem cell transplantation led not only to recovery of hematopoietic and lymphoid tissues but also facilitated recovery of the small intestinal mucosa, which was significantly damaged by ionizing radiation.

0341

FEASIBILITY OF A NEW RQ-PCR APPROACH FOR QUANTITATIVE AND SENSITIVE MONITORING OF CHIMERISM POST-BMT

G. Cazzaniga, F. Colnaghi, A. Colombo, V. Rossi

Clinica Pediatrica Univ. Milano-Bicocca, MONZA, Italy

Background. Allogeneic-BMT is extensively used to treat patients with hematological and metabolic diseases. Monitoring post-transplantation by chimerism analyses predicts negative events and allows to set up the appropriate preventive therapeutics before clinical symptoms. In this context, a quantitative analysis of chimerism kinetics, instead of simple qualitative detection, would permit an accurate evaluation of the kinetics of host cells in the post-BMT period. Until now, fluorescent-based PCR analysis of short tandem repeats (STRs) is the technique most used to document chimerism after allo-BMT. However, the sensitivity of this method is relatively low (detection level of a minor genotype is around 1%), mainly as a consequence of PCR competition biases. *Aims.* We evaluated the feasibility of a new approach for determining mixed chimerism based on real-time quantitative PCR (RQ-PCR) detection of insertion/deletion (ins/del) polymorphisms by TaqMan technology. *Methods.* Ins/Del polymorphisms of 2 to 5 nucleotides were selected to have a high minor allele frequency; one TaqMan Genotyping Assay has been developed, and two allele-specific PCR assays plus an internal control reference were used for post-BMT monitoring. The genotype screening was based on the delta-delta-Ct method by a dual probe TaqMan Genotyping Assay. Twenty-four ins/del sequence polymorphisms belonging to human biallelic loci were selected with an average minor allele frequency of 0.35, that will contain at least one marker that can distinguish between >99.5% of unrelated sample pairs. Markers with completely different genotype between donor and recipient, or heterozygous in recipient will have high $\Delta\Delta$-Ct, and thus can be considered as informative. The most informative marker can then be selected to be used in the post-transplantation quantitative chimerism monitoring. The quantification assays was run by allele-

specific oligonucleotide (ASO) PCR assay in two separate tubes (one per allele), and a third assay, specific for RNAse P, was used for the DNA normalization and run in a separate tube using the same DNA. *Results.* We evaluated the 24 markers' informativity on DNA samples from 5 recipient/donor pairs, 3 unrelated and 2 related. In all of them discrimination between recipient and donor genetic profile was possible, and thus quantification of mixed chimerism feasible. By using serial dilutions of mixed DNAs, the method showed a linear correlation with r higher than 0.98 and a sensitivity of 0.1%. *Summary*. The main advantage of the new ins/del RQ-PCR method over STR-PCR chimerism assays, in addition to the quantification, is the absence of PCR competition and plateau biases, thus resulting in greater sensitivity and linearity. The chimerism detection and monitoring workflow is simple, and it appears promising for the detection and quantification of a minor DNA genotype in a major one, as required for chimerism quantitative evaluation. Fluorescent-based PCR of short tandem repeats (STR-PCR) and real-time PCR ins/del chimerism assay will be compared for a panel of post-BMT follow up patients samples and presented. In conclusion, this new assay has the potential to provide an accurate quantitative assessment of mixed chimerism that can be useful in guiding early implementation of additional treatments in hematopoietic and non-hematopoietic stem cell transplantation.

0342

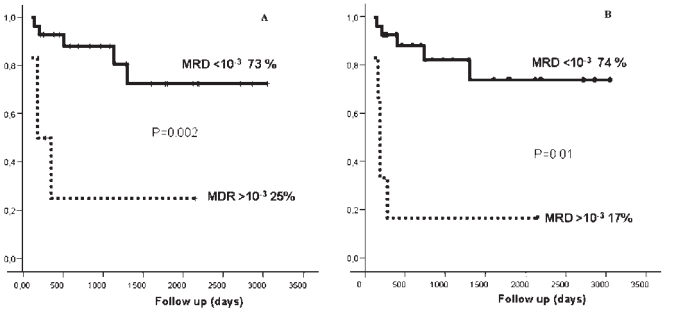
MINIMAL RESIDUAL DISEASE MONITORING AFTER ALLOGENEIC TRANSPLANTATION MAY HELP TO INDIVIDUALIZE POST-TRANSPLANT THERAPEUTIC STRATEGIES IN ACUTE MYELOID MALIGNANCIES

M. Díez-Campelo,[1] J.A. Pérez-Simón,[2] J.J. Pérez,[2] M. Alcoceba,[2] M.J. Arcos,[2] I. Graciani,[2] J. Richtmon,[2] C. Encinas,[2] M.D. Caballero,[2] F. Sánchez-Guijo,[2] M.C. López-Berges,[2] B. Vidriales,[2] J.F. San Miguel[2]

[1]Hospital Universitario, SALAMANCA; [2]Hematology, Hospital Universitario CIC, IBMCC (USAL-CSIC), SALAMANCA, Spain

Background. Allogeneic hematopoietic stem cell transplantation is a potentially curative treatment option for many patients with acute myeloid malignancies (acute myeloid leukemias (AML) and myelodisplastic syndrome (MDS)). However, relapse may occur in 15-50% of patients. Once relapse has occurred, rescue therapy usually remains unsuccessful. One of the most important factors which influence on the efficacy of the rescue therapy is tumor load at the time of relapse. In this regard, the monitoring of minimal residual disease (MRD) after transplant would allow to identify patients with impending relapse. *Aims.* The current study evaluates in 41 patients diagnosed with AML or high risk MDS the use of MRD monitoring before and after transplant in order to identify patients at high risk of relapse. *Results*. MRD assessment before and after transplant (days +21-56 and +100) allowed to discriminate different risk populations. Accordingly, the higher the MRD value, the worse the outcome, with MRD $</\geq 10^{-3}$ being the most significant cut-off values. Regarding relapse, results confirmed the importance of the negative predictive value (NPV) of MRD monitoring. Thus, the percentage of non relapses among patients with low MRD ($<10^{-3}$) at pre-transplant and at day +100 were 87 and 93%, respectively. Among 8 patients with positive MRD ($\geq 10^{-3}$) before transplant, all but 2 had died. Regarding 6 patients who displayed positive MRD on day +100 there were 5 events. The patient without event was the only one developing cGVHD. Fifty out of the 41 patients are currently alive. Overall TRM was 32% at 4 years. Projected overall and event free survival (EFS) at 4 years were 57% and 56%.

Figure 1. Impact of MRD on Overall (A) and Event free (B) survival at day +100.



Projected relapse free survival (RFS) at 4 years was 79%. Overall survival was 73% *vs* 25% at 4 years among patients with low ($<10^{-3}$) *vs* high MRD ($\geq 10^{-3}$) at day +100 after transplant, p=0.002; regarding EFS 74% of patients with low MRD were event free at 4 years as compared to 17% among patients with high MRD at day +100 (p=0.01) (Figure 1); finally, in terms of RFS 89% of patients with MRD $<10^{-3}$ were event free as compared to 40% among those with the higher value (p=0.003). Similar results were obtained with the same cut-off value at different days post-transplant (+21-56) and also in the pre-transplant analysis in terms of OS, EFS and RFS) (data not shown). In multivariate analysis MRD monitoring prior to transplant (HR= 6.3; (95% CI; 1.6-23.8), p=0.006) and at day +100 (HR= 30.2; (95% CI; 4-224.3), p=0.001) as well as chronic GVHD (HR=9.2; (95% CI; 2.1-39.3), p=0.003) had a favorable impact on EFS. *Summary*. In the present study we show that multiparametric flow cytometry evaluation of MRD during pre and early post-transplant period is a valuable tool for outcome prediction in patients with myeloid malignancies undergoing HCT and this method may allow early therapeutic intervention among patients identified as high risk.

0343

SURVIVAL IMPROVEMENT OF UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH REDUCED INTENSITY REGIMENS IN HIGH-RISK PATIENTS FOR AGE OR DISEASE (ON BEHALF OF GITMO)

A. Rambaldi,[1] A. Carobbio,[1] L. Lombardini,[2] T. Lamparelli,[3] S. Pollichieni,[4] R. Oneto,[3] R. Fanin,[5] F. Bonifazi,[6] P. Corradini,[7] G. Lambertenghi,[8] F. Ciceri,[9] P.E. Alessandrino,[10] P. Iacopino,[11] W. Arcese,[12] R. Scimè,[13] M. Falda,[14] F. Benedetti,[15] A. Bacigalupo,[3] A. Bosi[16]

[1]Ospedali Riuniti di Bergamo, BERGAMO; [2]Divisione di Ematologia, Policlinico Careggi, FIRENZE; [3]Azienda Ospedaliera Universitaria San Martino, GENOVA; [4]Italian Bone Marrow Donor Registry (IBMDR), GENOVA; [5]Clinica Ematologica DIRM, A.O. Universitaria di Udine, UDINE; [6]Istituto di Ematologia ed Oncologia Medica Seragnoli, Università di Bologna, BOLOGNA; [7]Istituto Nazionale dei Tumori, MILANO; [8]Fondazione IRCCS Ospedale Maggiore, MILANO; [9]Ospedale San Raffaele, MILANO; [10]Policlinico S. Matteo, PAVIA; [11]Azienda Ospedaliera Bianchi-Melacrino-Morelli, REGGIO CALABRIA; [12]Policlinico Universitario Tor Vergata, ROMA; [13]Azienda Ospdaliera V. Cervello, PALERMO; [14]Azienda Sanitaria Ospedaliera San Giovanni Battista, TORINO; [15]Ospedale Policlinico Giambattista Rossi - Borgo Roma, VERONA; [16]Policlinico Careggi, FIRENZE, Italy

Background. Despite reports on the feasibility of reduced intensity conditioning (RIC) transplants from unrelated donors (UD), there's no clear evidence of a survival advantage in a cohort of consecutive and unselected high-risk patients activating a donor search. *Aims.* To test prospectively survival rates of patients activating an UD search leading or not to a transplant (with two different RIC regimens) in a program involving all Italian transplant centers. *Patients and Methods*. Two categories of patients were eligible for this program. Category A) included elderly patients (age between 55-65) with a diagnosis of Acute Myeloid Leukaemia (AML, n=62), Acute Lymphoblastic Leukaemia (ALL, n=5), Chronic Myeloid Leukaemia (CML, n=11), Primary Myeloid Fibrosis (PMF, n=17) and Myelodysplastic Syndrome (MDS, n=20) while category B) included patients of any age with Hodgkin Disease (HD, n=105), Non Hodgkin Lymphoma (NHL, n=73) and Chronic Lymphocytic Leukaemia (CLL, n=34). Accordingly, 327 UD searches were activated between September 2002 and December 2004. UD transplant was performed in 121 cases (36%), and only two RIC regimens were allowed: one based on fludarabine, low dose TBI and alemtuzumab, and the other based on thiotepa and antithymocyte globulin. No relevant differences in terms of median age (48.4 *vs* 51.9 years) and diagnosis (HD 34% *vs* 31%, NHL 23% *vs* 22%, AML 21% *vs* 18%, CLL 7% *vs* 12%, MDS 4% *vs* 7%, CML 2% *vs* 4%, PMF 4% *vs* 2%, ALL 2% *vs* 1%) were registered among patients who eventually underwent UD transplant versus those who did not. *Results*. 121 patients were transplanted at a median interval of 168 days from the UD search. Of the 206 who did not receive an UD transplant, 169 (82%) stopped the UD search and 37 (18%) still have an ongoing search. The major causes for the 169 search interruptions were death (52%), disease progression (20%) or other treatments (19%). With a median follow up of 1.5 years (range 0-4.7), the 3-year Overall Survival (OS), calculated from the time of UD search activation to death or last follow-up visits, was 47% and 28% for transplanted and not-transplanted patients, respectively. Cox multivariable analysis considering UD transplant as time-dependent covariate was performed according to the different diagnoses. UD transplant was associated with a significant sur-

vival improvement in acute leukemia patients (HR=0.56, p=0.04), whereas only a favorable trend was observed for NHL, HD and CML/PMF patients (p=0.7, p=0.3 and p=0.9, respectively). Patients' outcomes and survival were not different according to the two RIC regimens adopted. *Conclusions*. This study confirms that UD transplants is feasible in high risk or elderly patients. A significant survival advantage is shown for UD transplants only for patients with acute leukemia. The impact of UD transplants on survival in patients with chronic lymphoid and myeloid malignancies is not statistically significant, and should be considered before activating an unrelated donor transplant program.

0344

BONE AS A REGULATOR OF HEMATOPOIETIC STEM CELL TRAFFICKING: BIOCHEMICAL MARKERS OF BONE REMODELING AND ANGIOGENIC CYTOKINES IN STEM CELL MOBILIZATION

M.K. Angelopoulou,[1] E. Terpos,[2] P. Tsirkinidis,[3] K. Anargyrou,[3] V. Pappis,[3] M. Moschoyiannis,[3] E. Chatzileonida,[3] O. Tsopra,[3] E. Papakostas,[3] M.-C. Kyrtsonis,[3] E. Dimitriadou,[3] F.N. Kontopidou,[3] T.P. Vassilakopoulos,[3] G.A. Pangalis[3]

[1]National and Kapodistrian University of Athens, ATHENS; [2]251 Air Force General Hospital, ATHENS; [3]National And Kapodistrian University of Athens, ATHENS, Greece

Background. Bone has been long considered as a structural, non-functional tissue supporting bone marrow hematopoietic stem cells (HSC). However recent evidence indicates an active interplay between bone and HSC. Endosteum-lining osteoblasts are an important component of the HSC *niche*. The mobilization and collection of peripheral blood stem cells (PBSC) is a standard procedure in the treatment of many hematologic malignancies, both in the autologous and allogeneic stem cell transplantation setting. The mechanisms of HSC mobilization implicate adhesion molecules, cytokines, chemokines and enzymes that lead to the disanchorage of HSC from bone marrow, through the endothelium to the circulating blood. However little is known regarding the significance of bone remodeling and metabolism in HSC mobilization in humans. *Aims.* To study biochemical markers of bone remodeling, osteoclast/osteoblast regulators and angiogenic cytokines in the process of PBSC mobilization of patients with lymphoma and myeloma. *Methods.* Twenty-four patients (10 with multiple myeloma, 5 with Hodgkin's Lymphoma and 9 with non-Hodgkin's lymphoma) were studied. Serum samples from each patient were collected at two different time points: before the initiation of mobilization (pre-mobilization sample) and on the day of PBSC collection, which coincided with the highest circulating CD34 counts (collection sample). The following molecules were measured by ELISA in patients' sera: i) osteoclast regulators: soluble receptor activator of nuclear factor kappa-B ligand (sRANKL), osteoprotegerin (OPG), and osteopontin; ii) osteoblast inhibitor dickkopf 1 (Dkk-1); iii) markers of bone resorption: C- and N- cross-linking telopeptide of collagen type I (CTX and NTX, respectively) and tartrate-resistant acid phosphatase isoform 5b (TRACP-5b); iv) markers of bone formation: osteocalcin (OC), and bone-specific alkaline phosphatase (bALP); and v) angiogenic cytokines: angiopoietin-1 (Ang1), angiopoietin-2 (Ang2) and angiogenin. Values between the two different time points were compared with non-parametric methods. Patients who had a successful PBSC collection ($CD34^+$ cells>2.0×10^6/kg) were considered as *good mobilizers*, while the remaining ones were classified as *poor mobilizers*. *Results.* The comparison of the molecules under study between the pre-mobilization and collection samples revealed the following: Soluble RANKL (p=0.000), OPG (p=0.003), bALP (p=0.000) and Ang2 (p=0.001) increased significantly, while OC (p=0.000), CTX (p=0.014) and Ang1 (p=0.001) decreased significantly between pre-mobilization and collection. The increase in sRANKL was more prominent than OPG, leading to an increased sRANKL/OPG ratio, indicating stimulation of osteoclast activity. The reduction of Ang1 and the concomitant increase of Ang2 resulted in a significant reduction of Ang1/Ang2 ratio indicating vessel destabilization. The comparison between good and poor mobilizers revealed that poor mobilizers had significantly higher CTX and NTX levels both at pre-mobilization (p=0.001 and p=0.02 respectively) and collection samples (p=0.001 and p=0.001 respectively), lower Ang-1 pre-mobilization (p=0.02) and higher OC at collection (p=0.03) compared to good mobilizers. *Summary and Conclusions.* Bone metabolism seems to be altered during PBSC mobilization, pointing to a more dynamic role of bone tissue in the trafficking of HSC. The results of our study show for the first time that vessel endothelial cell destabilization and osteoclast stimulation are two important events associated with the mobilization process. Moreover several of these markers, may identify poor mobilizers.

0345

CHRONIC KIDNEY DISEASE AFTER NON-MYELOABLATIVE STEM CELL TRANSPLANTATION IN ADULTS

S. Kersting, L. Verdonck

UMC Utrecht, UTRECHT, Netherlands

Background. Chronic kidney disease (CKD) after myeloablative stem cell transplantation (SCT) is a well-established problem. Little is known about CKD after non-myeloablative SCT. *Aims.* The aims of the present study were to evaluate the prevalence of CKD and to analyse risk factors for CKD in a large cohort non-myeloablative SCT recipients. Moreover, we wanted to study whether CKD influenced survival in these patients. *Methods.* We performed a retrospective cohort study of 108 adults who received non-myeloablative SCT with fludarabine (30 mg/m^2/day for 3 days) and/or total-body irradiation conditioning (200cGy). Renal function was assessed by estimating glomerular filtration rate (GFR) with the MDRD equation. CKD was defined as GFR <60 mL/min/1.73 m^2 if it persisted until death or last follow up. *Results.* CKD developed in 15% of patients after a median of 15 months. None of the patients required dialysis. Cumulative incidence of CKD was 7% at 12 months, 14% at 24 months, 16% at 36 months and 22 % at 48 months. Risk factors for CKD were female gender (p=0.021), older age (p=0.040) and lower GFR pre-transplant (p<0.001). Complications after SCT (graft-versus-host disease, cytomegalovirus reactivation, admission to intensive care unit, hypertension) were not associated with CKD. Stem cell transplantation nephropathy, a cause of CKD after myeloablative SCT, did not occur. Overall survival was 66%. There was no difference in survival between patients with or without CKD. *Summary and Conclusions.* CKD is a frequent complication after non-myeloablative SCT and is not related to SCT nephropathy. Women, patients above 50 years of age and patients with slightly decreased kidney function pre-transplant have the greatest risk of development of CKD. Identifying these patients is important, since CKD is associated with hypertension, anaemia and reduction in functional status.

0346

HEPATITIS B REACTIVATION IN PATIENTS UNDERGOING AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION: A SINGLE CENTRE EXPERIENCE

M. Esposito,[1] M.R. Villa,[1] S. Improta,[1] A. Lucania,[1] G.P. Izzo,[1] P. Correale,[1] A. Marrone,[2] L. Mastrullo[1]

[1]P.O. San Gennaro U.O.C. Ematologia, NAPOLI; [2]Medicina Interna e Epatologia II Università degli Studi di Napoli, NAPOLI, Italy

Background. Hepatitis due to reactivation of hepatitis B virus (HBV) is an important cause of liver related desease in patients undergoing autologous hematopoietic cell transplantation. It has been observed in patients positive for hepatitis B surface antigen (HBsAg) and in HBsAg negative patients who had HBV infection in the past (HBsAb and HBcAb positive). *Aims*. We investigated the incidence of hepatitis B reactivation in patients undergoing autologous hematopoietic cell transplantation in our centre between April 2005 and December 2007. *Methods*. We have studied 30 patients, 19 out of 30 were seronegative for HBV, 9 were anti HBs (HBsAb) and 2 were healthy carriers of hepatitis B surface antigen (HBsAg). Prior to transplant, all patients had normal levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase and total bilirubin. Liver function tests were carried out thrice weekly during the first 30 days of transplant, once a week during the next 30-60 days, and at 2 to 12 week intervals until their last follow up. The patients were followed for a median of 16 months after transplantation. *Results*. Hepatitis B reactivation developed in 3 patients after transplantation. One of these patients was HBsAg positive and two were HBsAb positive before transplantation. Hepatitis flare-up occurred at a median of 4-6 months after autologous transplantation. These patients become HBsAg positive, HBeAg and anti HBc IgM positive. HBV DNA was positive for all three patients. In two patients hepatitis was documented with an elevation of transaminase and bilirubin levels. Lamivudine treatment was started in all these patients. Two patients recovered completely after this treatment. One patient are still treated with lamivudine. *Conclusions*. Our result indicate that HBV reactivation post autologous hematopoietic cell transplantation is possible not only in HBsAg positive patients, but also in HBcAb o HBsAb positive patients. We suggest that a careful monitoring of HBV-DNA levels is important for prevent hepatic damage caused by HBV reactivation.

0347

SUCCESSFUL OUTCOME AFTER NON-MYELOABLATIVE ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH RENAL DYSFUNCTION

S. Kersting, L. Verdonck

UMC Utrecht, UTRECHT, Netherlands

Background. Non-myeloablative allogeneic HSCT is a transplantation approach that enables patients with co-morbid conditions to undergo allogeneic HSCT. Little is known about patients with reduced renal function as a single co-morbidity before HSCT. *Aims.* The aim of the present study was to assess the outcome of patients with mildly reduced renal function before non-myeloablative HSCT. *Methods.* Fifteen patients with a glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m^2 before non-myeloablative HSCT were matched on gender, age and type of transplant to 30 controls with normal renal function. All patients received a non-myeloablative HSCT with fludarabine (30 mg/m^2/day for 3 days) and/or total-body irradiation conditioning (200cGy). Graft-versus-host disease (GVHD) prophylaxis consisted of mycophenolate mofetil and cyclosporine. Data on renal function, cyclosporine dose, cyclosporine trough levels, hypertension and GVHD were collected. *Results.* Of the 15 patients with impaired renal function, 8 patients (53%) improved or stabilized to a GFR ≥60mL/min/1.73 m^2 at last follow up. Five patients (33%) developed chronic kidney disease stage 3 (GFR <60 mL/min/1.73 m^2) compared to 5 patients (17%) in the control group (p=0,031). Survival was similar between cases and controls. There were no differences in complications after HSCT (acute renal failure, hypertension, acute GVHD, chronic GVHD or nephrotic syndrome). Furthermore, there were no differences in cyclosporine dose and trough levels. *Summary and Conclusions.* Non-myeloablative HSCT can be safely offered to patients with mildly reduced renal function. Cyclosporine can be administered at the same dose as patients without renal dysfunction, as long as cyclosporine trough levels and creatinine are monitored and dose adjustments are made if necessary.

0348

THE STUDY OF $CD3^+CD4^+CD25^+$ T CELLS LEVEL AND IMMUNE RECONSTITUTION AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION IN HEMATOLOGICAL DISEASES

A.D. Moicean, A.M. Dumitrescu, V.M. Teleanu, D.N. Colita

Fundeni Clinical Institute, BUCHAREST, Romania

T regulatory (Treg) cells are an immunoregulatory cell type with suppressive effect in the course of immune response that the body uses to control autoimmunity in periphery through *dominant tolerance* By naturally occurring $CD4^+$ T regulatory (Trn or nTreg) which constitutively display CD25 several different Treg cell populations have been described in the past decade. These include cross-regulatory $CD4^+$ Th1 and Th2 cells, IL-10 producing $CD4^+$Tr1 cells and TGFb-producing $CD4^+$ Tr2/Th3 cells. Both Tr1 and Tr2/Th3 Treg cells can acquire CD25 expression. We have studied the $CD3^+CD4^+CD25^+$ T cells level at 1-24 months after hematopoietic stem cell transplantation on 32 consecutive patients with hematological malignant diseases. All patients received peripheral stem cells mobilized with G-CSF. Two patients received related myeloablative allotransplant for acute myeloblastic leukemia (AML), one patient received MUD myeloablative allotransplant for acute leukemia too, one patient received related nonmyeloablative allotransplant for AML and 28 received autotransplant (1 for AML, 5 for nonhodgkin lymphoma (NHL), 7 for Hodgkin Disease and 13 for multiple myeloma). All but NHL patients after autotransplant had an increase level of $CD3^+CD4^+CD25^+$ T cells (media 49.11%/Th, 31.95 - 80.52). The NHL patients had media 28.72% $CD3^+CD4^+CD25^+$ T cells/Th. Both patients with myeloablative allotransplant had a high level of $CD3^+CD4^+CD25^+$ T cells (media 77.19%/Th, 69.15 - 85.23). The patient with nonmieloablative allotransplant had low level of $CD3^+CD4^+CD25^+$ T cells (15.25%/Th). In conclusion, the level of $CD3^+CD4^+CD25^+$ T cells after HSCT seems to be different with stem cell source and conditioning regimen. For nonmieloablative allotransplant which creates a more immunological than hematological space for hematological stem cells homing the light suppressive activity of immune cells is the decisive factor for engrafting.

0349

HHV-6 AND HHV-7 ACTIVATION IN EARLY PERIOD AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

G. Lejniece,[1] I. Puga,[2] A. Sultanova,[2] M. Murovska,[2] S. Chapenko[2]

[1]*The National center of Hematology, Riga East Hospital, Clinic Linezers, RIGA;* [2]*A. Kirchenstein Institute of Microbiology and Virology Riga Stradins University, RIGA, Latvia*

Background. Despite great advances in stem-cell transplantation, opportunistic infections remain a major cause of morbidity and mortality for transplant patients. Beta-herpesviruses - Human herpesvirus-6 and -7 (HHV-6 and HHV-7) are considered the immunomodulatory and immunosuppressive viruses that infect individuals persistently during lifetime and in a case of reactivation may be cause for subsequent development of different complication after transplantation. Although, the pathogenesis of HHV-6 is not well understood, recent evidence suggests that the virus is a potential life-threatening pathogen in the post-transplant period. The significance of HHV-7 activation in transplant patients remains poorly understood. The aim of this study was to examine activation of HHV-6 and HHV-7 before and in early period after auto-peripheral blood stem cell transplantation (auto-PBSCT). Methods. Forty patients (22 male, 18 female, mean age 31.6 years [range: 17 - 54]) with Hodgkin's lymphoma (26/40), non-Hodgkin's lymphoma (7/40) and myeloma (4/40) were examined for beta-herpesviruses activation before and a 10 - 14th day after auto-PBSCT. All patients had received prophylactic therapy with Valtrex (500 mg x 2 in the day) one month after PBSCT. Nested polymerase chain reaction (nPCR) with peripheral blood mononuclear cells and cell-free blood plasma DNAs as the templates were performed to detect of latent/persistent and active viral infection, respectively. Results. Latent/persistent HHV-6 and/or HHV-7 infection before transplantation was detected in 35 out of 40 patients (87.5%): HHV-6 - in 15/40 (37.5%) and HHV-7 - in 33/40 (82.5%) patients. From these 35 patients dual (HHV-6+HHV-7) viral infection in 13 (37.0%) patients was revealed. Active virus infection (HHV-7) was detected only in 3/35 (8.6%) patients. Activation of beta-herpesviruses after transplantation was detected in 17/35 (48.6%) patients. From these 17 patients HHV-6 activation alone was not revealed, HHV-7 activation alone was found in 14/17 (82.4%) and HHV-6 + HHV-7 activation - in 3/17 (17.7%) patients. The analysis of the data showed significantly higher frequency (p=0.0004) of beta-herpesviruses activation in early period after auto-PBSCT (48.6%) in comparison to the period before transplantation (8.6%). The frequency of HHV-7 activation alone was significantly higher (p=0.0043) after auto-PBSC (14/35, 40.0%) in comparison with those in the period before transplantation (3/35, 8.6%). Conclusion. The activation of HHV-6 and/or HHV-7 in the patients with Hodgkin's, non-Hodgkin's lymphoma and myeloma are not frequently observed after treatment with high-dose of chemotherapy before transplantation. However, in early period after auto-PBSCT, the frequency of viruses' activation, HHV-7 activation in particular, becomes significantly higher. HHV-7 reactivation after engraftment can be a risk factor for the activation of other herpesviruses and for subsequent development of viral associated complications in transplant recipients.

0350

ALTERED EXPRESSION AND FUNCTION OF THE CHEMOREPELLENT/RECEPTOR PAIR SLIT/ROBO UPON MYELOID DIFFERENTION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS

S.B. Geutskens,[1] W.A. Andrews,[2] S.E. De Haan,[1] J.G. Parnavalas,[2] P.L. Hordijk,[1] P.B. Van Hennik[1]

[1]*Sanquin Research and Landsteiner Laboratory, AMSTERDAM, Netherlands;* [2]*University College London, LONDON, UK*

Slit is an extracellular matrix molecule originally identified in the developing central nervous system (CNS). Here it transmits a repulsive cue to neuronal progenitors preventing axonal outgrowth via the transmembrane receptor Roundabout (Robo). Four Robo and three Slit homologues have been identified in vertebrates. While Slit1 is confined to the CNS, Slit2 and Slit3 are widely expressed and have been reported to inhibit the CXCL12-induced migration of breast cancer cells, myocardial progenitors and of mature hematopoietic cells, i.e. T-lymphocytes and monocytes. CXCL12 and its receptor CXCR4 are critical in the regulation of hematopoietic stem/progenitor cell (HSPC) migration to the bone marrow (BM) upon transplantation. We have set out to investigate whether a negative migratory cue, i.e. Slit and its receptor Robo, can modulate HSPC migration. We show that Slit2 and -3 were expressed in BM-derived endothelial and stromal cell lines, whereas Robo1 was

expressed by CD34+ HSPC derived from different sources. Interestingly, Robo1 mRNA and surface protein expression levels in HSPC were significantly higher as compared to CD14+ monocytes. Moreover, Robo1 expression was reduced during differentiation of HSPCs towards CD14+ cells *in vitro*. Slit3 inhibited the CXCL12-induced migration of Robo1-expressing HL60 and U937 cells, while it enhanced the directional migration of monocytes. HSPC migration was not affected under these conditions, but lower Slit3 concentrations inhibited directional HSPCs migration, which is in agreement with their higher Robo expression levels. Thus, the outcome of Slit responsiveness appears to be dependent on Robo expression levels. s.geutskens@sanquin.nl

0351

CRITERIA FOR INTENSIVE CARE UNIT ADMISSION OF CRITICALLY ILL HAEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS - A SCORING SYSTEM

C.L. Wong, J. Apperley, E. Kanfer, D. Marin, D. Milojkovic, A. Rahemtulla, K. Patel, E. Olavarria

Hammersmith Hospital, LONDON, UK

Background. There is only general guidance within our Haematology Department relating to patients referred to Intensive Care Unit (ICU). On average, approximately 15 patients are admitted into the ICU every year after undergoing Haematopoietic Stem Cell Transplant (HSCT) in our institution. There is a need for consistent criteria to be used in the assessment of critically ill patients so that appropriate referral pathways to ICU can be followed. *Aims.* The overall aims of the study were: 1) To identify critically ill Haematology patients using consistent criteria. 2) To assess the degree of illness severity according to the number of organ failures and the new SAPS II score (Simplified Acute Physiology Score). 3) To set a standard, using the data collected above, against which a patient can be assessed and referred to ICU appropriately. *Method*. The first phase of the study was conducted on 9 patients with haematological malignancies who were admitted into the ICU. It showed that critically ill patients could be defined as patients who fulfilled ≥2 of the following criteria: sepsis, haemodynamic instability, respiratory failure, acute renal failure, use of inotropes, liver abnormality, Glasgow Coma Scale <6 and/or acute grade III-IV GVHD. The number of organ failures and SAPS II score needed to be explored further to assess their reliability in discriminating patients who need to be referred to ICU and their outcome. Therefore, the second phase of the study was subsequently conducted on 40 consecutive patients who had undergone autologous or allogeneic HSCT over a period of six months. *Results*. There were 25 autografts and 15 allografts. 14 (35%) patients became critically ill as defined by the above criteria. 8 out of 14 (57%) critically ill patients were referred for ICU management, of which 4 were admitted into ICU. The mortality amongst patients fulfilling the definition of critically ill was 43%. All 14 critically ill patients had ≥2 organ failures although this was not found to be a useful factor for discriminating patients who needed to be referred to ICU. The SAPS II score, however, was very useful in predicting outcome. All patients with a SAPS II score of <56 points survived the critically ill episode without transfer to ICU. Patients with a SAPS II score >65 points had 100% mortality regardless of whether they were transferred to ICU or not. Finally, patients with a SAPS II score between 45-65 points were better managed in ICU, although the SAPS II score was not predictive of their survival. *Summary and Conclusions*. Critically ill patients after HSCT should be identified using ≥2 criteria as per study proforma. SAPS II score should be performed on all critically ill patients to help discriminating patients who need to be referred to ICU. A *window* of between 45 and 65 points was defined as the optimal range in which patients should be considered for ICU transfer.

0352

COMPARISON OF IV BUSULFAN AND FLUDARABINE VS IV BUSULFAN AND CYCLOPHOSPHAMIDE AS CONDITIONING REGIMEN IN MYELOABALTIVE ALLOGENEIC STEM CELL TRANSPLANTATION

S. González de Villambrosia, A. Moretó, J. Núñez, A. Bermúdez, L. Yáñez, A. Insunza, A. Iriondo

Hospital Universitario Marqués de Valdecilla, SANTANDER, Spain

Objetives. To evaluate the effectiveness and toxicity of once-daily iv busulfan and fludarabine (BUFLU) compared to busulfan and cyclophosphamide (BUCY) as conditioning regimen for myeloablative allogeneic stem cell transplantation. *Methods and material*. Retrospective study of 40 patients who underwent allogeneic transplantation in our centre from May 2005 to January 2008. 20 patients received busulfan (3.2 mg/kg x 4 days; days -5 to -2) and fludarabine (40mg/m^2 x 4 days; days -5 to -2) as conditioning regimen. Busulfan (3.2 mg/kg x 4 days; days -7 to -4) and cyclophosphamide (60 mg/kg x 2 days; days -3 to -2) was given in 20 patients. There were no statistical significant differences between both groups comparing age, sex, diagnosis, comorbidity, lines of treatment, disease status, histocompatibility, donor type (55% unrelated in BUFLU *vs* 40% in BUCY), stem cell source (bone marrow 95% *vs* 80%) and G-CSF use (10% *vs* 15%). *Results.* Considering hematopoietic recovery, there were no differences in days to 100 and 500 neutrophil engraftment (13.55 *vs* 13.6; 16 *vs* 18) and to 20000 and 50000 platelet engraftment (16.40 *vs* 18.95; 26.5 *vs* 38) between BUFLU and BUCY groups. However, in the BUFLU group we found a shorter total duration of neutropenia below 100 (4.5 *vs* 8.9 days p=0.0001) and 500 (9.2 *vs* 14.77 days p=0.0015) and lower red blood cell and platelet transfusion requirements (2.15 *vs* 5.85 p=0.006 and 4 *vs* 14 p=0.029 respectively). Complete donor chimerism in day +30 was observed in 19 (95%) patients with the BUCY regimen and 8 (40%) patients with BUFLU (p=0.0002). In the latest group, more than 75% of donor chimerism was found in 9/12 patients with mixed chimerism and all patients, except one, reached complete donor chimerism by day +120. No patient developed neurological toxicity. Considering gastrointestinal toxicity (nausea, vomiting and early diarrhea), liver toxicity (OMS) and veno-oclussive disease (5% *vs* 5%) there were not significant differences between both groups. The incidence of severe mucositis was similar with both regimens (55% *vs* 50%) but median duration of parenteral nutrition was shorter with BUFLU (4 *vs* 7.5 days). There was less grade III/IV acute graft versus host disease (aGVHD) in the BUFLU group (25% *vs* 55% p=0.05). Mortality before day 100 was 13% (2/15) in the BUFLU group (both of them due to early relapse) and 5% (1/20) in the BUCY group (due to refractory aGVHD). No patient died because of toxicity in the BUFLU group. With a median follow up of 160 days in the BUFLU group and 381 days in the BUCY group, we found no significant differences in overall survival (90% *vs* 75% respectively) and relapse rate (20% *vs* 15% respectively). *Conclusions.* In our experience, iv BUFLU as mieloablative conditioning regimen in allogeneic stem cell transplantation is associated with lower early toxicity, neutropenia duration, transfusion requirements and severe aGVHD than iv BUCY. It is necessary a longer follow up to evaluate survival and relapse.

0353

COMPARING TOXICITY PROFILES OF INTRAVENOUS BUSULFAN IN PRETRANSPLANT CONDITIONING -DIVIDED DOSES VERSUS SINGLE DAILY DOSE

S. Narayan, E. Thoulouli, G.S. Lucas, J. Burthem, J. Lee, M. Waller, H. Lenehan, J.A.L. Yin

Manchester Royal Infirmary, MANCHESTER, UK

Background. Oral busulfan has been used in pretransplant conditioning for many years. Its safety in clinical use however has been compromised by its unpredictable intestinal absorption, erratic bioavailability and high incidence of hepatic veno-occlusive disease (VOD). Introduction of a parenteral formulation ensuring 100% bioavailability with predictable pharmacokinetics has led to its increased use in clinical practice. Although pharmacokinetic monitoring allows individualization of drug dosage, it is not universally available. Intravenous Busulfan has demonstrated a good safety profile when administered at 0.8 mg/kg 6-hourly. Recent studies have used busulfan as once daily dosing at 3.2 mg/kg/day with no increased toxicity or end-organ damage despite higher plasma concentration times. We retrospectively analysed patients treated at Manchester Royal Infirmary with an intravenous busulfan-based conditioning regimen and compared toxicity profiles between 6-hourly and once daily dosing. *Patients and Methods.* Between May 2005 and January 2008 25 patients with haematological malignancies were transplanted using intravenous busulfan either at 0.8mg/kg/dose 6-hourly (group I; n=13) or 3.2 mg/kg once daily (group II; n=12). Regular clinical and laboratory indices of organ function and toxicity were monitored. No pharmacokinetic studies were performed. Conditioning regimens used were BuCy or FluBuCamp as per standard protocols. Phenytoin and LMWH prophylaxis were used in all patients. Azoles and Paracetamol were avoided until 48hours after completion of busulfan infusion. Organ toxicity was documented according the NCI-CTCAE version3 (National Cancer Institute's Common Toxicity Criteria for Adverse Events). Hepatic VOD was diagnosed based on Baltimore criteria. *Results and Discussions*. All patients engrafted. Median time to neutrophil engraftment in both groups was 12.5days. In group I, one patient developed secondary graft failure following an HLA-Cw mismatched unrelated donor trans-

plant. No infusion-related toxicities were observed. All patients developed mucositis, most commonly grade2-3. No neurotoxicity was seen. 4 patients in group I and 11 in group II developed transiently impaired renal function. Temporary renal replacement therapy was required in one patient in group II who had ongoing renal impairment following cadaveric renal transplant. In both groups 90% of patients developed hepatotoxicity (grades 1-3), most commonly manifesting as transient elevation of ALT and bilirubin. Hepatic VOD was suspected in one patient in group I, who later succumbed to accelerated CML. In group II, two patients developed VOD. One patient with multiple risk factors developed severe disease with hepatorenal syndrome which was fatal. *Conclusions*. Intravenous busulfan may be used with once daily dosing safely and efficaciously. Careful patient selection and choice of transplant regimen together with minimal use of concomitant hepatotoxic drugs may reduce the incidence and severity of regimen related toxicity. More studies are needed to evaluate safety of once daily busulfan, especially in heavily pretreated patients with multiple risk factors for organ toxicity.

0354

IMPACT OF MTOR INHIBITION ON LYMPHOID HOMING AND TOLEROGENIC FUNCTION OF NANOPARTICLE LABELED DENDRITIC CELLS FOLLOWING ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

R. Zeiser,[1] R. Reichardt,[1] C. Dürr,[1] D. Von Elverfeldt,[2] E. Jüttner,[3] B. Smith,[4] R.S. Negrin[4]

[1]Albert-Ludwigs University Freiburg, FREIBURG, Germany; [2]Department of Diagnostic Radiology and Medical Physics, FREIBURG, Germany; [3]Department of Pathology, Freiburg University, FREIBURG, Germany; [4]Stanford University School of Medicine, STANFORD, USA

Background. Dendritic cells (DC) play a major role in the pathogenesis of graft-versus-host disease (GvHD). Directed modification of surface molecules on DC that provide instructive signals for T cells may create a tolerogenic DC phenotype that affects GvHD severity. *Aims.* To investigate the impact of the mTOR inhibitor rapamycin (RAPA) on *in vivo* migratory capacities, tolerogenic function and B7 family member surface expression on DC following allogeneic hematopoietic cell transplantation (aHCT). *Methods.* To generate a platform for magnetic resonance imaging (MRI) and bioluminescence imaging (BLI) based cell trafficking studies we labeled luciferase transgenic DC with superparamagnetic iron oxide (SPIO) nanoparticles bound to a murine IgG antibody that allowed for Fc γ (R) receptor mediated endocytosis. *Results.* Locally injected luc+ DC could be tracked within their anatomical context at high resolution by BLI and MRI after aHCT, based on stable intracellular localization of SPIO-IgG complexes. RAPA preconditioned DC (DC-R) displayed reduced expression of MHC class II, B7-1 (CD80) and B7-2 (CD86) but not B7-H4 whose ligation of T cells has a profound inhibitory effect on their proliferation and cytokine secretion. DC-R of recipient genotype reduced GvHD severity as evidenced by improved survival and reduced allogeneic T cell expansion, which is compatible with their tolerogenic phenotype. CCR5, CCR7 and CD62L expression was not affected by mTOR inhibition which allowed for DC-R *in vivo* trafficking to secondary lymphoid compartments where immunregulation is required. *Conclusions.* This study is the first to delineate the impact of RAPA on DC migration and tolerogenic function after aHCT. Modification of the DC phenotype by mTOR inhibition may have therapeutic potential in an attempt to reduce GvHD following aHCT.

0355

RECONSTITUTION OF T CELLS AFTER *IN VIVO* T CELL DEPLETION WITH ALEMTUZUMAB IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

E.M. Wagner,[1] T. Schmitt,[2] S. Wenzel,[2] J. Hemmerling,[2] A. Konur,[2] K. Kolbe,[2] C. Huber,[2] W. Herr,[2] R.G. Meyer[2]

[1]Haematology and Oncology, MAINZ; [2]Johannes Gutenberg-Universität, MAINZ, Germany

Introduction. Targeting CD52 by alemtuzumab is frequently used for *in vivo* T cell depletion (TCD) in allogeneic stem cell transplantation (HSCT). We have recently introduced a protocol applying alemtuzumab-mediated TCD allogeneic HSCT followed by prophylactic application of CD8-depleted donor lymphocyte infusions (DLI) [Meyer, Blood 2007; 109: 374]. Here we provide data on the CD52-expression of reconstituting lymphocytes and monocytes as well as on the influence of DLI-application in patients in following this protocol. *Methods and Results*. Peripheral blood mononuclear cells of 20 patients after HSCT following conditioning with fludarabine, melphalan and alemtuzumab were monitored for the expression of CD52 by flow cytometry. Of these patients, 9 were treated with CD8-depleted DLI. Eleven patients did not receive DLI because of mild graft-versus-host disease (GVHD, n=9) or unavailability of the donor (n=2). The CD52-expression in monocytes, B and NK cells remained unaltered after transplantation and was not influenced by the application of CD8-depleted DLI. The majority of reconstituting CD4 T cells were CD52-negative after transplantation. In those patients who did not receive DLI, the majority of CD4 T cells remained CD52-negative throughout the first year after HSCT. This cannot be explained by a direct effect of alemtuzumab, because earlier studies have shown that the antibody is not present in active plasma concentrations beyond day +60 after HSCT. If CD8 depeted DLI were applied, they lead to the rapid re-establishment of CD52-positive CD4 T cell-populations. Hence, the proportion of CD52-positive CD4 T cells was significantly higher in patients who had received DLI compared to those who had not. Although the DLI were depleted from CD8 T cells, there was also a trend towards more CD52-positive CD8 T cells in patients who had received DLI. We further analysed the donor chimerism of CD52-positive and -negative CD4 T-cell subpopulations in two patients who received DLI as well as in one patient who did not. In all three patients, the CD52-positive subpopulations were mainly of host origin, whereas the majority of donor T cells were among the CD52-negative subpopulation. After the application of DLI, the CD52-positive CD4 T cell-populations switched to donor-type, indicating the proliferation of the transferred CD4 T cells *in vivo*. In contrast, in the patient who did not receive DLI, the percentage of donor chimerism of the CD4pos/CD52pos population remained considerably lower than in the CD4pos/CD52neg fraction. *Conclusions*. T cell depletion applying alemtuzumab leads to the reconstitution of CD52-negative T cell-populations, especially in the CD4-positive population. The majority of T cells remain CD52-negative beyond the presence of the antibody. By following the CD52-expression before and after CD8-depleted DLI, we demonstrated the *in vivo* proliferation of DLI-derived T cells. Spontaneously reconstituting CD52-positive CD4 T cells were mainly of host origin which might indicate a differentiated effect of alemtuzumab on CD4 T cell subsets.

0356

PROGNOSTIC FACTORS OF CHRONIC GRAFT VERSUS HOST DISEASE AFTER ALLOGENEIC BLOOD STEM CELL TRANSPLANTATION: NIH SCALE PLUS TYPE OF ONSET PREDICT SURVIVAL AND DURATION OF IMMUNE SUPPRESSIVE THERAPY

J.A. Pérez-Simón, C. Encinas, F. Silva, M.J. Arcos, M. Díez-Campelo, F. Sanchez-Guijo, E. Colado, J. Martín, L. Vazquez, C. Cañizo, D. Caballero, J.F. San Miguel

Hospital Universitario, SALAMANCA, Spain

Background. Chronic graft-versus-host disease (cGVHD) is a major complication after peripheral blood stem cell transplantation (PBSCT). Several grading systems have been developed trying to predict survival among patients with cGVHD. Nevertheless, most of these studies have been developed in the bone marrow transplantation (BMT) setting while the characteristics of cGVHD differ between PBSCT and BMT. The National Institutes of Health (NIH) have proposed a new clinical scoring system for assessment of cGVHD severity based on the number of organs involved and the degree of functional impairment. Nevertheless, this scoring system requires to be validated to assure its prognostic impact. *Aims.* In the current study we have evaluated the prognostic value of the NIH scoring system and searched for additional prognostic factors in a series of 171 patients consecutively undergoing PBSCT from a matched related donor. All patients received CsA plus MTX as GVHD propylaxis. 68 patients received myeloablative and 103 reduced intensity conditioning regimen. 37% were categorised as low risk according to disease status at transplant, 43% as intermediate and 20% as high risk. *Results.* Cumulative incidence of cGVHD was 70% among patients surviving > 100 days after transplant and 60% were cathegorized as having extensive cGVHD. Cumulative incidences of mild, moderate and severe cGVHD were 29%, 42% and 28%, respectively. Overall, sixty eight percent of patients were off immune suppression 5 years after transplantation. Absence of prior aGVHD [HR=2.7 (95% CI = 1.3-6), p=0.004] and mild cGVHD [HR=4.2 (95% CI = 1.4-12.12), p=0.007] significantly increased the probability of being off treatment at last follow up. With a median follow-up of 54 months overall survival at 5 years was 52%. Severe cGVHD according to NIH scoring system [HR=13.27 (95%

CI = 2.81 - 62.5), *p*=0.001] adversely influenced on outcome while *de novo* onset [HR=0.094 (95% CI = 0.02-0.43), *p*=0.003] had a favourable impact on survival. The combination of both variables allowed to identify 4 different subgroups of patients in terms of outcome, with OS of 82%, 70%, 50% and 25%, respectively. *Conclusions.* NIH scoring system is of prognostic value among patients undergoing PBSCT and, together with type of onset, must be considered in order to predict the outcome of patients who developed cGVHD. These parameters should be considered in order to adapt immune suppressive strategies to the risk of the patients.

0357

IMPACT OF NOD2/CARD15 POLYMORPHISMS ON TREATMENT OUTCOME IN *EX VIVO* T-CELL DEPLETED HAEMATOPOIETIC STEM CELL TRANSPLANTATION

W.J.F.M. van der Velden, N.M.A. Blijlevens, N.P.M. Schaap, H. Dolstra, J.P. Donnelly

Radboud University Nijmegen Medical Center, NIJMEGEN, Netherlands

Background. NOD2 is as an intracytosolic pattern recognition receptor sensing the microbial component muramyl dipeptide. It is expressed in Paneth cells, dendritic cells and monocytes and plays an important role in the innate immune defences and immune regulation at the epithelial surfaces of gut and lung. NOD2 polymorphisms (SNPs), resulting in dysfunction of NOD2, have been shown to influence the outcome of haematopoietic stem cell transplantation (HSCT). However, the impact of NOD2 polymorphisms in the setting of *ex vivo* T-cell depleted HSCT remains to be determined because contradicting data exist. Aims. To determine the impact of NOD2 polymorphisms on treatment outcome in *ex vivo* T-cell depleted HSCT. *Methods.* We performed a retrospective analysis in one hundred and twenty-five (n=125) Dutch patients and their donors admitted at our transplant unit between May 1996 and November 2005 for an HLA-identical sibling, *ex vivo* T-cell depleted allogeneic HSCT. All patients were treated according to the same protocol and received ciprofloxacin antimicrobial prophylaxis. We selected a homogenous group of eighty-five (n=85) patients all receiving a uniform conditioning regimen containing idarubicin, a regimen associated with the occurrence of severe mucositis. We assayed NOD2 polymorphisms SNP 8 (Arg702Trp), 12 (Gly908Arg) and 13 (Leu1007fsinsC) with the use of a Taqman real time PCR protocol. *Results.* The overall frequency of NOD2 SNPs was 18.8%. Allele frequencies were 6.2, 0.6 and 3.2% for SNP8, 12 and 13 respectively. In 22 pairs at least one mutation was present. In 4 pairs (4.7%) only recipients had a mutation, in 8 pairs (9.4%) only donors and in 10 pairs (11.8%) both donor and recipient. Mutations were associated with significant higher incidence of severe aGvHD (grade III-IV); mutation present 18.2% *vs* wt 3.2%, and in case of both recipient and donor mutated, 30% *vs* wt 3.2%. There was also a significant impact on the 1-year treatment related mortality (TRM) in pairs with both recipient and donor mutated; TRM 1- year 50% *vs* wt 11.1%. There seemed to be a trend towards a higher incidence in gram positive bacteraemia in the presence of these polymorphisms. No significant impact on disease relapse was found. *Conclusions*. We show a significant impact of NOD2 polymorphisms on the outcome of *ex vivo* T-cell depleted HSCT, regarding occurrence of aGVHD and TRM. Pre-transplant screening for these polymorphisms should be considered in order to select the most appropriate donor. Importantly, the impact of NOD2 polymorphisms seems to be determined by factors such as background incidence of aGVHD and mortality as well as the allele frequency of the NOD2 polymorphisms in the HSCT population.

Thrombosis I

0358

CLOTTING FACTOR VIII AND RISK OF VENOUS THROMBOEMBOLISM IN CANCER PATIENTS - RESULTS OF THE VIENNA CANCER AND THROMBOSIS STUDY

R. Vormittag, R. Simanek, A.L. Chiriac, C. Ay, D. Dunkler, P. Quehenberger, C. Marosi, C. Zielinski, I. Pabinger

Medical University Vienna, VIENNA, Austria

Background. Malignancy represents a complex acquired condition of thrombophilia. VTE is a main determinant of cancer morbidity and mortality. The identification of reliable markers of risk prediction and individual risk stratification will be needed to treat patients selectively according to their risk profile in view of preventing thrombosis and improving survival. Elevated levels of clotting factor VIII (FVIII) are an established risk factor for primary and recurrent VTE. Up to now, no prospective data on FVIII as predictive parameter for VTE in cancer patients are available. *Aims*. The aim of this prospective study was to assess whether FVIII was a risk marker for VTE in cancer patients. *Methods*. Patients with newly diagnosed cancer and those with disease progression that did not recently have chemotherapy, radiotherapy or surgery were included in the prospective observational CATS and followed prospectively. In case of overt infection patients were not enrolled. Study endpoint was symptomatic, objectively confirmed VTE. Factor VIII activity was measured on a Sysmex CA 7000 analyzer using factor VIII deficient plasma (Hyland Baxter Immuno, Vienna, Austria) and Dade Actin-FS (Dade Behring, Marburg, Germany). Kaplan-Meier and Cox regression analyses were used for statistical evaluation. Multivariable Cox regression analyses included (continuous or dichotomized) FVIII, age, sex and treatment regimen. Treatment regimen (surgery and/or radiotherapy and/or chemotherapy) was included as time-dependent parameter. Elevated FVIII was a dichotomized variable indicating plasma levels above or below 230% (95th percentile of a group of 75 healthy, age- and sex-matched control individuals).

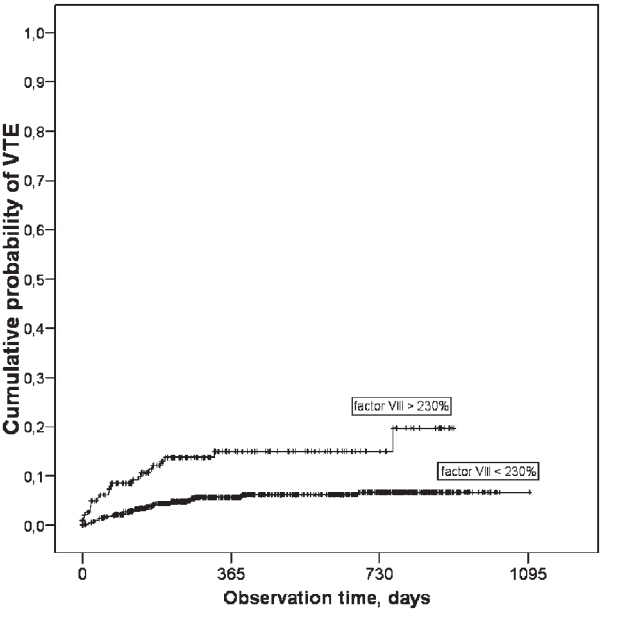


Figure 1.

Results. Data on 786 cancer patients (353 women/433 men) were available for analyses. Patients were followed for a median observation time of 422 days. 56 events were observed. Main tumour entities were malignancies of the breast (n=132), lung (n=100), gastrointestinal tract (n=141), pancreas (n=47), kidney (n=21), prostate (n=99) and brain (n=94); 109 patients had haematological malignancies and 43 other tumours. No relevant correlation between FVIII and the inflammatory marker fibrinogen was found (correlation coefficient 0.16). A twofold increase of FVIII was associated with a 3.0-fold [95% CI: 1.8-5.1] increased hazard ratio (HR) for VTE in univariate analysis and a 3.6-fold HR [2.1-6.2] in multivariable analysis. Elevated FVIII was present in 180 (22.9%) patients. Elevated FVIII was associated with a 2.7-fold increased HR [1.6-4.7] for

VTE in univariate analysis and a 3.0-fold [1.7-5.1] increased HR in multivariable analysis (Figure 1). *Conclusions.* FVIII was independently associated with an increased risk of VTE in cancer patients.

0359

HYPOFIBRINOLYSIS IS A RISK FACTOR FOR MYOCARDIAL INFARCTION IN YOUNG MEN

M.E. Meltzer,[1] C.J.M. Doggen,[2] P. De Groot,[3] F. Rosendaal,[2] T. Lisman[4]

[1]University Medical Center Utrecht/Leiden University Medical Center, UTRECHT / LEIDEN; [2]Leiden University Medical Center, LEIDEN; [3]University Medical Center Utrecht, UTRECHT; [4]University Medical Center Utrecht/University Medical Center Groningen, UTRECHT/GRONINGEN, Netherlands

Results of studies on the relation between the fibrinolytic system and arterial thrombosis have been conflicting. Previously, we demonstrated that hypofibrinolysis as measured by a plasma-based assay constitutes a risk factor for venous thrombosis. In the present study we investigated the effect of hypofibrinolytic activity on the risk of myocardial infarction (MI) using the same assay. We included 560 men with a first MI and 646 control subjects under the age of 70 who participated in the Study of Myocardial Infarctions Leiden (SMILE), a population-based case-control study. Participants filled in a questionnaire and provided a blood sample. Patients and controls using anticoagulants at time of blood draw were excluded, leaving 426 patients and 646 controls in present analyses. Lysis of a tissue factor-induced clot by exogenous tissue-type plasminogen activator was studied by monitoring changes in turbidity during clot formation and subsequent lysis. Using quartiles of clot lysis time (CLT) based on values found in control subjects, we found age-adjusted Odds Ratios (ORs) (95% confidence interval) of 1.7 (0.7-3.7), 3.1 (1.4-6.9), and 3.2 (1.5-6.7) for respectively the second, third, and fourth quartile compared with the first, in men below 50 years. After adjusting for lipid levels, blood pressure, C-reactive protein, body mass index, diabetes, smoking, and alcohol use, these ORs decreased to 1.2 (0.5-3.1), 2.1 (0.8-5.2), and 1.8 (0.7-4.8). In men above 50 years no clear association was found between CLT and MI-risk. The age-adjusted OR in these men with CLTs in the fourth quartile was 0.9 (0.6-1.3) compared with the first. After further adjustment for the cardiovascular risk factors described above, the OR was 0.7 (0.4-1.1). Our study shows that decreased fibrinolytic capacity increases the risk of a first MI in men under the age of 50.

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0360

THE RISK OF PULMONARY EMBOLISM DUE TO PROXIMAL DEEP VENOUS THROMBOSIS OF THE LEGS DIFFERS IN PATIENTS WITH DIFFERENT KINDS OF INHERITED THROMBOPHILIA

E. Rossi, T. Za, A. Ciminello, G. Leone, V. De Stefano

Institute of Hematology, Catholic University, ROME, Italy

Background. It is uncertain whether the presence of inherited thrombophilia influences the risk of developing pulmonary embolism (PE) and whether different thrombophilic alterations are associated with different incidences of PE. *Aims.* The present study is aimed to investigate the risk of PE among patients with deep vein thrombosis (DVT) and inherited thrombophilia. Patients and *Methods.* We studied 920 patients (M/F 397/523) with proximal DVT of the legs with or without symptomatic PE referred for thrombophilia screening, after exclusion of patients with overt cancer and antiphospholipid antibodies; DVT was unprovoked in 315 patients and provoked by a triggering circumstance in 605 ones. The median age at the time of DVT was 37 years (range 0 to 85).Three hundred and fifty-three patients (38.3%) had deficiency of antithrombin (AT, n=16), protein C (PC, n=26), protein S (PS, n=22), factor V Leiden (FVL, n=168), prothrombin G20210A (PT-GA, n=87), or multiple abnormalities (n= 35), and 566 had no known defect. We analyzed the incidence of PE at the time of the first DVT in the patients with thrombophilia in comparison with those with no known defect. *Results.* First DVT was complicated by symptomatic PE in 242 patients (26%); the risk of PE was increased in patients with AT deficiency (relative risk, RR, 2.4, 95% CI 1.6-3.6) or with PT-GA (RR 1.5, 95% CI 1.1-2.0) and decreased in those with FVL (RR 0.7, 95% CI 0.5-1.0) in comparison with those with no known inherited defect. The increase in risk for PE associated with AT deficiency or PT-GA was more pronounced in women (RR 4.0, 95% CI 2.4-6.5 and 1.8, 95% CI 1.1-2.7, respectively) and in patients younger than 45 years (RR 3.6, 95% CI 2.3-5.6 and 2.0, 95% CI 1.3-3.0, respectively). In the subgroup of younger women the risk associated with AT deficiency was 4.9-fold increased (95% CI 2.6-9.2) and the risk associated with PT-GA was 2.3-fold increased (95% CI 1.3-3.9). *Conclusions.* The design of our study is susceptible of underestimation of the rate of PE among DVT patients. However, there is no reason to think that the pattern of underestimation should have differed among the patient groups, having the diagnostic procedures been carried out before the referral to the Thrombosis Center. Therefore, the estimate of the relative risk between patients is quite reliable. Our data suggest that patients with DVT have different risks of PE according to the genotype and that AT deficiency or PT-GA can lead preferentially to PE. The risk associated with such abnormalities is further increased in women and in younger individuals.

0361

CANCER-RELATED VENOUS THROMBOSIS: RESIDUAL VEIN THROMBOSIS IMPROVES SCREENING FOR OCCULT CANCER

S. Siragusa, A. Malato, G. Saccullo, I. Abbene, D. Caramazza, L. Lo Coco

University Hospital of Palermo, PALERMO, Italy

Background. Clinical advantages of extensive screening for occult cancer in patients with idiopathic Deep Vein Thrombosis (DVT) is still debated since this approach improves early detection of cancer but not cancer-related mortality. Recently, we have demonstrated that patients with Residual Vein Thrombosis (RVT), 3 months after DVT, have a high risk for cancer in the subsequent 2 years (Siragusa S *et al.* Blood 2005;106(11):OC262). At the present it is unknown whether RVT assessment may be used to select patients, with idiopathic DVT, who require screening for occult cancer. *Objective of the study*. We conducted a prospective study evaluating whether a RVT-based screening for cancer is sensitive and influences cancer-related mortality. Study design. Prospective with two cohorts of DVT patients: the first cohort was monitored for clinical overt cancer only (Group A), while the second (Group B) received complete screening for occult neoplasm and subsequent surveillance. *Materials and methods*. Consecutive patients with a first episode of DVT who presented RVT after 3 month of anticoagulation and without signs and/or symptoms for overt cancer. Screening for occult cancer was based on: ultrasound and/or CT scan of the abdomen and pelvis, gastroscopy, colonoscopy or sigmoidoscopy, hemoccult, sputum cytology and tumor markers. These tests were extended with mammography and Pap smear for women and ultrasound of the prostate and total specific prostatic antigen (PSA) for men. All investigations had to be completed within four-weeks from the assessment of RVT. All patients were followed-up for at least 2 years. Incidence and cancer-related mortality was compared between the two groups by survival curves (Kaplan-Mayer) and related Breslow test for statistics. *Results*. Over a period of 6 years, 345 patients were included in the analysis: first cohort included 213 patients (Group A), second cohort 132 (Group B). Clinical characteristics between groups were homogenous. During the follow-up, 8.4% of patients developed overt cancer in group A; in group B, 8.3% of patients had diagnosed cancer at the moment of extensive screening while one new case (0.7%) occurred during the follow-up (Table). The sensitivity of this approach was 91.6% (95% confidence intervals 74.7-108.5). Cancer-related mortality was 6.5% in group A and 3.0% in group B ($p< 0.001$) (Table 1). *Conclusions*. Our study demonstrates that RVT-based screening for occult cancer improves early detection as well as cancer-related mortality.

Table 1. Cancer events.

Characteristic	Group A (n= 213)	Group B (N= 132)
Cancer, No (%) at screening time*	- -	10 (8.3)
Cancer, No (%) during clinical surveillance**	18 (8.4)	1 (0.7)
Density incidence (cases x 1000 p/y)	41.9	40.3
Mean time cancer diagnosis (months ±SD)	6.9 (1.3)	3.6 (0.7)
Cancer-related mortality, No (%)	14 (6.5)	4 (3.0)
Cancer-related mortality, mean time (months ±SD)	19 (3.4)	18 (2.8)

0362

EFFECT OF FONDAPARINUX 2.5 MG ONCE DAILY ON ALL CAUSE MORTALITY: A META-ANALYSIS OF THROMBOPROPHYLAXIS TRIALS

J.W. Eikelboom,[1] A.G.G. Turpie[2]

[1]Hamilton Health Sciences-General Hospital, HAMILTON, ON; [2]Hamilton Health Sciences - General Division, HAMILTON, ON, Canada

Background. Compared with placebo, unfractionated heparin or low-molecular-weight heparins (LMWH), fondaparinux 2.5 mg once daily significantly reduced overall mortality by about 15% at 30 days in patients with acute coronary syndromes (OASIS-5 and -6). *Aims.* In order to determine whether this benefit was observed in other settings, we analyzed the effect of fondaparinux 2.5 mg on all-cause mortality in all phase III fondaparinux thromboprophylaxis trials. *Methods.* We performed a meta-analysis of 8 randomized, double-blind trials in patients undergoing major orthopedic (EPHESUS, PENTHIFRA, PENTAMAKS, PENTATHLON 2000, PENTHIFRA-PLUS) or abdominal (PEGASUS, APOLLO) surgery or in medical patients (ARTEMIS). In 5 trials, fondaparinux 2.5 mg once daily was compared with approved regimens of LMWH; in 3 trials, the comparator was placebo. Fondaparinux and comparators were administered for up to 31 days. The efficacy outcome was all-cause mortality up to day 32. *Results.* A total of 13,085 patients were analyzed. Compared with placebo or LMWH, fondaparinux demonstrated a non-significant reduction in all-cause mortality (p=0.058; test for heterogeneity: p=0.58). Consistent results were observed irrespective of the type of comparator (Table) and in subgroups according to gender, age, body-mass index, and a history of venous thromboembolism, myocardial infarction, stroke or cancer. In surgical trials, consistent results were also observed in patients in whom fondaparinux was initiated at least six hours after surgery. Fondaparinux tended to reduce similarly cardiovascular (OR 0.76, 95% CI: 0.55 to 1.06) and non- cardiovascular deaths (OR 0.81, 95% CI: 0.53 to 1.24). *Summary and Conclusions.* In thromboprophylactic settings, fondaparinux 2.5 mg once daily given for up to 31 days tended to reduce all-cause mortality relative to placebo/LMWH. This result is consistent with the long-term reduction in mortality observed with fondaparinux 2.5 mg in patients with acute coronary syndromes.

Table 1. Effect of fondaparinux on mortality.

Day 32	Fondaparinux 2.5 mg	Comparator	Odds ratio (95% CI)
versus placebo	2.0% (28/1405)	2.6% (36/1409)	0.77 (0.46 to 1.26)
versus LMWH	1.5% (77/5133)	1.9% (98/5138)	0.78 (0.58 to 1.06)
All studies	1.6% (105/6538)	2.1% (134/6547)	0.79 (0.60 to 1.01)

0363

GESTATIONAL AGE-RELATED D-DIMER IN LMWH TREATED PREGNANT WOMEN WITH THROMBOPHILIA

U. Harbrecht, B. Enste, S. Flommersfeld, E. Höbert, H. Seidel, J. Oldenburg

Institute for Experimental Haematology and Transfusion Medicine, BONN, Germany

Background. D-Dimer is increasingly used to guide anticoagulation in pregnant women with thrombophilia and/or previous thrombosis. However, evidence in terms of onset, intensity and duration of anticoagulation is not well-established. Furthermore, gestational reference values for single D-dimer-tests are lacking. *Aims.* We investigated the course of D-dimer in low-molecular-weight heparin (LMWH) treated pregnants related to gestational age and intensity of anticoagulation. *Methods.* The study population comprised 292 women who had completed 323 pregnancies; 59% of patients had a history of thrombosis (n=171), 21% inherited (n=63) and 20% acquired (n=58) thrombophilia without thrombotic events. A subgroup of 24% (n=69) suffered from (recurrent) fetal loss. After risk stratification LMWH was given in prophylactic doses (n=303) at 40-100 AXaU/kg/d s.c. during entire or limited period of pregnancy and after delivery to achieve AXa-levels of 0.1-0.4 U/mL or in therapeutic dosis (100-200 AXaU/kg/d) in a few patients (n=20) with an AXa-target of 0.4-1.0 U/mL. D-dimer (Vidas D-Dimer®, Bio Merieux) and anti-factor-Xa (Heparin®, Instrumentation Laboratory) were measured at least once per trimester and in puerperium if possible. *Results.* Mean D-dimer (normal range 0-0.49 µg/mL) increased continuously under prophylactic LMWH regimen and was 0.44±0.29, 0.77±0.57 and 1.19±0.65 µg/mL in the first (week 8-13), second (week 14-26) and third trimester (week 27-40) and 0.57±0.65 (0.10-4.44) in puerperium (3-6 weeks after delivery). The corresponding AXa-levels were 0.27±0.12, 0.24±0.13, 0.26±0.13 and 0.40±0.17 U/mL, indicating antithrombotic therapy within the target range. In the therapeutic patient group D-dimer was considerably suppressed by LMWH. Two patients (0.6%) suffered from new thrombosis during LMWH therapy. *Conclusions.* D-dimer increases continuously with gestational age in heparin treated pregnants. Heparin appears to attenuate the natural increase of D-dimer dose-dependently.

0364

HEREDITARY AND ACQUIRED THROMBOTIC RISK FACTORS FOR CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION

C.L. Wong, R.M. Szydlo, S. Gibbs, M.A. Laffan

Hammersmith Hospital, LONDON, UK

Background. The role of thrombosis in the pathogenesis of chronic thromboembolic pulmonary hypertension (CTEPH) and non-CTEPH is not clear. Factor VIII (FVIII) is frequently elevated but previous studies have failed to identify a role for hereditary thrombotic risk factors. *Aims.* To analyse the frequency of both hereditary and acquired thrombotic risk factors in CTEPH compared to non-CTEPH. *Methods*. Between December 2005 and June 2006, 245 patients were investigated for pulmonary hypertension (PH) at Hammersmith Hospital. 45 had CTEPH and 200 had non-CTEPH. Patients were tested for antithrombin, protein C, protein S, Factor V Leiden (FVL) and prothrombin (PTG20210A) gene polymorphisms, antiphospholipid antibody (APA), lupus anticoagulant (LA), FVIII, von Willebrand factor (VWF) and fibrinogen. Ethnic group, blood group, WHO functional status and oxygen saturation were also recorded. *Results*. 9 out of 31 (29%) Caucasian patients with CTEPH *vs* only 10 out of 129 (5.15%) Caucasian patients with non-CTEPH were heterozygous for FVL (p=0.001). For the PTG20210A polymorphism, the frequency was not significantly different (p= 0.325) between the two groups, although the prevalence of 7.89% in CTEPH is similar to that in patients with thrombosis. In contrast to other studies, we have not found a significant increase in APA and LA. The incidence of elevated FVIII and VWF (>150 u/dL) was significantly increased in patients with CTEPH and non-CTEPH, although they were significantly higher (p<0.012) in the CTEPH group as compared to the non-CTEPH group. The ratio of blood group O to non-O between the 2 groups was found to be not significant (p=0.13). There was no correlation between fibrinogen and VWF levels in the CTEPH group (p=0.5), in contrast to the strong correlation between fibrinogen and VWF in the non-CTEPH group (p=0.001). There was no correlation between FVIII-VWF with WHO functional status in the CTEPH group although there was a strong correlation (p<0.001) between the two parameters in the non-CTEPH group. For equivalent WHO functional class, FVIII levels were higher in the CTEPH group. We have found no correlation between oxygen saturation and levels of FVIII and VWF in both groups of patients. *Summary and Conclusions*. The results indicate different pathophysiologies for CTEPH and non-CTEPH. This is the first study to find significantly increased prevalence of FVL polymorphisms and in association with the higher FVIII levels imply a role for thrombosis in the development of CTEPH. Higher levels of FVIII-VWF are not explained by differences in WHO functional class or oxygen saturation. The association between VWF and fibrinogen in the non CTEPH group suggests that elevation of VWF may be secondary to inflammation in this group and may also reflect associated disorders. The elevation of FVIII in the CTEPH group appears to be a primary phenomenon similar to that seen in patients with thrombosis and together with the increased prevalence of FVL in this group supports a primary role of thrombosis in aetiology.

0365

LEPTIN INDUCES THE EXPRESSION OF FUNCTIONAL TISSUE FACTOR IN HUMAN NEUTROPHILS AND PERIPHERAL BLOOD MONONUCLEAR CELLS THROUGH JAK2-DEPENDENT MECHANISMS AND TNFα INVOLVEMENT

S. Rafail,[1] I. Kourtzelis,[2] I. Mitroulis,[2] M. Speletas,[3] M. Doumas,[2] S. Giaglis,[4] K. Kambas,[2] G. Kartalis,[2] S. Konstantinides,[5] K. Schaefer,[5] K. Ritis[2]

[1]Democritus University of Thrace, ALEXANDROUPOLIS, Greece; [2]First Division of Internal Medicine, Medical School, Democritus University, ALEXANDROUPOLIS, Greece; [3]Department of Immunology and Histocompatibility, School of Medicine, University, LARISSA, Greece; [4]Foundation for Biomedical-Research of the Academy of Athens, Centre of Immunology, ATHENS, Greece; [5]Department of Cardiology and Pulmonary Medicine, Georg August University, GOETTINGEN, Germany

Background. Leptin is an adipocyte-derived cytokine primarily involved in the regulation of body weight and energy balance. Increased levels of plasma leptin have been observed in obese individuals and it is believed that hyperleptinemia may contribute to the atherothrombotic risk associated with obesity. *In vivo* studies suggest that leptin also promotes platelet aggregation and thrombosis in mice models. Furthermore, tissue factor (TF), the primary *in vivo* initiator of the extrinsic coagulation cascade, is expressed, among others, by inflammatory cells (activated endothelial cells and monocytes). Recently, polymorphonuclear (PMN) cells were also shown to produce functional TF. *Aims.* Inflammation and thrombosis are linked in many clinical disorders. Leptin is involved in the regulation of inflammation. We examined the effects of leptin on the expression of tissue factor in blood polymorphonuclear and peripheral blood mononuclear (PBMC) cells in an effort to elucidate the role of leukocytes as a source of circulating TF in hyperleptinemic disorders. *Patients and Methods.* PMN and PBMC from healthy donors (n=7) were incubated with leptin and the effects on TF expression were assayed functionally for TF activity, using a modified prothrombin time (mPT) as well as at mRNA and protein levels. Cell signalling inhibition studies were also performed in order to identify the molecular pathway involved in signal transduction. *Results.* Leptin stimulated PMN and PBMC induced a dose-dependent procoagulant activity as verified by mPT which could be completely blocked by the addition of a specific neutralizing antibody against TF. Moreover, leptin increased TF mRNA and protein expression levels in stimulated leukocytes, as determined by real-time RT-PCR, western blot, flow-cytometry and immunocytochemistry. Inhibition studies revealed that the effect of leptin on TF expression is mediated, at least in part, by JAK2 and PI3 kinase. At the same time, our findings, after neutralizing TNFα in supernatants of leptin treated cells, also suggest the involvement of TNFα in the leptin induced TF expression in leukocytes. *Conclusions.* Leptin appears to be capable of triggering the extrinsic coagulation cascade by upregulating the expression of TF in human leukocytes. These effects appear to be mediated by the leptin receptor and the JAK2-PI3 kinase intracellular signalling pathway and they may require, at least in part, the presence of TNFα. The contribution of PMN as a source for circulating TF via leptin suggests a possible novel link between obesity, inflammation and thrombosis.

0366

ENDOTHELIUM PROCOAGULANT AND ANGIOGENIC ACTIVITIES INDUCED BY ACUTE PROMYELOCYTIC LEUKEMIA (APL) CELLS ARE INHIBITED BY LOW MOLECULAR WEIGHT HEPARINS (LMWH)

A. Vignoli, M. Marchetti, L. Russo, D. Balducci, A. Falanga

Ospedali Riuniti, BERGAMO, Italy

Background. APL cells can stimulate endothelial cells to express procoagulant (PCA) and proangiogenic activities, which in turn contribute to the progression of the disease. LMWH have anti-inflammatory and antiadhesive properties on tumor cell/vascular cell interactions *in vitro*. Little is known on the possible effects of heparins on APL cell-induced endothelium procoagulant and angiogenic activities. Since endothelial Tissue Factor (TF) and its inhibitor TFPI are involved in the angiogenesis process, in a previous study, we demonstrated that LMWH dalteparin regulates the expression of TF and TFPI of microvascular endothelial cell (HMEC-1) stimulated by bacterial endotoxin (Vignoli *et al.*, Haematologica 2006). *Aims*. In this study we wanted to evaluate whether: 1. Two different LMWH (i.e. dalteparin and enoxaparin) may affect TF and TFPI of HMEC-1 exposed to APL cells; and 2. this effect may be associated to modulation of tumor-induced EC angiogenic response. *Methods.* HMEC-1 were incubated with tumor conditioned medium (CM) from the human APL cell line NB4, in presence or absence of LMWH dalteparin or enoxaparin (0.01, 0.1 and 1 IU/mL). After incubation, endothelial HMEC-1 were tested for TF activity by the one-stage clotting assay, released TFPI antigen by ELISA, and capillary-like tube formation by Matrigel assay. VEGF acted as standard proangiogenic factor. *Results*. NB4 CM induced a 47% increase in EC TF activity compared to control cells ($p<0.05$): this increase was dose-dependently inhibited by both LMWH, reaching a maximum of 70% and 78% mean inhibition with 1 IU/mL dalteparin and enoxaparin, respectively ($p<0.05$ for both LMWH vs NB4 CM alone). In addition, dalteparin and enoxaparin dose-dependently increased the release of TFPI by EC exposed to NB4 CM. In the same setting, both LMWH significantly ($p<0.05$) inhibited the capillary-like tube formation induced by NB4 CM, as well as by standard VEGF, giving a >80% inhibition at 1 IU/mL LMWH concentration against both stimuli. *Conclusions.* LMWH simultaneously counteract the prothrombotic features and the angiogenic response of microvascular endothelium elicited by APL cells. These results support a possible role for LMWH in controlling APL cell interaction with the vascular wall.

0367

PERIOPERATIVE MANAGEMENT OF ANTICOAGULATION WITH TAPERED DOSE WARFARIN

S. Schulman, B. Earl, M. Robinson

McMaster University, HAMILTON ON, Canada

Background. Controversy exists regarding optimal management of anticoagulation with warfarin before and after surgical procedures and randomized controlled trials are lacking. *Aims*. We have evaluated in a feasibility study tapered dose warfarin, which would eliminate the need for pre- and postoperative (low-molecular-weight) heparin injections. The intent was to achieve an International Normalized Ratio (INR) of 1.5 (range 1.2-1.7) on the day of the procedure, which maintains a certain degree of thromboprophylactic effect during the entire preoperative period, thereby obviating the need for bridging anticoagulation. *Methods*. We recruited at the HHS-General Hospital in Hamilton 70 patients on chronic treatment with warfarin and scheduled to undergo a variety of surgical procedures. Instead of stopping warfarin we tapered the dose by administering 50% of the maintenance warfarin dose for 5-6 days in patients with a baseline INR 3.0-3.5, for 4-5 days in patients with a baseline INR 2.1-2.9, for 3-4 days in patients with a baseline INR 1.9-2.0 and for 2 days in patients with a baseline INR 1.7-1.8. We obtained a new INR on the day before surgery and if possible also in the morning of surgery. In the evening of the day of surgery the patient took a double dose of warfarin, compared to maintenance, and thereafter the usual dose. The main outcome was the INR at the time of the procedure. *Results.* The baseline INR was 2.3±0.5. We attained a mean INR of 1.5±0.2 on the day before or the day of surgery with 86% within the desired range. Three patients (4%) had an INR of 2.0 or higher on the day of surgery. In a historical material with 200 patients stopping warfarin 5 days before surgery this happened in 3 cases (1.5%). Contributing to failure of reaching the intended INR range for surgery may have been misinterpretation of our instructions for dosing, since in 3 cases the INR increased and in 2 cases it remained unchanged from baseline.

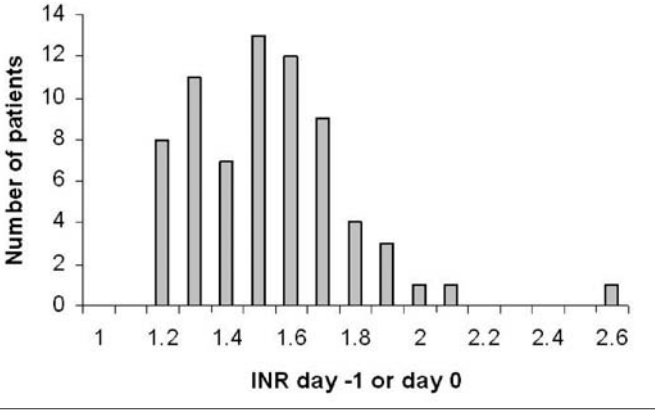

Figure 1.

Conclusions. Tapered dose of warfarin is an inexpensive alternative to bridging with heparins for invasive procedures. A small proportion of patients will remain in the therapeutic INR range by the time of surgery. This may be avoided by an INR the day before surgery and vitamin K 1 mg orally in case of unacceptable level, a strategy that we are now testing formally in a randomized controlled trial.

0368

LEONURUS HETERORHYLLUS SWEET (CHINESE MOTHERWORT HERB) EXTRACT INHIBITS TISSUE FACTOR EXPRESSION INDUCED BY THROMBIN ON HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

J. Yin,[1] GG Shi,[1] Y.J. Shen,[1] Z.W. Zhang,[1] X.G. Luo,[1] X.F. Wang,[2] H.L. Wang[2]

[1]Medical College of Shantou University, SHANTOU; [2]Shanghai Institute of Hematology, Ruijin Hospital, SHANGHAI, China

Background. Tissue factor (TF) is an initiator of coagulation and thrombosis as the receptor of clotting factor VII. Injured blood vessel endothelial cells release TF, which initiates thrombosis. In our previous study, we found that Chinese Leonurus Heterorhyllus Sweet (LHS) could inhibit thrombosis during heart ischemia. *Aims.* In this article, we investigated how LHS affected TF expression under the induction of thrombin on human umbilical vein endothelial cells (HUVECs). *Methods.* Twenty (20) grams of the dried herb (aerial part) was extracted. A stock solution of 250 mg raw material per milliliter was prepared from the freeze-dried powder of the herbal extract, from which a series of the diluted solutions were prepared in phosphate-buffered saline (PBS). Subconfluent proliferating HUVECs in 100-mm plastic dishes were incubated with thrombin (50 IU/mL) for 10 hours, and were sham-treated (control) or treated with the herbal extract (50 mg/mL for 48 hr at 37°C) in complete culture medium later. HUVECs were then washed twice with PBS to remove the herbal extract and harvested with help of trypsin for TF test. *Results.* LHS extract was effective in inhibiting TF transcription and expression induced by thrombin on HUVECs. The inhibitory action of the herbal extract was time- and dose-dependent, which reached its maximum when the final concentration of the herbal extract was 50 mg/mL and incubated with HUVECs for 48 hours at 37°C, a significant decrease of inhibitory action was shown when the drug exposure time was over 48 hours. TF activity was 31.17±3.78 U/10,000 cells (vs 58.19±4.17U/10,000 cells without LHS extract incubation, $p<0.01$), and TF antigen was 53.95±3.85pg/10,000 cells (*vs* 99.17±4.02pg/10,000 cells without LHS extract incubation, $p<0.01$) when the drug exposure time was 48 hours at 37°C and LHS extract final concentration was 50 mg/mL. Run-on assay showed that LHS extract down-regulated significantly TF gene transcription induced by thrombin on HUVECs. *Conclusions*. Leonurus Heterorhyllus Sweet was able to down-regulate TF expression induced by thrombin on human umbilical endothelial cells.

0369

ELASTASE DEGRADATION PRODUCTS OF FIBRINOGEN AND VENOUS THROMBOEMBOLISM

F.J.L.M. Haas,[1] P. Meijer,[2] R.E.G. Schutgens,[3] D.H. Biesma,[3] K Cluft[2]

[1]St. Antonius Hospital, NIEUWEGEIN; [2]Gaubius Laboratory, TNO, LEIDEN; [3]UMC Utrecht, UTRECHT, Netherlands

Background. A specific enzyme immune assay (EIA) for degradation of fibrinogen (FgEDP) by proteases from polymorphonuclear leukocytes is capable of quantifying very low levels of split products. The levels have been found to be stable in individuals, but elevated during acute vascular events. The predictive value of elevated FgEDP for coronary and cerebral infarction is significant (odds between 3,8 -7,5 for highest quartiles). *Aims*. To evaluate FgEDP in patients with acute venous thrombosis. *Methods*. In 438 symptomatic outpatients, participants in a management study, investigating the use of D-dimer and a clinical decision rule in the exclusion of DVT, the FgEDP assay was tested and compared with the D-dimer EIA assay using the Moab FDP-14 (FbDP). From all patients the values of erythrocyte sedimentation rate (ESR) and white blood cell count (WBC) were known. *Results*. The relation between FgEDP and FbDP was significant in the thrombosis negative group (T^-) group ($p=0.03$), but not in the thrombosis positive (T^+) group. The area under the curve (AUC) of the Receiver Operation Characteristic (ROC) curve, as measure of the clinical accuracy of both assays, were 0.62 (95% CI 0.57 -0.67) for FgEDP and 0.90 (95% CI 0.88-0.93) for FbDP. From the ROC curve the optimal FgEDP cut-off point for exclusion of DVT was 200 µg/L with a sensitivity 96.9% and specificity 13.2%. The calculated negative and positive predictive values were 83% and 41%. There was no significant relation between FgEDP and age in the whole group ($p=0.17$) and in the T^+ group ($p=0.66$), but this relation was significant in the T- group ($p=0.007$). The relation FgEDP and ESR was significant at a 0.001 level in the whole group and both T^+ and T- groups. The relation with WBC was significant for the whole group ($p=0.01$), but not for the T+ ($p=0.37$) and T^- group ($p=0.07$). There was a significant difference ($p<0.01$) between the values of the FgEDP assay in the T+ and T- group: 306 (IQR 123) µg/L and 272 (IQR 114) µg/L respectively. Unadjusted logistic regression for quartiles of FgEDP, ESR and WBC showed odds ratio's of 3.05 (95% CI 1.67-5.55), 1.49 (95% CI 0.78 - 2.81) and 3.40 (95%CI 1.77-6.56) for the highest quartiles. After adjustment for age, gender and ESR and each other, the odds for FgEDP and WBC remained significant: 3.83 (95% CI 1.76-8.34) and 2.68 (95% CI 1.31-5.45). *Conclusions.* A significant odds ratio for elevated FgEDP and WBC indicate a role of inflammation in VTE. FgEDP appears the best discriminatory variable in this study. For clinical decision, however, inflammation variables are not useful and the accuracy of the FgEDP is too low for the exclusion of DVT, just as the predictive values.

0370

REVERSIBILITY OF THE ANTICOAGULANT EFFECT OF HIGH DOSES OF THE DIRECT THROMBIN INHIBITOR DABIGATRAN, BY RECOMBINANT FACTOR VIIA OR ACTIVATED PROTHROMBIN COMPLEX CONCENTRATE

J. van Ryn, D. Ruehl, H. Priepke, N. Hauel, W. Wienen

Boehringer Ingelheim Pharma GmbH & Co KG, BIBERACH, Germany

Background. Dabigatran is a potent, direct and reversible inhibitor of thrombin. Its antithrombotic and safety profile has been demonstrated in numerous *in vivo* models and in phase III clinical trials in DVT prevention after orthopedic surgery. However, concern regarding potential overdosing or uncontrolled bleeding has led to the testing of agents known to reverse hemostatic defects. *Aims.* This study investigated the effects of recombinant Factor VIIa (Novoseven®) or activated prothrombin complex concentrate (Feiba®) on the experimentally-induced bleeding by a high dose of dabigatran in anaesthetized rats. Reversal of anticoagulant activity monitored as the aPTT was also measured. *Methods.* Saline or dabigatran (1 µmol/kg bolus + 0.5 µmol/kg/h) was infused for 25 min. Novoseven® or Feiba® were administered as a bolus injection 20 min after infusion begin, i.e. 5 min prior to the bleeding determination. Bleeding was induced by a standardized incision with a Surgicutt® blade in the tail of anaesthetized rats. Blood sampling for aPTT was performed at the end of the infusion period. *Results.* Bleeding was 125±8 s (mean±SE) in control animals. High dose dabigatran elevated this to 1455±352 s. High doses of Novoseven® (1 mg/kg i.v.) or Feiba® (100U/kg i.v.) did not affect blood loss as compared to control. When administered with high dose dabigatran, Novoseven® (0.1 or 0.5 mg/kg) significantly reduced bleeding to 186±49 and 135±13 s, respectively. Similarly, Feiba® (50 or 100 U/kg) also significantly reduced bleeding to 146±11 and 174±18 s, respectively. Control aPTT was 7±0.5 s, after dabigatran this increased to 58±8 s. Addition of Novoseven® dose-dependently reversed the aPTT to 31±1.9 or 27±2 s. The aPTT was not shortened in the presence of Feiba®. *Conclusions*. Novoseven® as well as Feiba® at therapeutically relevant doses reversed the prolonged bleeding time in rats treated with a high dose of the direct thrombin inhibitor dabigatran. Novoseven® also reversed the prolonged aPTT, whereas Feiba® did not. This is consistent with previous data reported by others (Thromb Res 2001,101, 145). These data suggest that Novoseven® and Feiba® have the potential as antidotes for the oral direct thrombin inhibitor dabigatran etexilate in case of severe bleeding complications.

0371

EFFECTS OF DABIGATRAN, A DIRECT THROMBIN INHIBITOR, AS COMPARED TO ENOXAPARIN AND THE DIRECT FACTOR XA INHIBITOR, RIVAROXABAN, ON TISSUE FACTOR-INDUCED PLATELET AGGREGATION IN HUMAN PLATELET RICH PLASMA

J. van Ryn, M. Kink-Eiband, N. Hauel, H. Priepke, W. Wienen

Boehringer Ingelheim Pharma GmbH & Co KG, BIBERACH, Germany

Background. Direct thrombin inhibitors are potent inhibitors of platelet function when platelets are activated with thrombin. This action does not occur by direct binding of the DTI to the platelet PAR-1/-4 receptor, but indirectly, by inhibiting thrombin and thereby reducing its interaction with the receptor on the platelet. *Aims.* It was hypothesized that both direct thrombin and factor Xa inhibitors and enoxaparin could inhibit platelet aggregation, if the stimulus to initiate aggregation was higher in the cascade than factor Xa, such as tissue factor. Thus, the

direct thrombin inhibitor, dabigatran, the direct factor Xa inhibitor, rivaroxaban and enoxaparin were tested. *Methods.* Free flowing whole blood (60 mL) was obtained from an antecubital vein using an 18 gauge needle from healthy human volunteers and collected into 3.13% sodium citrate (1/10 dilution). This was centrifuged to obtain platelet rich plasma (PRP). Samples (300 µL PRP) were placed in a 6-channel light transmission aggregometer. Photometric tracings were continuously digitally recorded over 5 min following the addition of tissue factor and curves were evaluated as AUC and time to aggregation. PRP was incubated with 2 mg/mL Pefabloc®FG (Gly-Pro-Arg-Pro) to prevent fibrin polymerisation, 5 mM $CaCl_2$ and increasing concentrations of dabigatran, enoxaparin or rivaroxaban. Tissue factor (5-27 µl of 10 mL Innovin solution) was tailored for each individual, so that the minimum concentration resulting in maximum aggregation was used. As positive controls, aggregation was also performed after stimulating with ADP (10 µM), collagen (2 µg/mL), TRAP (20 µM) or ecarin (0.1 U/mL). *Results.* All substances inhibited tissue factor-induced platelet aggregation in a concentration-dependent manner. Dabigatran was the most potent inhibitor of platelet aggregation among the substances tested, with an IC50 of 35 nM, rivaroxaban followed with an IC_{50} of 312 nM and enoxaparin with 13µM. All substances had no effect on platelet aggregation induced by ADP, collagen and TRAP. Dabigatran was a potent inhibitor of ecarin-induced platelet aggregation, while rivaroxaban and enoxaparin had no effect, as expected from their mechanism of action. *Conclusions.* Thus, this study demonstrates that direct thrombin inhibitors (by inhibiting thrombin) and direct factor Xa inhibitors (by preventing thrombin generation) inhibit platelet aggregation, though dabigatran was more potent than rivaroxaban and enoxaparin under these experimental conditions. Thus, these substances may not only be effective in venous / stasis thrombotic episodes where fibrin formation plays an important role, but may also be effective in more platelet dominant, arterial thrombosis settings.

0372

CIRCULATING PROCOAGULANT MICROPARTICLES IN PATIENTS WITH VENOUS THROMBOEMBOLISM

C. Ay,[1] J.M. Freyssinet,[2] T. Sailer,[3] R. Vormittag,[3] I. Pabinger[3]

[1]Division of Haematology and Haemostaseology, Department of Medicine I, VIENNA, Austria; [2]Université Paris-Sud, Le Kremlin-Bicêtre; Université Louis Pasteur, STRASBOURG, France; [3]Division of Haematology and Haemostaseology, Medical University of Vienna, VIENNA, Austria

Background. Circulating microparticles (MPs), small and phospholipid-rich vesicles released from the membrane of many cells upon activation or apoptosis, have been suggested to have an important role in coagulation. However, data from patients with venous thromboembolism (VTE) are scarce. *Aims.* The aim of our present study was to investigate the association of circulating procoagulant MPs with VTE in a population of high risk patients. *Methods.* We investigated procoagulant MPs in patients with a history of objectively confirmed recurrent VTE and in an age- and gender-matched control group of healthy individuals. All patients had at least one unprovoked event of deep venous thrombosis or pulmonary embolism. Plasma was obtained at least 3 months after the most recent event of VTE. The measurement of circulating procoagulant MPs was performed after capture onto immobilized annexin V and determination of their procoagulant potential with a prothrombinase assay. Comparison of categorical parameters among groups was done by Chi^2-test. T-test and Mann-Whitney-test were applied to compare metric variables with a normal or skewed distribution between patients and controls, respectively. Binary logistic regression analysis was applied to calculate the odds ratio (OR). *Results.* Hundred-sixteen patients (53 female / 63 male; mean age ±SD: 56±12 yrs) and 129 controls (66 female / 63 male; mean age ±SD: 53±11 yrs) were enrolled. Levels of MPs (nanomolar phosphatidylserine [nM PS]) were not statistically significantly elevated in patients compared to controls (median [interquartile range]: 5.35 [4.03-7.18] *vs* 5.30 [3.55-7.05], p=0.63). The OR of elevated MPs (cut-off at the 75th percentile of MPs level in controls) was 1.06 [95% CI: 0.59-1.88], p=0.85). *Conclusions.* In conclusion, we did not find an association between elevated procoagulant MPs and the risk of VTE, if studied during a chronic stage of the disease.

0373

INVESTIGATION OF THE ROLE OF PLATELETS IN CLOT BOUND THROMBIN ASSAYS AND THEIR CONTRIBUTION TO CLOT PROCOAGULANT ACTIVITY

P.M. Player, R. Luddington, S. MacDonald

Addenbrooke's Hospital, CAMBRIDGE, UK

Background. Thrombin bound to fibrin during the process of clot formation retains its activity and is still able to initiate further coagulation in the surrounding plasma as 95% of thrombin generation occurs after the moment of clotting. This is potentially of major clinical significance as a significant number of patients treated with anticoagulants following venous thromboembolism (VTE) have a second thrombotic event. By measuring the endogenous thrombin potential (ETP) the total amount of thrombin that a sample is capable of generating can be measured. *Aims*. The aim of this study is to determine Clot Bound Thrombin (CBT) activity and to investigate the procoagulant effects that these clots have on a plasma system. It has been shown that thrombin generation is initiated in platelet poor plasma (PPP) when in the presence of a plasma clot. In this study the role of platelet rich plasma (PRP) will be investigated to determine the role of platelets in the system and whether it is necessary to include them in an assay of CBT activity. Comparisons will also be made between thrombin generation assays with patients with a previous history of VTE and patients with known bleeding disorders. *Methods*. The experiment was a modification of the method described by Hemker, *et al.* (2003) (Pathophysiology of Haemostasis and Thrombosis 33, 4-15) using CBT to initiate thrombin generation. *Results.* The ETP relative fluorescence is greater in PRP (1088.75 FLU, SD=124.11) than in PPP (1011.5 FLU, SD=75.49) whereas the lagtime for PPP (18.81 min, SD=3.939969) is greater than PRP (10.53 min, SD=1.484192). The peak amount of thrombin produced for PPP (113.34 FLU, SD=9.077527) is greater than PRP (98.36 FLU, SD = 15.28028). The thrombin generation plot clearly shows that the ETP (area under the curve) is less for patients with a bleeding disorder (531.5 FLU.min, SD=91.92388) than the patients with a history of VTE (962 FLU, SD=158.8147). The VTE patients produce thrombin at an earlier time (14.80 min, SD=2.784526 *vs* 15.95 min, SD=1.774838) and at the greatest amount (124.3783 FLU, SD=20.60447 *vs* 53.315 FLU, SD=15.78969). *Summary/conclusions*. From this study platelets were shown to affect the single point and thrombin generation assays and represent a more physiological model. Varying the platelet count has also been shown to affect the results. The differences in platelet counts between patients will not give inaccurate results because this represents the patient's coagulation physiology and hence actual risk of bleeding or thrombosis. The results from the bleeding disorder patients show a decreased ETP level compared with patients with a history of VTE.

0374

THE INCIDENCE OF POSITIVE HIT ANTIBODIES IN A LARGE ICU, COMPARING THE USE OF UFH OR NORMAL SALINE FOR THE PATENCY OF THE ARTERIAL LINES

M. El-Ali, Th. Theodoridis, Ch. Kalpodimou, A. Pappas, B. Gerovasili, E. Angelopoulos, S. Poulaki, B. Markaki, D. Zervakis, S. Nanas, B. Christopoulou-Cokkinou

Evangelismos General Hospital, ATHENS, Greece

Aims. To compare the incidence of positive HIT antibodies during a period of 6 months of normal saline(NS) use instead of unfractionated heparin (UFH) for the patency of the arterial lines. *Material and Methods.* Two groups of patients (pts)were registered. The first group included pts hospitalized in the ICU from 01-01-07 to 30-06-07and UFH was routinely used. The second group included pts hospitalized from 01-07-07 to 31-12-07 and NS was used instead of UFH. HIT antibody detection was performed in cases of a clinical or laboratory suspicion. The ELISA method was used for the detection of HIT antibodies (Asserachrom HPIA, Diagnostica Stago, Asnieres, France). *Results.* In the first group 193 pts were included (64,2% survived), 38 pts (19,7%) had a clinical/laboratory suspicion of HIT(males: 23, females: 15, median age:63,3 years, median duration of hospitalization: 39 days, 38,9%survived),14pts of the 193 (7,3%) had positive HIT antibodies 232pts were included in the 2nd group (69,8%survived), 43 pts(18,5%) had a clinical or laboratory suspicion of HIT (males: 24, females: 19,median age: 62,9 years,median duration of hospitalization: 30,1 days and survival percentage 60,1%) ,positive HIT antibodies were detected in11pts of the232 (4,7%). *Conclusions.* The substitution of UFH by NS for arterial line flushing leads to a reduction in positive HIT antibodies. The difference is not statistically signif-

icant (p=0,18) but it is encouraging. Carefully planed studies of a larger scale are needed to confirm our results. Such a study has already been initiated by our research team.

0375

SYMPTOMATIC VENOUS THROMBOEMBOLISM IN MEDICAL IN-PATIENTS: A MULTICENTER ITALIAN SURVEY ON PREVALENCE, RISK ASSESSMENT, AND ATTITUDE TOWARDS PROPHYLAXIS

G. Gussoni,[1] M. Campanini,[2] M. Silingardi,[3] G. Scannapieco,[4] A. Mazzone,[5] G. Magni,[6] I. Iori[3]

[1]FADOI Study Centre, ROMA; [2]Internal Medicine, Maggiore Hospital, NOVARA; [3]Internal Medicine I, Arcispedale S. Maria Nuova, REGGIO EMILIA; [4]Internal Medicine, Cà Foncello Hospital, TREVISO; [5]Internal Medicine, Civile Hospital, LEGNANO; [6]QBGroup SpA, PADOVA, Italy

Background. Precise indications on epidemiology and prevention of venous thromboembolism (VTE) in medical in-patients are difficult to define because of the heterogeneity of design among available studies, and the complexity of patients population, frequently characterized by comorbidities and patient-specific multiple risk factors. The majority of in-hospital VTE occurs in medical services, and this trend is increasing over time, hence the need for up-to-date, real-world data on prevalence, risk profile and prophylaxis of VTE in medical in-patients. *Aims.* Objective of our study (GEMINI) was to contemporarily assess the epidemiology of symptomatic VTE in patients hospitalized in Internal Medicine (IM), to evaluate the impact of potential risk factors, and the attitude towards thromboprophylaxis. *Methods.* GEMINI was a prospective, observational study performed from March to September 2006 in 27 Italian Departments of IM adhering to FADOI (Italian Federation of Internal Medicine). Data from consecutive unselected patients admitted to IM were recorded during hospital stay, and a three-month follow-up was scheduled in patients with diagnosis of VTE and onset of symptoms later than 48 hours after admission (*hospital-acquired* events, primary study end-point). A sample size of 4500 patients was considered appropriate to document a reliable estimate of *hospital-acquired VTE,* by hypothesizing an event rate of 0.75±0.25%. *Results.* A total of 4846 patients were included (mean age 71.0±15.9, male 45.4%); the majority of patients (54.6%) had more than two active concomitant diseases at the time of admission. Symptomatic VTE was registered in 177 (3.65%) patients; of these, 26 cases (0.55%) occurred with onset of symptoms later than 48 hours after admission. In this group of patients no recurrent VTE was recorded, however a high rate of all-cause mortality (26.9%) occurred, at three-month follow-up. By means of multivariable regression analysis, previous VTE (Odds Ratio 8.52, 95% confidence interval 4.14-17.53) and bed resting (2.99, 1.91-4.71) were significantly associated with venous thromboembolism, while a trend for increased risk was documented in cancer patients. During hospital stay antithrombotic prophylaxis was administered in 41.6% of patients without VTE at admission (31.9% low-molecular-weight heparin, 8.7% oral anticoagulants, 0.8% unfractionated heparin, 1.0% other methods). Globally, 41.8% of GEMINI patients had indication to thromboprophylaxis according to 2004 Guidelines by the American College of Chest Physicians, and 58.7% of them actually received it. The choice of administering or not thromboprophylaxis appeared qualitatively adherent to indications from randomized clinical trials and international guidelines. As a specific finding, cancer patients were however less likely to receive thromboprophylaxis. Obesity and severe renal failure (conditions for which concerns have been raised on the appropriate use of heparins) were frequent in our unselected population (18.7% and 15.1%, respectively), therefore supporting the need for specific data on these patients, usually excluded from randomized clinical trials on thromboprophylaxis. *Summary and Conclusions.* VTE is a quite common finding in patients admitted to IM Departments, and recommended thromboprophylaxis is still under-used in this setting. Tough a difficult task to achieve, due to complexity of this population, further efforts are needed to reach a more systematic and appropriate use of prophylaxis in at-risk medical in-patients.

Vascular biology, bleeding disorders and transfusion medicine

0376

REDUCTION IN PLASMA PROTEIN C & PROTEIN S LEVELS DURING VASO-OCCLUSIVE CRISIS IN CHILDREN WITH SICKLE CELL DISEASE

A. Piccin,[1] C. Mc Mahon,[2] C. Murphy,[3] E. Eakins,[3] O.P. Smith,[4] W.G. Murphy[5]

[1]Dept of Haematology Our Lady's Children Hospital, [2]Irish Blood Transfusion, DUBLIN; [2]Haematology Dept. Our Lady's Children Hospital, DUBLIN; [3]Irish Blood Transfusion Service, DUBLIN; [4]Dept of Haematology, Our Lady's Children Hospital, DUBLIN, Ireland

Background. Vascular occlusion is the main cause of crises in sickle cell anaemia (SCA). Recent studies suggest that the expression of phosphatidylserine and annexin V on sickle erythrocyte membranes together with increased micro-particle formation, may promote pro-coagulatant and inflammatory events that include: thrombin generation, endothelial cell adhesion, and vaso-occlusion. The protein C anticoagulant pathway serves as an *on demand* anticoagulant system. Thrombin binds to thrombomodulin results in a complex that rapidly converts protein C to activated protein C (APC). Activated protein C in complex with protein S functions as an anticoagulant by inactivating factors Va and VIIIa. Defects of this pathway are the most common basis for hereditary thrombophilia and infants born with homozygous protein C deficiency usually develop microvascular thrombosis of the skin (purpura fulminans). More recently, APC has also been shown to be cytoprotective to many cell types *in vitro* and *in vivo*. *Aims*. To compare plasma protein C and protein S levels in SCA patients in crisis with protein C and protein S levels in normal (age/ethnicity) matched controls and in SCA patients in steady state who were or were not receiving hydroxyurea or blood transfusion therapy. *Methods*. All patients and controls were of sub-Saharan African ethnicity. All SCA patients (n=119) had haemoglobin SS: 58 patients without history of crisis in steady state (mean age 5.3 years), 15 patients in sickle cell crisis (mean age 5.4 years), 31 patients on hydroxyurea (mean age 7.2 years) and 15 patients on transfusion therapy (6.5 years). Seventeen children of sub-Saharan African ethnicity of similar age (mean 4.6 years) were used as control group (haemoglobin AA). Protein C was analysed by chromogenic method and protein S by latex based assay. Data analysis was performed by a one-way analysis of variance. *Results*. Protein C values were significantly reduced in patients in crisis (mean=0.51 IU/mL; range 0.45-0.58; SD±0.11) compared to all other groups: controls (mean 0.70 IU/mL; range 0.64-0.76; SD± 0.12 p<0.001); hydroxyurea (mean= 0.66 IU/mL; range 0.62-0.70; SD± 0.12; p<0.001); steady state (mean =0.61 IU/mL; range= 0.58-0.64; SD± 0.11; range; p<0.05); and transfusion therapy (mean =0.63 IU/mL; range =0.59-0.68; SD±0.07; p<0.05). PS values showed a statistically significance between the control group (mean=0.77 IU/mL; range =0.68-0.86; SD± 0.17) vs crisis (mean= 0.54 IU/mL; range =0.45-0.64; SD± 0.17; p<0.001); control vs hydroxyurea (mean= 0.65 IU/mL; range =0.60-0.69; SD± 0.12; p<0.05); control vs transfusion(mean =0.62 IU/mL; range =0.54-0.69; SD±0.13; p<0.05) and crisis vs steady state (mean =0.67 IU/mL; range =0.64-0.71; SD±0.13; p<0.05). *Conclusions*. Plasma levels of protein C and protein S were significantly reduced in children during sickle cell crises but were normal in those patients on hydroxyurea and transfusion therapy. Protein C levels decrease dramatically in septic shock and the extent of the decrease is correlated with the risk of mortality and multi-organ failure. Although the absolute decrease protein C and protein S seen in sickle cell crises is not as dramatic as that seen in patients with sepsis, further studies are warranted to determine the clinical significance of these findings.

0377

ELECTIVE SPLENECTOMY IN CHILDREN WITH SICKLE CELL ANAEMIA: IS IT A CAUSE OF FURTHER CRISES?

A. Piccin,[1] R. Geoghegan,[2] H. Conroy,[2] H. Rizkalla,[3] M. Mc Dermott,[3] M. Corbally,[4] O.P. Smith,[2] C. Mc Mahon[5]

[1]1Dept of Haematology Our Lady's Children Hospital, 2Irish Blood Transfusion, DUBLIN; [2]Dept of Haematology, Our Lady's Children Hospital, DUBLIN, Ireland; [3]Dept of Pathology, Our Lady's Children Hospital, DUBLIN, Ireland; [4]Dept of Surgery, Our Lady's Children Hospital, DUBLIN, Ireland; [5]Haematology Dept. Our Lady's Children Hospital, DUBLIN, Ireland

Background. Acute splenic sequestration is a major cause of mortality in children with sickle cell anaemia (SCA). It is caused by intrasplenic trapping of red cells and is defined as a haemoglobin decrease of at least 2g/dL associated with a markedly elevated reticulocyte count and acutely enlarging spleen. Recurrent splenic sequestration is common and occurs at least in 50% of those who survive the first episode. Sequestration can occur in children as young as 5 weeks but is seen most often in children between the ages of 3 months and 5 years. Splenectomy (SP) is an accepted treatment for patients with recurrent splenic sequestration. Previous reports have suggested that SP increases the risk of developing further sickle cell events. *Aims*. Evaluating the outcome following splenectomy in SCA. *Methods*. We present a retrospective national study on children with SCA treated with elective SP. The medical notes of all children with SCA undergoing open SP at Our Lady's Children's Hospital, Dublin, between 2001-2007, were reviewed. The number of crises were recorded pre and post splenectomy. Blood values, obtained 4 weeks before SP in the absence of transfusion (T) therapy, were compared with values 3 months and 6 months after SP. White cell count (WCC), neutrophils (N), platelets (PLT), haemoglobin, haematocrit, mean corpuscular volume, bilirubin, lactic dehydrogenase (LDH), reticulocytes were recorded and variation (Δ) analyzed using Wilcoxon test (Graph Pad statistical package®). *Results*. Fifteen patients with SCA were identified: M=10, F=5 (M/F ratio=2) the median age was 5 years (range 13 months-15 yrs). All were of African ethnicity: 13 (86%) Nigerian, 1 (7%) from Congo and 1 (7%) from Angola. The indication for elective SP was recurrent splenic sequestration. Four patients had been started on hydroxyurea (HU) or Transfusion (T) before SP: one because cerebral stroke (T), 3 because chest crises (HU). All patients were transfused for 4 weeks prior to surgery to reduce HbS <30%. Sixty-six crises were recorded pre and 29 post SP. When splenic sequestration was excluded, there were 16 crises pre, and 36 post. The majority of crises were bone (62%) pre SP and abdominal (36%) post SP. Patients on HU/T did not develop further crisis. At 3 months post SP WCC, N and PLT values were all statistically significantly raised (ΔWCC p=0.03; ΔN p=0.03; ΔPLT p=0.0001). The trend continued at 6 months (ΔWCC p=0.001; ΔN p=0.002; ΔPLT p=0.002). Changes in haemoglobin, Hct and the markers of haemolysis (bilirubin, LDH and reticulocytes) were not statistically significant. *Conclusions*. Elective SP is a lifesaving therapeutic approach for children with recurrent splenic sequestration. However, this study demonstrates an increase in crises post SP. We suggest that the persistently elevated WCC, PLT, N values may be contributing factors. The adoption of disease modifying therapy, such as HU, may be needed to control these parameters in the future.

0378

A CORRECT GATING STRATEGY PLAYS AN IMPORTANT ROLE IN RARE EVENT DETECTION OF ENDOTHELIAL PROGENITOR CELLS

J.L.A.J. Rummens, A. Daniels, H. Jongen, R. Koninckx, M. Hendrikx, P. Dendale, D. Hansen, K. Hensen

Virga Jesse Hospital, HASSELT, Belgium

Aims. In the present study, we aimed to optimise a six-colour multiparameter flowcytometric panel for EPC detection. Since EPC's are very rare in circulation, it is technically challenging to count such rare events and several layers of control were build in our analysis. Additionally, we investigated whether exercise could acutely increase the numbers of endothelial progenitor cells in circulation. Numbers of EPCs were compared between healthy volunteers and patients that underwent coronary artery bypass graft (CABG). Flow cytometric data were compared by the functional and morphological CFU-EC assay. *Methods*. Healthy volunteers (n=10) and post-op CABG patients (n=10) underwent an exhaustive bicycle exercise test. Age between groups were comparable. Blood (20 mL) was taken just before and 10 min after exercise. Mononuclear cells were isolated and samples were incubated using following combinations of monoclonal antibodies: CD3-FITC; CD15-FITC; CD133-PE; CD14-PerCP; CD34-PE-Cy7; VEGFR2-APC and CD45-APC-Cy7. In order to have a good estimate of non-specific binding of antibodies to cells, fluorochrome matched isotype controls were used in combination with fluorescence minus one (FMO). In addition, the FITC channel was reserved as dump channel using anti-CD3- and anti-CD15-FITC conjugated monoclonal antibodies. Approximately 2×10^6 events were acquired. Absolute cell numbers were determined with the single-platform method using TruCount Tubes (BD). To determine CFU-EC numbers, cells were grown in Endocult® following manufacturer's instructions. Statistical analysis was performed using *Analyse-it®* software. Pre- and post-exercise cell numbers were compared using Wilcoxon signed-rank test. Numbers between volunteers and patients were compared using the mann-whitney-U test. A p-value<0.05 was considered statistically significant. *Results*. An increase of EPC numbers before and after exercise could be obtained. The absolute number of $CD34^+/CD133^+/VEGFR2^+$ cells per mL blood in healthy volunteers changed nearly 2-fold (from 5,92±6.09 before exercise to 11.01±9.60 after exercise (p=0.04)). However, this increase was not statistically different for CABG patients (1.71±1.99 *vs* 7.19±15.14 p=0.38). Interestingly, the absolute EPC cell numbers in healthy patients is higher compared to CABG patients but only statistically significant after exercise (p=0.04).Different results were obtained when comparing the amount of CFU-EC colonies. There was almost no increase in the number of CFU-EC colonies after exercise. For healthy volunteers a mean of respectively 11.44±19.99 and 13.8±22.18 CFU-EC/106 cells before and after exercise was obtained (p=0.16). For CABG patients this number was only 1.03±1.00 and 1.39±1.45 (p=0.54). However the number of colonies between healthy volunteers and patients was significantly higher both before (p=0.008) and after (p<0.001) exercise. *Conclusions*. In conclusion, we developed a useful six-colour flowcytometric assay to detect EPCs in circulation. Our results show that physical exercise does increase circulating EPC numbers in both healthy volunteers and CABG patients. Moreover, our findings suggest that coronary arterial disease has a negative impact on the numbers of circulating EPC's and that after CABG operation numbers do not normalise. This study provides a basis to investigate the EPC numbers as a biomarker of cardiovascular status.

0379

RETROSPECTIVE EVALUATION OF DYSFIBRINOGENAEMIC PATIENTS AT A SINGLE CENTER: CLINICAL FEATURES AND LABORATORY FINDINGS

C. Santoro, A. Leporace, F. Biondo, P. Pignoloni, C. Mercanti, R. Foà, M.G. Mazzucconi

Hematology, ROMA, Italy

Background. Dysfibrinogenaemia is a very rare bleeding disorder in which the clinical phenotype is unpredictable. The literature consists predominantly of collections of case reports. A relatively recent compilation of over 250 patients, revealed that 53% were asymptomatic, 26% had haemorrhage and 21% had thrombosis, some of whom also had haemorrhage. *Aims.* To retrospectively investigate clinical features and laboratory findings of our dysfibrinogenaemic patients and to compare our data to literature's. *Methods.* Over the last ten years, 17 dysfibrinogenaemic patients were diagnosed at our centre [7 males, 10 females, median age at diagnosis 46.6 years (6.1-80.9), 8 families]. The reasons for admission were: finding of a reduction of fibrinogen activity in 12 patients, familial study in 5. Laboratory findings are shown in the accompanying Table 1.

Patients	Fibrinogen activity/antigen (mg/dL)	PT ratio 0.90-1.14	PTT ratio 0.92-1.16
1	112/380	1.07	1.1
2	Undetectable/250	1.3	1.2
3	36/192	1.2	1.3
4	66/250	1.17	1.12
5	52/140	1.2	1.06
6	57/140	1.1	1.02
7	62/380	1.16	0.9
8	70/250	1.04	1.08
9	50/470	1.3	0.9
10	98/226	0.9	1.09
11	Undetectable/195	1.14	1.1
12	Undetectable/250	1.2	1.04
13	Undetectable/250	1.09	1.32
14	Undetectable/250	1.2	1.06
15	56/192	1.2	0.9
16	Undetectable/215	1.28	1.04
17	Undetectable/400	1.28	1.15

Figure 1.

Results. Ten/17 (59%) patients experienced hemorrhagic symptoms, mostly mild: traumatic cutaneous bleedings in 7 patients; gastro-intestinal bleeding in 2; epistaxis in 3; gum bleeding in 2. One/17 patient (6%) experienced a cerebral ischemia (concomitant disease: acleisto-cardia). Twelve patients underwent surgery, eleven underwent dental extractions. Tranexamic acid was used as prophylaxis treatment of surgery in 2 patients. Only 1 patient bled after dental extraction. Eleven spontaneous deliveries and 3 cesarian sections were performed in 9 women without any prophylaxis treatment. No hemorrhagic or thrombotic complications were reported. No spontaneous abortions occurred. None of the 17 patients was transfused. *Conclusions.* We confirm that dysfibrinogenemia is a rare coagulation disorder. The prevalence of asymptomatic patients is inferior to literature data (35% vs 53%). Just 1 patient (6%) had a thrombotic event. Hemorrhagic patients (59%) experienced mostly mild symptoms. No one of them needed transfusion therapy.

0380

FIBRINOGEN OSTRAVA I AND OSTRAVA II

R. Kotlín,[1] A. Sobotková,[1] J. Suttnar,[1] T. Riedel,[1] B. Blazek,[2] P. Salaj,[1] J.E. Dyr[1]

[1]Institute of Hematology and Blood Transfusion, PRAHA; [2]Children`s Medical Clinic, University Hospital Ostrava, OSTRAVA, Czech Republic

Background. Fibrinogen is a 340 kDa glycoprotein which is composed of six polypeptide chains. It plays a crucial role in blood coagulation. Serine protease thrombin cleaves fibrinogen to fibrin monomer and releases N-terminal parts of Aalpha and Bbetachains, termed fibrinopeptide A and fibrinopeptide B, respectively. Hereditary dysfibrinogenemia is a disease wherein an inherited abnormality in the fibrinogen molecule results in defective fibrin clot formation. *Aim.* Aim of our study was to characterize a case of a gene defect causing dysfibrinogenemia in two unrelated families from Ostrava, Czech Republic. *Methods.* Fibrin polymerization and fibrinolysis were measured by turbidimetrical method. Kinetics of fibrinopeptide release was measured by HPLC method according to Suttnar *et al.*[1] In-gel digestion of separated fibrinogen chains was performed as described by Shevchenko *et al.*[2] Mass spectrometry was performed using Biflex IV mass spectrometer as described earlier.[3] Gene sequencing was performed by dideoxysequencing method. Scanning electron microscopy (SEM) was performed on VEGA Plus TS 5135 electron microscope as described earlier.[4] *Results.* Fibrinogen Ostrava I: The patients - 8 yr-old boy and his 2-yr-old brother - have prolonged thrombin time and low Clauss fibrinogen level (Table 1).

Table 1. Coagulation tests results .

	aPTT	PT	TT	fbg (Clauss)	fbg (Immuno)
Ostrava I	37,9 s	19,1 s	79,5 s	0,5 g/l	2,52 g/l
Ostrava I Brother	41,3 s	18,0 s	59,7 s	0,5 g/l	2,56 g/l
Ostrava I Mother	35,8 s	13,2 s	14,5 s	2,27 g/l	2,12 g/l
Ostrava II	n.d.	n.d.	156,9 s	0,68 g/l	3,58 g/l
Ostrava II Mother	159,5 s	n.d.	>180 s	0,75 g/l	1,72 g/l
Ostrava II Father	n.d.	n.d.	n.d.	2,1 g/l	2,82 g/l
Ostrava II Aunt	43,1 s	n.d.	30,2 s	0,83 g/l	2,68 g/l
Ostrava II Grandmother	170,7 s	n.d.	n.d.	1,17 g/l	4,13 g/l
Normal values	28,8 - 37,2 s	12 - 15 s	13 - 17 s	2,0 - 4,2 g/l	2,0 - 4,2 g/l

Fibrin polymerization was impaired and measurement of fibrinopeptide release showed higher rate of released fibrinopeptide B. The patient and his brother bear a heterozygous point mutation in exon 2 of FGA causing substitution of Aalpha 16 Arg to Cys. SEM revealed thicker fibres than control. Fibrinogen Ostrava II: The patient - 2 yr-old boy and his relatives have prolonged thrombin time and low Clauss fibrinogen level (Table 1). Fibrin polymerization was impaired and measurement of fibrinopeptide release showed higher rate of released fibrinopeptide B. The patient and his relatives bear a heterozygous point mutation in exon 2 of FGA causing substitution of Aalpha 16 Arg to His. SEM revealed thicker fibres than control. *Conclusions.* The patients were found to bear a point mutation in the fibrinogen Aalpha chain. Arg 16 is necessary for right fibrinopeptide A release by thrombin. Substitution Aalpha 16Cys was found to be more serious than substitution Aalpha 16His. Those mutations lead to impaired fibrinopeptide release and polymerization, abnormal fibrin clot and pathophysiologic coagulation.

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0381

TRANEXAMIC ACID EFFECTS ON POSTOPERATIVE BLEEDING AFTER CARDIOPULMONARY BYPASS SURGERY: INFLUENCE OF PAI-1 GENE POLYMORPHISM

P. Machado, J.M. Raya, J.J. Jimenez, J.L. Iribarren, M.J. Rodriguez-Salazar, T. Martin, L. Morabito, M. Brouard, Y. Barrios, R. Martinez, M.L. Mora, J.M. Rodriguez-Martin

Hospital Universitario de Canarias, LA LAGUNA, Spain

Background. Excessive perioperative bleeding continues to complicate cardiopulmonary bypass (CPB) surgery and in some hospitals more than 25% of all blood products are assigned to open heart surgery. Postoperative hemorrhage is related to increased morbidity and mortality in patients undergoing cardiac surgery. Excessive postoperative bleeding (EPB) has been attributed to acquired platelet dysfunction, impaired coagulation, and increased fibrinolysis. The characterization of the hemostatic defects responsible for EPB is crucial for specific treatment and optimal clinical management of the patients. The plasminogen activator inhibitor-type1 (PAI-1) 4G/5G polymorphism is known to influence plasma levels of PAI-1, the main endogenous regulator of fibrinolysis, the 4G allele being associated with high levels and the 5G allele with low levels. *Aims.* To determine the effects of tranexamic acid (TA) on postoperative bleeding in CPB surgery according to 4G/5G PAI-1 gene polymorphism. *Methods.* We performed a prospective analysis on the postoperative bleeding effect of TA prophylaxis (2g), administered before and after CPB, according to PAI-1 polymorphism. We recorded data related to coagulation, fibrinolysis and bleeding, preoperatively, at admission (0-hr) to the intensive care unit (ICU), 4-hr and 24-hr postoperative. Excessive blood loss is defined as that above 1L/24 hours. For gene analysis, blood samples (3 mL) were collected before surgery in EDTA-containing tubes and subjected to DNA purification using proteinase K, phenol-chloroform extraction, and ethanol precipitation. Genotyping was performed in a blinded manner, without knowledge of any clinical data. Gene analysis of PAI-1 4G/5G polymorphism was performed in all patients using primers and restriction endonuclease digestion. In addition, neutral markers were genotyped to follow genomic control strategies that would detect spurious associations due to population substructure. SPSS v.15 was used for statistical analysis. *Results.* We studied fifty patients (24 with TA and 26 without TA). In patients not receiving TA, significant differences were found between PAI-1 genotype groups (4G/4G; 4G/5G; 5G/5G) in chest tube blood loss at 0-hr (p=0.03); at 4-hr (p=0.001) and at 24-hr (p=0.009). Fresh frozen plasma was required during ICU stay in 50% of 5G/5G, 25% of 4G/5G and none of 4G/4G carriers, (p=0.021). 4G/4G patients did not show significant differences in blood loss between TA and placebo groups. 4G/5G patients receiving TA had lower blood loss than placebo group at 0-hr (p=0.012), and at 24-hr after surgery (p=0.014). In contrast, 5G/5G patients receiving TA had significantly lower blood loss compared to placebo group at 0-hr (p=0.028), at 4-hr (p=0.008), and at 24-hr (p=0.004) after surgery. Fifty-five percent of 5G/5G patients in the placebo group received fresh frozen plasma during ICU stay compared with no patients in the TA group (p=0.014). *Conclusions.* In our experience, PAI-1 5G/5G homozygote patients who did not receive TA showed significantly greater postoperative bleeding than patients with other PAI-1 genotypes. 5G/5G homozygote carriers who received TA showed the greatest blood-sparing benefit. Finally, we consider that the main limitation of the study is the relatively small sample size, and that these findings require confirmation in larger series.

0382

THE COLLAGEN BINDING ASSAY IN THE DIAGNOSIS OF VON WILLEBRAND'S DISEASE

P.N. Badenhorst, S.M. Meiring, M. Kelderman

University of the Free State, BLOEMFONTEIN, South Africa

Background. Von Willebrand's disease (VWD) is a commonly inherited bleeding disorder caused by either a qualitative or quantitative defect of Von Willebrand factor (VWF). The standard assessment of VWF functional activity is the ristocetin cofactor assay (VWF:RCo), where dilutions of the patient's plasma are tested for their ability to promote platelet agglutination in the presence of the antibiotic ristocetin. Recently measurement of the collagen binding function of VWF (VWF:CB) was introduced as an additional functional test. Because VWF:RCo and VWF:CB measure different binding properties of VWF, the place of the VWF:CB assay in the diagnostic workup of VWD is still debatable. Furthermore, only one patient with a collagen binding defect of VWF has been reported in the literature. *Aims.* To determine the role of the collagen binding assay in the diagnosis VWD and to see how many patients in our comprehensive VWD testing facility had defective collagen binding as the only abnormality. *Methods*. For the first part of the study we tested 40 normal subjects; 10 with type 2A; 10 with type 2B; 14 with type 2M and 10 with type 1 VWD. We performed all prescribed laboratory tests for the diagnosis of VWD. These tests included the VWF antigen levels, ristocetin cofactor activity, collagen binding activity, multimer analysis with densitometric tracing, ristocetin induced platelet agglutination and the factor VIII binding assay. For the latter part of the study we performed the same tests on 105 patients referred to us with a history of bleeding. *Results.* We found the VWF:CB assay to be very sensitive to large VWF multimers with decreased values type 2A and 2B disease. The VWF:CB assay correlated well with the antigen levels in type 2M and type 1 VWD and contributes to the diagnosis of these sub-types. We found two patients with a collagen binding defect as the only abnormality. Both patients had a history of bleeding with normal VWF antigen levels; normal binding to platelets (ristocetin cofactor levels) and normal multimer patterns. However, the collagen binding activity of both patients was low compared to the antigen levels (CB/AG <0.3). An acquired defect due to the presence of autoantibodies to the collagen binding site of VWF was excluded by mixing studies with normal plasma. *Conclusions*. Our results suggest a definite place for the VWF:CB assay in the diagnosis of VWD and that it should be regarded as complementary rather than an alternative assessment of VWF functional activity. We also report two more patients with collagen binding defects that may be caused by mutations in the collagen binding (A3) domain of VWF. Genetic analysis is needed to confirm this.

0383

REGRESSION OF ACQUIRED VON WILLEBRAND SYNDROME AFTER SUCCESSFUL TREATMENT OF LOW GRADE GASTRIC MALT LYMPHOMA

F. Biondo, A. Matturro, C. Santoro, P. Pignoloni, A. Leporace, A. Pulsoni, R. Foà, M.G. Mazzucconi

Hematology, ROME, Italy

Background. Acquired Von Willebrand syndrome (AVWS) is a rare bleeding disorder which mimics most of the clinical symptoms and laboratory features of the hereditary disease. Usually, this acquired syndrome occurs in persons with no personal or family history of bleeding; it is particularly frequent in lymphoproliferative or myeloproliferative diseases and it can also be associated with solid tumors, immunologic and cardiovascular disorders, and other miscellaneous conditions. Clinical history: A 48-year old woman was diagnosed with Von Willebrand Disease (VWD) in 2003 at another Institution following an episode of gastric bleeding. In that occasion, the endoscopy and biopsy showed a corrosive gastritis, without Helicobacter Pylori (HP) infection and no evidence of lymphoproliferative disease. The patient came to our attention in 2005 because of a second episode of gastric bleeding. She had no family history of hemorrhagic disorders and she had no personal history of bleeding (spontaneous or post-surgery) before 2002, when mucocutaneous symptoms (ecchimoses and menhorragia) occurred. Laboratory tests were as follows: aPTT ratio 1.15 (n.v. 0.92-1.16); PT ratio 0.90 (n.v. 0.90-1.14), bleeding time (BT) >13 minutes (n.v. <9 minutes), Ristocetin Cofactor Activity (VWF:RCo) 20% (n.v. 50-150), FVIII:C 52% (n.v. 48.5-120%), VWF:Ag 34% (50-126%), Collagen Binding Assay (CBA) 19% (n.v. 50-146%), Ristocetin Induced Platelet Agglutination (RIPA) 1.4 mg/mL (n.v. 0.8-1.2 mg/mL), VWF:RCo/Ag ratio 0.6 (discrepant <0.7); the diagnosis of VWD subtype 2A was therefore confirmed. A new gastric mapping endoscopy with multifocal biopsies was performed with desmopressin prophylaxis therapy and it showed an infiltration of low grade gastric MALT lymphoma B cells. Negativity of HP infection was demonstrated by serology and gastric biopsies. A bone marrow biopsy showed a <10% involvement; CT scan excluded other involved sites. The patient was classified as stage IVE according to the Ann-Arbor classification of extranodal non-Hodgkin's lymphomas (NHL). The patient underwent Rituximab immunotherapy at a dose of 375 mg/m^2 once weekly for four weeks. To prevent gastric bleeding, prophylactic treatment with Desmopressin 0.3 μg/Kg for 3 times a week was performed. *Results.* Restaging of the disease after Rituximab treatment indicated a partial remission; gastric biopsies showed the persistence of MALT lymphoma while the bone biopsy was negative. Maintenance treatment with Rituximab 375 mg/m^2 every three months for four doses was performed. Restaging documented a complete remission (absence of disease in the gastric biopsies and bone marrow). Since the start of Rituximab no gastric bleeding was recorded. On July 2007, reassessment of VWD was performed. Laboratory tests showed the following results: aPTT ratio: 0.87, BT 3 minutes, VWF:RCo 106%, FVIII:C 158%, VWF:Ag 110%, VWF:RCo/Ag ratio 0.96. To confirm these data, laboratory tests were repeated in October 2007. The results were: aPTT ratio 0.91, VWF:RCo 76%, FVIII:C 174 %, VWF:Ag 78%, VWF:RCo/Ag ratio 0.97. *Conclusions.* The normalization of the coagulative parameters in concomitance with a complete remission of the primary lymphoproliferative disorder allowed us to reach a diagnosis of acquired VWD in association with a low grade gastric MALT lymphoma. The successful treatment of the underlying primary disease was followed by a regression of the VWD.

0384

EPIDEMIOLOGY OF TRANSFUSION RELATED ACUTE LUNG INJURY IN E-FIT, THE FRENCH HEMOVIGILANCE DATABASE : 1994 TO 2006. A STUDY OF THE FRENCH HEMOVIGILANCE NETWORK

P. Renaudier,[1] M.P. Vo Mai,[2] N. Ounnoughene,[2] P. Breton,[3] S. Cheze,[4] A. Girard,[5] L. Hauser,[5] J.F. Legras,[2] H. Odent-Malaure,[5] D. Rebibo,[5] S. Schlanger,[3] C. Waller,[5] B. Willaert,[2] C. Caldani[2]

[1]Unité d'Hémovigilance, LYON CEDEX 04; [2]AFSSaPS, SAINT DENIS; [3]DRASS, ROUEN; [4]CHU, CAEN; [5]EFS, CAEN, France

Background. Transfusion Related Acute Lung Injury (TRALI) is a life-threatening complication of allogenic blood transfusion, manifested typically by a non-cardiogenic pulmonary edema. However, the magnitude of the risk of TRALI remains unknown at this time, all the more so as a variety of other clinically similar respiratory complications can be associated with transfusion. Hemovigilance had been implemented in France since 1994. All adverse effects (AE) regardless of their severity should be notified on a normalized form to AFSSaPS, the French health products safety agency, as required by the French law. *Objective*. to describe TRALI observed in e-FIT, the French hemovigilance database. *Population and Methods.* AE possibly, probably or definitely associated with transfusion were considered for inclusion. As the item *TRALI* was explicitly present since 09/01/2001, we screened the database from 07/01/1994 to 09/01/2001 with *pulmonary edema AND fluid overload excluded*, or with free comments including the words *TRALI*, *anti-granulocyte antibodies*, *non-cardiogenic pulmonary edema*, *white lung syndrome*. The subsequent AE were considered for inclusion only if TRALI was the final diagnostic. *Results.* From 07/01/1994 to 12/31/2006, 85 812 AE were registered in e-FIT, corresponding to 35,423,172 labile blood products (LBP) transfused. 139 AE fulfilled the above criteria (1/81,000 LBP transfused). There were 74 men and 65 women, age = 0 to 86 (median 58). 111 patients presented with with dyspnea, 64 with chills and/or hyperthermia and 67 with a typical pulmonary edema. 15 TRALI were fatal, 101 were life-threatening and 23 were benign. 49 patients received only packed red cells, 8 only fresh frozen plasma and 51 only platelet concentrates. 29 patients received fresh frozen plasma either alone or associated with an other LBP ; the type of plasma was secured by quarantine for 27 (one donor) and treated by solvent-detergent (100 donors) for 2 (but associated with packed red cells and apheresis platelet concentrates and excluded). In no case, FFP treated with SD were involved. *Conclusions*. (1) TRALI is more frequent in elderly patients, as noted in other reports ; (2) Platelets concentrates remains the more frequently LBP involved, but packed red cells also contribute to nearly half of the TRALI reported ; (3) Plasma secured by quarantine is the only FFP involved ; (4). Besides the classical life-threatening presentation, a benign form of TRALI seems to exist

0385

THE DECLINING RISK OF ABO INCOMPATIBILITIES: TWELVE YEARS OF HEMOVIGILANCE IN FRANCE

P. Renaudier,[1] M.P. Vo Mai,[2] N. Ounnoughene,[2] P. Breton,[3] S. Cheze,[4] A. Girard,[5] L. Hauser,[5] J.F. Legras,[2] H. Odent-Malaure,[5] D. Rebibo,[5] S. Schlanger,[3] C. Waller,[5] B. Willaert,[2] C. Caldani[2]

[1]Unité d'Hémovigilance, LYON CEDEX 04; [2]AFSSaPS, SAINT DENIS; [3]DRASS, ROUEN; [4]CHU, CAEN; [5]EFS, CAEN, France

Background. ABO incompatibilities (ABOi) usually result from the failure to comply with Standard Operating Procedures (SOP). Continuous training (CT) is the main way to ensure their proper use in ultra-high security systems like civil aviation but little is known for transfusion. In France, SOP include a Beth-Vincent test at the bedside before the transfusion of Packed Red Cells (PRC). At the hospitals, Hemovigilance Officers (HO) are in charge of transfusion safety along with the settlement of traceability and the notification of all Adverse Effects (AE). If SOP and CT are requested by Law since 1993 for blood banks, they are only advised for hospitals even though the bedside Beth-Vincent test requires a special training. However, HO have more and more transposed the model of blood banks in hospitals especially since 2000. *Objective*. To describe time-trends of ABOi observed in e-fit, the French Hemovigilance database. *Population and Methods.* All AE with *acute ABOi* as the final diagnostic were considered. Time-trends were studied according to the Box and Jenkins model (ARMA). *Results.* From 07/01/1994 to 12/31/2005, 79 106 AE were registered in e-fit, corresponding to about 30,000,000 Labile Blood Products (LBP) transfused. 304 (0.4 %) were ABOi (1/107,850 LBP transfused). There were 183 men and 121 women with 81.3 % of whom more than 40 years old. 205 patients (67.4 %) received only PRC, 73 (24.0 %) Platelet Concentrates (PC), 22 (7.2 %) Fresh Frozen Plasma (FFP) and 4 (1.3 %) more than a single LBP. 18 ABOi were fatal, possibly (4 cases) probably (4 cases) or definitely (9 cases) associated with a PRC, and 1 doubtfully with a FFP. Time-trends displayed 3 periods : (1) from 1994 to 1997 ABOi increased concurrently with the frequency of all AE notifications, (2) from 1997 to 2000 reached a steady-state with 35 ABOi/year on average (1/70,000 LBP), (3) since 2001 regularly decreased of 14 %/year on average (13 ABOi for 2005 = 1/198,450 LBP). The localization of the failure to comply with SOP (i.e. hospital, blood bank or both) was studied for PRC. From 1997 to 2005, the liability of blood banks alone or along with hospitals remained stable (resp 0.42 and 2.58/year on average) whereas those of hospitals dramatically decreased (16.25/year from 1997 to 2000 and 8.6/year from 2001 to 2005). *Conclusions*. (1) ABOi are now 3 times less frequent than in 2000 ; (2) a notification bias is unlikely because both hospitals and blood banks are aware of traceability data that include the ABO phenotype for both the recipient and the LBP issued ; (3) CT applied on a nationwide basis seems to be the main factor to reduce ABOi in hospitals ; (4) a residual risk related to human errors seems to exist ; (5) the way to overcome that risk remains a matter of debate.

0386

EFFICACY OF RECOMBINANT HUMAN AND RHESUS THROMBOPOIETIN STIMULATED BLOOD TRANSFUSIONS IN COMPARISON TO UNSTIMULATED WHOLE BLOOD OR THROMBOCYTE TRANSFUSIONS IN A NON-HUMAN PRIMATE MODEL

F.S.F. Aerts Kaya,[1] T.P. Visser,[1] S.C.C. Hartong,[1] Z. Agur,[2] G. Wagemaker[1]

[1]Erasmus University Medical Center, ROTTERDAM, Netherlands; [2]Institute for Medical BioMathematics (IMBM), Bene-Ataroth & Optimata Ltd, RAMAT GAN, Israel

Background. Pre-treatment of allogeneic blood donors with thrombopoietin or other c-mpl ligands to increase thrombocyte counts would reduce apheresis volume, increase transfusion efficacy, decrease volume donated per recipient and the number of donors needed, and reduce the number of thrombocyte transfusions required and thus the cumulative risk of immune responses. *Aims.* We tested the efficacy of recombinant rhesus TPO (rrTPO) and human TPO (rhTPO) treatment of non-human primate blood donors on the total number of thrombocytes per transfusion and its effect on platelet and hematocrit increments in irradiated recipient monkeys. In addition, we used a pre-treatment regimen predicted by biomathematical modeling[1] for optimal efficacy and reduced immunogenicity, and measured the humoral immune response to TPO after up to 4 times challenging of donors. *Methods.* Healthy male rhesus monkeys (n=11) were treated with 5 µg/kg/d rrTPO or rhTPO (Genentech Inc.), administered subcutaneously for 4 consecutive days and served as donors up to 4 times for lethally or sublethally irradiated pancytopenic monkeys (n=21). Data were compared to regular thrombocyte transfusions or whole blood transfusions obtained from untreated animals and infused into irradiated control monkeys (n=23). Transfusions were collected in citrate and exposed to 20 Gy Á-rays delivered by a 137Cs source. Antibodies to TPO were demonstrated using a sensitive ELISA with a detection limit of 10 pg/mL. *Results.* Each transfusion was standardized to contain on average 1.0E+10 thrombocytes, independent of the source or stimulation status of the platelets. The average transfused volume was 10.3±4.2 mL for rrTPO stimulated transfusions, 14.6±3.4 mL for rhTPO, 32.1±6.4 mL (from 100 mL of blood) for regular platelet transfusions and 38.1±10.8 mL for whole blood transfusions. Transfusion with rrTPO or rhTPO stimulated blood products resulted in platelet increments of 33.4±26.0 and 28.0±19.7E+09/L, respectively, the former significantly different from 25.4±19.2E+09/L observed for regular platelet transfusions ($p<0.05$). Hematocrits were not affected by infusions of TPO stimulated platelet transfusions, contrasting an average 6.1% unstimulated whole blood transfusion. The average increment of thrombocytes per mL infused volume was 0.8±0.6E+09/L for both whole blood and thrombocyte transfusions and was significantly higher for both rrTPO with an increase of 3.4±2.4E+09/L ($p<0.01$) and rhTPO with an increase of 2.0±1.6E+09/L ($p<0.01$). Even after 4 cycles of rr or rhTPO pretreatment, antibodies to TPO were not detected using the regimen predicted by the biomathematical model. *Conclusions.* A mathematically predicted non-immunogenic TPO administration regimen adapted for multiple pre-treatment of blood donors resulted in a significant increase of blood platelets and in well-tolerated collection of the required 1.0E+10 thrombocytes per transfusion unit. Despite the 7- to 10-fold lower total volume obtained from the donor monkeys after TPO stimulation, platelet increments in recipients were significantly higher. These data demonstrate that use of TPO or a TPO-mimetic can considerably reduce blood volume required per recipient while significantly promoting transfusion efficacy.

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0387

VESICULATION DURING STORAGE OF RBCS - OXIDATION, PHAGOCYTOSIS AND FAS-RELATED SIGNALLING MOLECULES

A. Kriebardis,[1] M. Antonelou,[1] K. Stamoulis,[2] E. Economou-Petersen,[3] L. Margaritis,[1] I. Papassideri[1]

[1]University of Athens, ATHENS; [2]Blood Transfusion Center, NIKEA, PIRAEUS; [3]National Blood Center, ATHENS, Greece

Background. Red blood cells (RBCs) lose membrane *in vivo*, under certain conditions *in vitro* and during *ex vivo* storage. The vesiculation influences the viability of stored RBCs and the post transfusion performance. *in vivo*, the RBC-derived microvesicles expose PS and are rapidly removed from the circulation by means of scavenger receptors. At least a part of the apoptotic machinery that is active in nucleated cells exists in mature RBCs. They further suffer protein oxidative damages during storage and progressively exhibit most of the *aging* characteristics of signalling potential, namely the activation of caspases and membrane binding of IgGs. *Aims.* The concept of the protein oxidation, phagocytosis-associated and Fas-related signaling as a part of the RBC storage lesion has been supported by previous reports. Despite that, there is not an established connection between them and vesiculation during storage. The presently reported work aimed to investigate this probable connection. *Methods.* Vesicles were isolated from the plasma of twenty-one non-leukoreducted packed RBCs (pRBCs) units in CPDA, at eight different time-points of the storage period and shortly afterwards. The oxidation status and the composition of the vesicles were evaluated by means of immunoblot. Ghost membranes were also investigated as controls. *Results.* IgGs-specific bands were detected in vesicles beginning from the first days of storage while the CD47 membrane marker was found in them at two weeks onward. The vesicles contain Fas/CD95, FADD and full length caspase 3 and caspase 8. Low MW cleavage products of caspase 8 and caspase 3 were also increasingly detected in the vesicles at 10 days onwards. The protein oxidative indexes of vesicles stored up to 21 days were significantly greater compared to those of the corresponding membranes of origin. Some of the oxidized protein bands have been identified as actin, band 3 and stomatin. *Summary and Conclusions.* The IgGs composition of the vesicles closely resembles that of

pRBCs stored for long periods in CPDA. The presently reported high protein oxidation index of vesicles provides direct evidence for a link between the storage-induced protein oxidation and vesiculation processes. The findings suggest that the vesicles (i) represent a microenvironment of high oxidative stress or of gathering oxidatively damaged proteins and (ii) have the same mechanism of recognition with their parent and/or senescent RBCs, including the binding of autoantibodies. The presence of CD47 showed that the vesicles contain more than one phagocytosis-related signals. Hence, the effectiveness of their clearance could be related to both the type and the amount of signals exposing on their surface. Our novel findings commit substantiating data for the existence of Fas-dependent signaling components inside the vesicles released during storage and circumstantial evidence for the action of a Fas/FADD/caspase 8/caspase 3 complex. The gathering of oxidative proteins and reactive signalling components into vesicles strongly suggests that the vesiculation further operates as an effective way of discharging damaged or *dangerous* proteins produced in the pRBC units during storage.

0388

RELATION OF PRESENTING FEATURES WITH CLINICAL AND LABORATORY OUTCOMES OF TTP/HUS PATIENTS

M.M. Yilmaz,[1] B. de Laat,[2] J.L. Kerckhoffs,[3] R.R.P. de Vries,[1] A. Brand[1]

[1]Dept of Immunohematology and Bloodtransfusion,Leiden University, LEIDEN; [2]Dept of Blood Coagulation,Sanquin Diagnostic Services, AMSTERDAM; [3]Dept of Hematology,Haha Hospital,, THE HAGUE, Netherlands

*Background.*Thrombotic thrombocytopenic purpura (TTP) is an uncommon hematological disorder characterized by severe thrombocytopenia, coombs negative hemolytic anemia with fragmented eritrocytes, fever and with renal and/or neurologic findings. Pathology for most forms of TTP is due to a severe defect in protease that cleaves von Willebrand factor (VWF). This metalloprotese is referred to as ADAMTS-13. However cases with acquired TTP, may have severe ADAMTS-13 deficiency secondary to the development of anti ADAMTS 13 antibodies. *Aims.* In the current study we analyzed retrospectively the ADAMTS-13 activity, antigen and inhibitor levels in plasma of TTP-HUS patients and thereby evaluated the possible association between ADAMTS-13 antigen, activity antibody and plasma exchange and the other presenting clinical features. *Methods*. This study is a retrospective analysis of TTP/HUS patients treated between 1984 and 2005 in Leiden University Medical Center and Haga Hospital ,the Netherlands. Measurement of the ADAMTS-13 activity in plasma was performed with TRETS assay (j. Thromb Haemost 2007,5:1330-1). Both for the activity and antigen assay a value of <30 was considered as decreased. The Antigen assay was newly developed by one of us (BdeL). Tecnozym ADAMTS13 inhibtor assay was used antibody assay. For the antibody data a titer of >15 is considered as positive. *Results.* We investigated 65 adult TTP-HUS patients, Fourty six (70.8 %) out of 65 TTP/HUS patients had a complete remission. The ADAMTS-13 activity in plasma were measured in 39, antigen and antibody in 37 patients. The ADAMTS-13 activity in 16 of 39 (41%) and antigen for 14 (37.8%) TTP-HUS patients had low (<30) levels. 24 of 37 (64.8%) TTP-HUS patients had high level of ADAMTS-13 antibody (>15). Three of them were secondary TTP-HUS patients. There was no statistical difference for antibody levels between early, moderate and late responder groups (p=0.642). And also there was no statistical difference for TPE volume between early and late responders group and early and moderate responders group (p=0.916) (p=0.262). But it was determined that in relapsed patients antibody levels were higher than in non-relapsed patients. This was statistically significant (p<0.0001). *Conclusions*. ADAMTS-13 antibody level at the beginning of the disease did not differ between early moderate and late responder groups but may help to predict patients who will possibly relapse.

0389

PREOPERATIVE INDIVIDUALIZED PROTOCOL REDUCE THE NEED FOR TRANSFUSION IN ELECTIVE TOTAL HIP OR KNEE ARTHROPLASTY

E. Colado, J.R. González-Porras, M.P. Conde, J. Martín-Sánchez, J. Olazabal, J. Alonso, L. López-Corral, M.P. Nieto, M. Corral

Hospital Universitario de Salamanca, SALAMANCA, Spain

Background. Individualized, patient based protocols can reduce Allogeneic Blood Transfusion (ABT) in elective surgery. *Aims.* To obtain a preoperative hemoglobin (Hb) level ≥14 g/dL and normal iron stores, and to reduce the number of ABT in major elective orthopedic surgery patients. *Methods.* Between January 2005 and September 2007, 305 patients were enrolled in an optimal use/blood-saving program (OU/BSP) based on the correction of preoperative iron-deficiency anemia or chronic inflammation anemia as soon as surgery was scheduled. Patients were classified according to Hb levels. Surgery was delayed in anemic patients with Hb ≤ 10 g/dL until the cause had been identified and properly treated. Patients with Hb 10-13 g/dL received recombinant human erythropoietin (rHuEPO) and/or oral or intravenous (IV) iron, depending on their iron metabolism parameters and time to surgery. Patients with Hb 13-15 g/dL were treated with IV or oral iron and autologous predonation was considered. Patients with Hb higher than 15 g/dL were given no treatment. We used the chi-square test and Student's t-test in the univariate analysis, and multivariate analysis was performed using multiple logistic regression (Forward method). P values < 0.05 were considered statistically significant. A cohort of 305 patients was enrolled and was compared to a matched historical control. No differences were observed when comparing age (68.1±10.6 *vs* 68.8±9.9 years), sex (Male 124 / Female 181 in both groups), weight (72±12.6 *vs* 74±10.5 kg), surgical procedure (46,6%, hip arthroplasty; 54,4%, knee arthroplasty in both groups), ferritin level (127±227 *vs* 129±126 ng/mL) or Hb levels (13.8±1.32 *vs* 13.8±1.4 g/dL) nor time to surgery. 80 patients (26.2%) required no therapeutic intervention, 145 patients (47,5%) were treated on oral iron therapy, 49 patients (16.1%) were treated with IV iron, 9 patients (3%) were treated with rHuEPO and iron, autologous predonation was performed in 20 patients (6.6%), loss of follow-up occurred in two patients (0.7%). *Results.* 80% of patients in our program obtained a preoperative Hb of 14 g/dL or more, compared with 60% of our historical cohort (p< 0.001). Subsequently, 18% of our patients received ABT whereas 31.8% of our historic control did (p<0.001). As expected, 40% of patients on autologous predonation program received blood transfusion. In the multivariate analysis, the variables independently associated with a higher risk of red blood cell transfusion were: weight > 70 kg (*vs* < 70 kg) [52% *vs* 10%, p= 0.003], hip arthroplasty (*vs* knee) [38.2% *vs* 11.7%, p= 0.003] and presurgical Hb < 14 g/dL (*vs* presurgical Hb > 14 g/dL) [47.6% *vs* 16.5%, p= 0.002]. *Conclusions*. Strategies aimed at reducing ABT should be individualized on a patient basis. Autologous predonation increases the overall risk of transfusion.

0390

USE OF A NOVEL POST-OPERATIVE CELL SALVAGE SYSTEM IN CARDIAC SURGERY

I.M. Anderson, I. Quasim, M. Steven, L. Soutar

Western Infirmary, GLASGOW, UK

Background. Cardiac surgery uses approximately 20% of US national blood stocks. With pressure on blood supplies and well documented risks associated with transfusion, there has been increased interest in minimising blood use by pharmacological or mechanical means. Intraoperative cell salvage has been used in cardiac surgery for many years however a recently introduced system (CardioPat) has been specifically designed to address some of the technical challenges presented by post-operative cardiac surgery; the system accurately measures blood loss and is able to apply a variable amount of suction to the drains. Traditionally blood collected postoperatively in chest drains has been discarded however the CardioPat system collects blood lost from mediastinal drainage, washes it, haemoconcentrates to approximately 80% and resuspends the red cells for autotransfusion. *Aims.* We aimed to assess the effectiveness of this system in postoperative patients who were felt to be bleeding excessively on their return from theatre. *Methods*. Patients who bled more than 80 mL in at least two consecutive 15 minute periods on their return from theatre were targeted and connected to the CardioPat system. Demographic details were collected as per normal audit procedure, as were details on admission and discharge Haemoglobin (Hb) concentrations. Total mediastinal blood loss was noted as was the amount of washed red cells returned to the patient. Blood bank transfusion details were collected at discharge from hospital. Transfusion triggers and routine clinical care were observed as per unit protocols. *Results.* In our institution, mean red cell use is 1.7 units per patient and mean postoperative mediastinal drain losses are 960 mL. The CardioPat device has been used postoperatively in 34 patients to date. Mean mediastinal drain losses were 2394mL in this group (range 780-7400 mL). The device produced an average of 295 mL of blood for re-infusion (range 15-2340 mL). This is equivalent to an average of 1.7 units of red cells (range 0.1-14.2). Mean homologous transfusion in this group was 6.7 units. This is similar to a group defined as bleeding excessively in a previous audit however discharge Hb in our patient group was higher (9.7 g/dL v 9.2). Admission and discharge Hb in this group of patients were similar to oth-

er cardiac patients (13.4 g/dL and 9.7g/dL respectively). *Conclusions*. The CardioPat has proved useful in this postoperative setting and can lead to considerable reductions in homologous transfusion. The current UK cost of a unit of red cells is £150 therefore use of the CardioPat has been cost-neutral. Universal cell salvage has been criticised for increasing costs; targeting this higher risk group allows cell salvage to become cost-neutral and even cost-saving.

0391

CONTINUING EDUCATION IN TRANSFUSION MEDICINE: 12 YEARS OF EXPERIENCE IN THE UNIVERSITY HOSPITAL OF SALAMANCA

J. Martín-Sánchez,[1] E. Colado,[1] C. Encinas,[1] I. De la Fuente,[1] M.J. Rodriguez,[2] J.R. Gonzalez-Porras,[1] M.J. Nieto,[1] M. Corral[1]

[1]Hospital Universitario de Salamanca, SALAMANCA; [2]Hospital Nuestra Señora de Sonsoles, AVILA, Spain

Background. Demography and clinical context of our transfused population have radically changed in the past few years. These variables have influenced transfusion practice medicine in our setting. *Aims*. To analyze transfusion practice during the years 2004, 2005, 2006 and 2007, and compare it with our own experience since 1995. To assess the utility and impact of continuing transfusion education and analysis started in 1995, in the consecution of transfusion excellence and detection of new clinical problems related to transfusion. *Methods*. A prospective concurrent design was followed; a 2 month period was analyzed using a basic transfusion form. Local transfusion guidelines were created in 1995 and periodically reviewed afterwards. Widespread knowledge of the guidelines was guaranteed thanks to clinical sessions, speeches, personal case-by-case advice, department oriented clinical sessions, daily instruction, and the annual meeting of the hospital transfusion committee. Two independent analyzers reviewed every form and determined the adequacy of each transfusion event according to those guidelines. *Results*. We analyzed 1442 transfusion events in 726 patients. 1001 of the events corresponded to packed red blood cells (RBC) (289, 235, 218, 254 in 2004, 2005, 2006 and 2007 respectively), 305 to pooled or apheresis-obtained platelets (PT) (80, 118, 107 and 169; 2004, 2005, 2006 and 2007), and 8 to fresh frozen plasma units (FFP) (all in 2006). Median age of our population was 66 years (interquartilic semirange, IS, was 49-76 years), and 50.1% of our patients were older than 65. RBC receptors' median age was 71 (IS 57-78), *vs* 64 in 1995. PT receptors' median age was 55 (35-65). Hematology patients received 47.1% (680 transfusion events), and together with oncological patients accounted for 53.6% of all transfusions (764 events). Pretransfusional haemoglobin values have followed a declining trend during the last 3 years and since 1996: in 2007 they are 7.6g/dL. RBC transfusion inadequacy amounted to 3.8% (24 out of 733; 4.2%, 1.7%, 3.7%, 5,2% in 2004, 2005, 2006 and 2007 respectively). For PT the rate was 8% (38 out of 473 events), annual rates were 10% in 2004, 18.6% in 2005, 3.7% in 2006 and 2% in 2007. In 1995, the rate was 32% for RBC, but prompt educational intervention made it go as low as 5.8% with a first educational action in 6 months time (1996; $p<.0001$). This low rate of inadequate transfusions has been maintained as shown above with only one increase in 1998. A new educational intervention was effective again. *Summary and Conclusions.* We have demonstrated that a continuing education approach is the most valuable tool to achieve optimal use of blood components. Continuing transfusion education and assessment are unavoidable if we are to maintain our present quality standards, as new problems arise because of a changing clinical scenario. One of the problems is the risk of undertransfusion if haemoglobin transfusion thresholds descend even more. Another one, transfusing and ageing population. Thus, careful monitoring of haemoglobin trigger, quality of life and clinical state of the receptors must be a priority.

0392

TWO-YEAR AUDIT OF THERAPEUTIC APHERESIS ACTIVITY IN A TERTIARY REFERRAL CENTRE IN IRELAND: ANALYSIS BY 2007 AMERICAN SOCIETY FOR APHERESIS GUIDELINES

T. Swords,[1] R. Swords,[2] Z. Heetun,[2] C. Gibbons,[2] O. Langan,[2] K. Mulhall,[2] P. Hayden[2]

[1]University College Hospital Galway, GALWAY; [2]Galway University Hospital, GALWAY, Ireland

Aims. We performed a retrospective analysis of all patients undergoing therapeutic apheresis (TA) in a large tertiary referral centre over a 2 year period (2006-2007). The indications for TA were then classified using the 2007 guidelines of the American Society For Apheresis (ASFA) in order to determine the evidence base underlying current TA activity at our institution. *Methods*. The indications for TA were taken from the medical records of patients treated between January 2006 and December 2007 inclusive. Procedural details of each TA episode were also compiled from apheresis service records. *Results*. There were 27 patients treated during this 24 month period, 13 males (48%) and 14 females (52%). A total of 165 apheresis procedures were performed, of which 61 (37%) were for ASFA category 1 indications, 36 (22%) for category 2 and 68 (41%) for category 3 disorders. Of the procedures conducted, 85 were for neurological (52%) disorders, 36 for renal (22%), 20 for haematological (12%), 18 (11%) for autoimmune conditions and 6 (4%) for metabolic diseases. The most common conditions requiring plasmapheresis were Guillain-Barre syndrome and Myasthenia Gravis. The average procedure time was 72 minutes. A total of 128 (78%) of the TA procedures required the placement of Central Venous Access Devices. Peripheral venous access was sufficient in 37 (22%) of the cases. The main complications related to achieving venous access and fluctuating Access Pressures. Human Albumin Solution (4.5%) was the most commonly used replacement fluid (91% of procedures) and all patients had a single plasma volume exchange. *Summary and Conclusions*. The 2007 ASFA guidelines were useful in allowing for clear category assignment. The availability of fact sheets with details of the evidence base for TA in each given condition was helpful for the TA team, patients and clinical service users. The guidelines highlighted shortcomings in our practice particularly with respect to the number of category 3 procedures conducted. For individual cases, the choice of replacement fluid, number of apheresis procedures performed and plasma volumes exchanged in our practice were often at variance with the proposed guidelines. The authors have now adopted a revised approach to clinical apheresis, which closely follow the ASFA approach, in the hope of reducing the number of unnecessary procedures and improving cost effectiveness. This information will facilitate rational use of this limited resource in the future.

0393

MULTIPLATE IMPEDANCE AGGREGOMETRY AS A PREDICTOR OF POST-OPERATIVE BLOOD LOSS IN CARDIAC SURGERY

I.M. Anderson, S. Travers, I. Quasim, M. Steven, L. Soutar

Western Infirmary, GLASGOW, UK

Background. Surgery involving cardiopulmonary bypass is recognised to have a significant impact on platelet function both in terms of platelet dysfunction due to direct platelet damage from the bypass machine's roller pumps and also due to pharmacological effects of pre-operative antiplatelet medication. Accordingly there has been significant interest in point-of-care tests of platelet function. Various methods are available to assess platelet function. Impedance aggregometry was first described in 1979 but has recently been modified to allow multiple tests to be performed simultaneously and analysed by computer. The Multiplate analyser determines an area under the curve in response to stimulation of activation pathways and thus determines platelet function. *Aims.* We sought to assess the ability of Multiplate tests to predict post-operative bleeding in our cardiac surgical population. *Methods*. We tested 44 consecutive patients scheduled for elective cardiac surgery. Prior to cardiopulmonary bypass, all samples were submitted for a collagen activation test as an overall assessment of platelet function and a *TRAP* (thrombin receptor activating peptide) test to assess the effect of antiplatelet medication. An 'Aspi 'test±an ADP test was performed to specifically assess the effect of aspirin or clopidogrel where these drugs had been in use preoperatively; in our unit aspirin is continued until the day of surgery although clopidogrel is normally stopped 5 days before. On arrival in the Intensive Care Unit these tests were repeated postoperatively and the results noted. Post-operative drain losses were noted at 16 hours. *Results*. 44 patients were studied and included in the statistical database. Data was analysed by *Best Subsets* then multiple regression analysis using Minitab statistical software. No preoperative test was predictive of blood loss. The best predictor of excessive blood loss was a combination of the postoperative collagen and TRAP test results. However, these only accurately predicted blood loss in 43% of patients and were not independently significant. *Conclusions*. Multiplate analysis offers point-of-care testing. Initial results indicate better prediction in postoperative rather than pre-operative testing - perhaps not surprising in view of greater platelet dysfunction in this setting. Initial results have encouraged us to continue our study to obtain greater patient numbers.

0394

MONOCYTE SUBPOPULATIONS WITH DIFFERENT CYTOKINE RELEASE INDUCED BY LEUKOCYTAPHERESIS IN HEALTHY BLOOD DONORS

M. Hendelmeier, A. Oehring, E. Strasser, R. Zimmermann, R. Eckstein

University Hospital Erlangen, ERLANGEN, Germany

Background. Dendritic cells (DC) are the terminal stage of monocyte differentiation. Peripheral blood monocytes are a heterogenous population and consist of distinct subsets. The understanding of monocyte heterogeneity and the release of cytokines may have implications for the development of DC vaccines. *Aims.* Leukocytapheresis may be a stimulus for the release of cytokines by monocyte subpopulations. *Methods.* 12 healthy blood donors were investigated before and after leukocytapheresis procedures (COM.TEC cellseparator, Fresenius HemoCare, MNC program and COBE Spectra, Gambro, PBSC program). Additionally, apheresis products were examined and the release of cytokines was investigated by flow cytometry. *Results.* The leukocyte concentration of products ranged between 28×10^3 and 112×10^3 White blood cells (WBC) per microliter. The mean percentage of $CD14^{++}$ monocytes releasing IL-1 was 82.1 percent (range, 71.6% to 94.8%) and the mean percentage of $CD14^{+}CD16^{+}$ monocytes releasing IL-6 was 6.4 percent (range, 4.2% to 11.6%). The main population of $CD14^{++}$ monocytes showed a significant higher release of IL-1 in the pre- and postdonation blood counts compared to the $CD14^{+}CD16^{+}$ monocyte subpopulation releasing IL-1 (predonation: $p<0.001$ and postdonation: $p<0.002$). Subpopulations of $CD14^{+}CD16^{+}$ monocytes showed a significant higher release of IL-6 in the predonation blood counts compared to $CD14^{++}$ monocytes (4.8% *vs* 9.1%, $p<0.001$). The pre- and post-donation counts of $CD14^{+}CD16^{+}$ monocytes releasing IL-6 did not differed significantly, whereas the release of IL-6 by this subpopulation in the apheresis products increased significantly ($p<0.0001$). Compared to the initial concentration of $CD14^{++}$ monocytes releasing IL-1 in donors no cytokine release in apheresis products was found, whereas $CD14^{+}CD16^{+}$ monocytes releasing IL-6 were enriched by factor 2 in apheresis products (both $p<0.0001$). *Summary and Conclusions.* The $CD14^{++}$ monocytes releasing IL-1 were enriched in the postdonation blood counts. However, no $CD14^{++}$ monocytes releasing IL-1 were found in the apheresis products. $CD14^{+}16^{+}$ monocytes releasing IL-6 were increased in the apheresis products, but not in the postdonation blood counts. These results may suggest a cytokine release in different monocyte subpopulations induced by leukocytapheresis.

0395

PHARMACOKINETICS (PK) OF SUBCUTANEOUS FONDAPARINUX 1.5 MG IN PATIENTS WITH MODERATE RENAL IMPAIREMENT (MRI)

A.G.G. Turpie,[1] D. Boyle[2]

[1]Hamilton Health Sciences - General Division, HAMILTON, ONTARIO, Canada; [2]GlaxoSmithKline Upper Merion, KING OF PRUSSIA, PA, USA

Background. Fondaparinux is approved for the prevention of venous thromboembolism. Like low-molecular-weight heparins, fondaparinux is mainly eliminated via the kidneys. At the once-daily (od) dose of 2.5 mg, exposure to fondaparinux is increased in patients with MRI (creatinine clearance, CLcr: 20 to 50 mL/min). *Aims.* We looked for a reduced dose of fondaparinux able to achieve a similar level of exposure in patients with MRI and in patients with normal renal function (NRF).

Table 1.

AUC_{0-24} (mg*h/mL)	Day	1.5 mg od in MRI patients (n=3457)	2.5 mg od in MRI patients (n=3257)	2.5 mg od in NRF patients (n=16,543)
	1	2.9 (2.1-4.1)	4.8 (3.5-6.8)	3.2 (2.1-4.8)
	28	6.2 (3.9-10.5)	10.4 (6.4-17.2)	5.0 (2.8-8.9)

Geometric mean (5^{th}-95^{th} prediction intervals)

Methods. Using a population PK model based on data obtained in 756 major orthopedic surgery patients, we predicted the pharmacokinetics of fondaparinux based on the demographic characteristics and renal function of the patients enrolled in the Phase II/III thromboprophylactic trials. A total of 20,000 patients were simulated in order to predict exposure (AUC0-24) and peak plasma concentration (Cmax) on Days 1, 7 and 28 after the od administration of fondaparinux 1.5 mg or 2.5 mg in MRI or NRF patients. *Results.* Predicted AUC0-24 (Table 1) and Cmax in patients with MRI administered 1.5 mg od were very close to those in patients with NRF administered 2.5 mg od. In the low range of CLcr, the inter-individual variability in the plasma concentration of fondaparinux was lower with 1.5 mg than with 2.5 mg. *Summary and Conclusions.* Exposure to od fondaparinux was estimated to be similar in MRI patients administered 1.5 mg and in NRF patients administered 2.5 mg. Based on the results of these pharmacokinetic simulations, fondaparinux 1.5 mg od may be an appropriate thromboprophylactic dose in patients with MRI.

0396

NOVEL, GRADUALLY INCREASING AND LONG-LASTING ADHESIVE AND SECRETORY RESPONSES ELICITED BY LEUKOTRIENE B4 IN ENDOTHELIAL CELLS ARE MEDIATED VIA THE MAP KINASE PATHWAY

A.S.M. Johansson, J.Z. Haeggstrom, J. Palmblad

Karolinska Institutet, STOCKHOLM, Sweden

Leukotriene B4 (LTB4), a powerful chemotactic and immune modulating lipid, signals via distinct G-protein-coupled surface receptors, denoted BLT. The ensuing cellular responses are rapid in onset and short-lived. Recently, we reported that BLT1 is the predominating BLT expressed on human umbilical vein endothelial cells (HUVEC). LTB4 stimulation of HUVEC causes adhesion of neutrophils, up-regulation of E-selectin, ICAM-1 and VCAM-1, release of MCP-1 and IL-8, and of NO production. Since L-NAME inhibited the nitrite release, this is suggested to reflect NO production. The expected fast and transient adhesive and secretory responses (peaking after 15-30 min) were, unexpectedly, followed by sustained larger peaks after 3-7 h, similar in magnitude to those elicited by LPS. Moreover, LTB4 responses were mediated by the MAP kinase/Erk pathway, whereas no activation of NK-kB p65, c-jun or Elk-1 was observed. Thus, contrary to previous observations, LTB4 causes not only rapid but also long-lasting pro-inflammatory responses in endothelial cells, which is a novel observation. Since IL-8 and MCP-1 plays a role in neutrophil and monocytes recruitment our findings may have functional consequences in the early and sustained vascular responses to inflammation. Moreover, the results point to BLT receptors as potential targets for pharmacological intervention in vasculitides of various causes.

SIMULTANEOUS SESSIONS I

Non-Hodgkin's lymphoma - Clinical (aggressive)

0397

RANDOMIZED PHASE II TRIAL ON PRIMARY CHEMOTHERAPY (CHT) WITH HIGH-DOSE METHOTREXATE (MTX) ALONE OR ASSOCIATED WITH HIGH-DOSE CYTARABINE (ARAC) FOR PATIENTS (PTS) WITH PRIMARY CNS LYMPHOMA (PCNSL): THE INTERNATIONAL EXTRANODAL LYMPHOMA STUDY GROUP (IELSG) #20 TRIAL

J.M. Ferreri,[1] M. Foppoli,[1] M. Martelli,[2] G. Pangalis,[3] M. Frezzato,[4] G. Cabras,[5] A. Fabbri,[6] G. Corazzelli,[7] F. Ilariucci,[8] G. Rossi,[9] R. Soffietti,[10] C. Stelitano,[11] D. Vallisa,[12] F. Zaja,[13] L. Zoppegno,[14] G. Aondio,[15] G. Avvisati,[16] M. Balzarotti,[17] A.A. Brandes,[18] J. Fajardo,[19] H. Gomez,[20] A. Guarini,[21] G. Pinotti,[22] L. Rigacci,[23] C. Uhlmann,[24] M. Ponzoni,[1] M. Reni,[1] E. Zucca,[25] F. Cavalli[25]

[1]San Raffaele H Scientific Institute, MILAN, Italy; [2]University La Sapienza, ROMA, Italy; [3]Athens Medical School, ATHENS, Greece [4]Ospedale San Bortolo, VICENZA, Italy; [5]Ospedale Businco, CAGLIARI, Italy; [6]Università Senese, SIENA, Italy; [7]National Cancer Institute, NAPLES, Italy; [8]Ospedale Santa Maria Nuova, REGGIO EMILIA, Italy; [9]Spedali Civili, BRESCIA, Italy; [10]Ospedale San Giovanni Battista, TURIN, Italy; [11]Ospedale di Reggio Calabria, REGGIO CALABRIA, Italy; [12]Ospedale Civile, PIACENZA, Italy; [13]Ospedale di Udine, UDINE, Italy; [14]Hospital San Martìn, LA PLATA, Argentina; [15]Ospedale Moriggia-Pelisciani, GRAVEDONA, Italy; [16]Campus Bio-Medico, ROME, Italy; [17]Istituto Clinico Humanitas, ROZZANO, Italy; [18]Ospedale Busonera, PADOVA, Italy;; [19]Hospital Santa Maria, LISBOA, Portugal; [20]INEN, LIMA, Peru

Background. MTX-based CHT is the conventional approach to PCNSL, but superiority of polyCHT over MTX alone is unproven. A benefit of adding araC to MTX has been suggested by a meta-analysis and a large retrospective series. *Aims.* To assess if MTX-araC combination is more active than MTX alone as primary CHT for PCNSL in a randomized comparison. *Patients*. PCNSL pts (HIV-; 18-75 ys; PS≤3; 2004-2007) were randomly allocated to receive 4 courses (interval 3 weeks) of MTX 3.5 g/mq (arm M) or MTX (same dose) + araC 2 g/mq x 2/d, d 2-3 (arm MA). CHT was followed by radiotherapy (RT). Pts were stratified based on IELSG score and centre RT policy for pts >60 ys in complete remission (CR) after CHT. CR rate (CRR) after CHT was the primary endpoint; planned accrual (alpha=.05; betta=.2) for P0 30% and P1 50% was 39 pts/arm. *Results.* 79 pts (median age 58 ys) were randomly assigned to receive M (N=40) or MA (N=39). IELSG risk was low in 22 (28%) pts, intermediate in 48 (61%) and high in 9; 14% of pts had ocular lesions and 7% had meningeal disease; no differences in presentation between arms were observed. 231 (73%) of the 316 planned courses were actually delivered (M 71%; MA 76%). CHT was interrupted due to PD in 20 (50%) M and 8 (21%) MA pts (p<0.001), toxicity in 1 (3%) M and 7 (18%) MA pts (p=0.009) and refusal in 2 MA pts. ≥25% dose reduction was indicated in 1 M and 17 MA pts. G4 neutropenia (10% *vs* 74%), G4 thrombocytopenia (5% *vs* 64%) and infections (3% *vs* 23%) were significantly higher in MA arm. All G3-4 non-hematological toxicities were <5%. One M pt (3%, cardiotoxicity) and 3 MA pts (8%, sepsis - hepatotoxicity) died of toxicity. After CHT, 7 M and 18 MA pts achieved CR (18% *vs* 46%; p=0.0002); 10 M and 9 MA pts achieved PR (ORR: 43% *vs* 69%; p=0.0002). After CHT-RT, 11 M and 25 MA pts achieved CR (28% *vs* 64%; p<0.0001). At a median f-up of 16 mo, 29 M and 22 MA pts experienced failure (PD, relapse, death), with a 3-yr EFS of 24% *vs* 35% (p=0.02). *Conclusions.* This is the first randomized trial on PCNSL with completed accrual. The addition of araC to MTX resulted in significantly better outcome and acceptable toxicity. MTX+araC may be the control arm for future randomized trials.

0398

CNS RECURRENCE IN AGGRESSIVE LYMPHOMA TREATED WITH MODERN CHEMOTHERAPY (CHOP-14) WITH OR WITHOUT RITUXIMAB: AN ANALYSIS OF CNS-EVENTS IN ELDERLY PATIENTS TREATED IN THE RICOVER-60 TRIAL OF THE GERMAN HIGH-GRADE NON-HODGKIN'S LYMPHOMA STUDY GROUP (DSHNHL)

N. Schmitz,1 V. Boehme,[1] S. Zeynalova,[2] E. Lengfelder,[3] M. Reiser,[3] H. Steinhauer,[3] M. Clemens,[3] C. Nickenig,[3] M. Loeffler,[2] M. Pfreundschuh[4]

[1]ASKLEPIOS KLINIK St. Georg, HAMBURG; [2]Institute of Medical Informatics, Statistics & Epidemiology, University Leipzig, LEIPZIG; [3]On behalf of the DSHNHL, HOMBURG; [4]Department of Internal Medicine I, Saarland University, HOMBURG/SAAR, Germany

Background. CNS recurrence of aggressive lymphoma remains a distressing and usually incurable event. In an analysis of patients on protocols of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL) between 1990 and 2000, the CNS relapse rate was 2.2% in 1693 patients treated with modern chemotherapy but without rituximab (Boehme *et al.*, Ann. Oncol 2007;18:149-57). We now analyzed the incidence and prognostic factors for CNS recurrence in the RICOVER-60 trial (Pfreundschuh *et al.*, Lancet Oncol 2008;9:105-16). *Patients and Methods*. From 2000-2005, elderly patients (pts.) between 61 and 80 years with CD20$^+$ aggressive lymphoma (all risk groups according to the IPI) were randomized to receive 6 or 8 cycles of CHOP-14 chemotherapy with or without 8 courses of rituximab. For patients with lymphoma manifestation in testes, bone marrow, sinuses or other head and neck locations deemed at high risk for CNS recurrence, the protocol asked for intrathecal (i.th.) prophylaxis with 15 mg methotrexate (MTX) twice in the first two cycles of chemotherapy. *Results*. 1217 patients were evaluable, of whom 58 patients (4.8 %) after a median time of 8 months (1-39) developed relapse or progression to the CNS with a median time of survival of 3 months (0.1-38). Multivariate Cox regresssion analysis identified involvement of more than one extranodal site (RR = 3.4; p< 0.001), the presence of B-symptoms (RR = 1.9; p= 0.025) and increased LDH (RR = 1.5; p= 0.146) as predictors of CNS recurrence. Patients with three characteristics had a CNS relapse rate of approximately 24% at 2 years, about 6-fold the incidence rate observed in all other patients. The addition of rituximab to chemotherapy reduced the risk of CNS recurrence (RR = 0.5; p= 0.025), whereas the number of treatment cycles (6 *vs* 8) did have no influence on the CNS-specific outcome. Of 210 patients (17%) with lymphoma involvement of testes, bone marrow or head (sinuses, orbita, oral cavity, tongue, salivary glands) 120 pts. received i.th. prophylaxis as intended per protocol whereas the remaining 90 patients were not treated with MTX due to protocol violation. Only seven of these patients (7/210 = 3.3%) developed meningeosis in the course of the disease. There was no difference in the incidence of meningeal relapse between the patients with or without prophylaxis. *Conclusions*. The incidence of CNS relapse in 1217 patients treated for aggressive lymphoma with CHOP-14 with or without rituximab was low (4.8 %) although slightly higher than reported in other recent series. The prognostic factors for CNS-disease and the poor prognosis of CNS relapse remained largely unchanged. Rituximab significantly reduced the incidence of CNS disease (3.6 *vs* 5.9%) and the risk of CNS recurrence (RR 0.5; p= 0.025) for the most part due to improved overall response after combined chemo-immunotherapy. The number of patients who experienced meningeosis was low (7 patients) and prophylactic i.th. therapy given to patients deemed at high risk for meningeosis did have no influence on the incidence of meningeal relapse. I.th. prophylaxis should no longer be recommended because with modern immuno-chemotherapy the incidence of CNS recurrence is low even in *high-risk* patients and there is no indication that the incidence of meningeosis could be reduced.

0399

RESULTS FROM AN INTERNATIONAL STUDY INVESTIGATING THE EFFICACY AND SAFETY OF LENALIDOMIDE IN RELAPSED OR REFRACTORY AGGRESSIVE NON-HODGKIN'S LYMPHOMA

C. Haioun,[1] C.B. Reeder,[2] J. Polikoff,[3] N.M. Chowhan,[4] I. Esseessee,[5] R. Greenberg,[6] A. Ervin-Haynes,[7] D. Pietronigro,[7] J.B. Zeldis,[7] T.E. Witzig,[2] M.S. Czuczman[8]

[1]CHU Henri Mondor, CRÉTEIL, France; [2]Mayo Clinic, SCOTTSDALE, AZ, USA; [3]Kaiser Permanente Medical Group, SAN DIEGO, CA, USA; [4]Cancer Care Center, NEW ALBANY, IN, USA; [5]Hematology Oncology Associates of Central Brevard, ROCKLEDGE, FL, USA; [6]Center for Cancer and Hematologic Disease, CHERRY HILL, NJ, USA; [7]Celgene Corporation, SUMMIT, NJ, USA; [8]Roswell Park Cancer Institute, BUFFALO, NY, USA

Background. Previous trials have shown that lenalidomide is active with manageable side effects in non-Hodgkin's lymphoma (NHL). *Aims.* The present study aims to confirm the activity and safety of lenalidomide in patients with relapsed or refractory aggressive NHL in an international setting. *Methods.* Patients with relapsed or refractory aggressive NHL with measurable disease (≥ 2 cm) after at least 1 prior treatment regimen were eligible. Patients received 25 mg lenalidomide orally once daily on Days 1-21 every 28 days and continued therapy as tolerated or until disease progression. Response and progression were evaluated using the IWG criteria. *Results.* As of August 25, 2007, 46 patients were eligible for response assessment and 79 for safety evaluation. Median age was 65 (21-84) years, 74% were male, and 96% had received prior rituximab. Median time from diagnosis was 2 (0.2-12) years and median number of prior treatment regimens was 3.5 (1-13). Histologies included diffuse large B-cell lymphoma [DLBCL] (n=29), follicular lymphoma grade 3 [FL] (n=1), mantle cell lymphoma [MCL] (n=13) and transformed lymphoma [TSF] (n=3). An objective response was observed in 13 patients (28%), including a complete response in 1 patient (2%) and partial responses in 12 patients (26%). Ten patients (22%) had stable disease. Responses were seen in each of the aggressive histologic subtypes studied: DLBCL (6/29), FL (1/1), MCL (5/13), and TSF (1/3). Most common grade 3 or 4 adverse events were neutropenia (24%), thrombocytopenia (16%), leukopenia (9%), anemia (6%), dehydration (5%), and fatigue (5%). *Summary and Conclusions.* Lenalidomide has activity in relapsed or refractory aggressive NHL, resulting in a response in 28% of patients with manageable side effects. Updated data will be presented at the meeting.

0400

THE HOST PHARMACOGENETIC PROFILE IS AN INDEPENDENT PREDICTOR OF OUTCOME AND TOXICITY IN DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH R-CHOP21

D. Rossi,[1] S. Rasi,[1] S. Franceschetti,[1] D. Capello,[1] A. Castelli,[1] A. Chiappella,[2] L. De Paoli,[1] A. Ramponi,[3] E. Pogliani,[4] U. Vitolo,[2] A. Conconi,[1] G. Gaidano[1]

[1]Division of Hematology, Amedeo Avogadro University, NOVARA; [2]Division of Hematology, Azienda Ospedaliera S. Giovanni Battista, TURIN; [3]Division of Pathology, Amedeo Avogadro University, NOVARA; [4]Division of Hematology, University of Milano Bicocca, MONZA, Italy

Background. R-CHOP is the standard treatment for diffuse large B-cell lymphoma (DLBCL). Several tumor cell-related predictors of efficacy and toxicity have been so far identified for DLBCL treated with R-CHOP. Conversely, no information is available on the impact of the host's genetic background as a predictor of outcome and toxicity in this context. *Aims.* This study aimed at verifying whether relevant single nucleotide polymorphisms (SNPs) of the host may further refine the prognostic stratification and the prediction of toxicity in DLBCL patients treated with R-CHOP21. *Methods*. The study was based on a consecutive series of 106 DLBCL treated with R-CHOP21 and provided with a large and homogeneous dataset comprising clinical variables at diagnosis, chemotherapy doses and toxicity. All data were collected prospectively. Candidate SNPs belonged to genes known to be involved in the pharmacogenetics of cyclophosphamide, doxorubicin, vincristine, prednisone and rituximab, and included SNPs affecting: i) drug metabolism (cytochrome-P450: CYP2B6-25504C>T, CYP2B6-15630G>T, CYP2C19-19153G>A, CYP3A4--392A>G, CYP3A4-14365T>G, CYP3A5-6980G>A); ii) drug detoxification: (gluthatione-S-transferase: GSTA1--4621T>C, GSTM1-null, GSTP1-1374A>G, GSTP1-2264C>T); iii) drug transport (multidrug-resistance-related proteins: ABCC1-129623G>T, ABCC2-53394T>A, ABCC2-68692G>A, ABCB1-90855C>T, ABCB1-49691G>A, ABCG2-8824C>A, ABCG2-33G>A; and iv) drug pharmacodynamics (NADPH-subunits: NCF4--368A>G, RAC2-7418T>A, CYBA-4185C>T; TP53: p53-440G>C; Fcγ receptor: FCGR2A-4487A>G, FCGR3A-5092T>G, FCGR3A-1301T>G/A; Glucocorticoid receptor: GR-67G>A, GR-1087A>G, GR-1829C>G). Genotyping of candidate SNPs was performed on PBMC collected at DLBCL diagnosis by SNP-minisequencing (ABI Prism SNaPshot Multiplex kit, Applied Biosystem). Primary end points were event free survival (EFS) and toxicity. *Results*. Univariate log-rank analysis identified CYBA-4185C>T (3-year EFS CT/CC: 61.2% *vs* TT 41.7%; *p=0*.008), ABCC2-53394T>A (3-year EFS TT: 64.0% *vs* AT/AA: 34.1%; *p*=0.013), and GSTA1--4621C>T (3-year EFS CT/TT: 64.8% *vs* CC: 39.2%; *p*=0.027) as predictors of EFS. Multivariate analysis identified CYBA-4185TT (HR:2.57; *p*=0.010) and GSTA1--4621CT/TT (HR:0.44; *p*=.010), along with chemotherapy interruption (HR: 3.52; *p*=0.002), IPI3-5 (HR:2.81; *p*=0.006), and liver involvement (HR:3.07; *p*=0.006) as independent predictors of EFS after adjusting for ABCC2-53394T>A, marrow involvement, organ function (marrow, renal, liver, cardiac), comorbidities, dose intensity of cyclophosphamide and doxorubicin, and toxicity. The impact of SNPs was also evaluated for toxicity in 658 courses of R-CHOP21 by logistic regression analysis adjusted for age, sex, ECOG PS, IPI, comorbidities, organ function, and doses of cyclophosphamide, doxorubicin and vincristine. NCF4--368AG/GG was an independent predictor of low risk of hematologic toxicity G3-4 (HR:0.49; *p*=0.012), infection (HR:0.42; *p*=0.002), cardiac toxicity (HR:0.34; *p*=0.001), and neurological toxicity (HR:0.52; *p*=0.009). *Conclusions*. The implications of these results are twofold. First, host SNPs affecting doxorubicin and/or cyclophosphamide pharmacodynamics (CYBA-4185C>T) and detoxification (GSTA1--4621C>T) are independent predictors of outcome in DLBCL treated with R-CHOP21. Poor EFS heralded by CYBA-4185C>T, a nonsynonimous SNP of NADPH-oxidase p22phox, may be due to reduced production of reactive oxygen species (ROS) that mediate doxorubicin cytotoxicity. Favorable EFS associated with GSTA1--4621C>T may be related to reduced expression of glutathione-S-transferase A1, a phase II enzyme involved in cyclophosphamide and doxorubicin detoxification. Second, NCF4--368AG/GG, a SNP belonging to NADPH-oxidase p40phox and regulating ROS generation and granulocyte burst, has an independent protective role against both hematologic and non-hematologic toxicity.

0401

PRIMARY DIFFUSE LARGE B-CELL LYMPHOMA OF THE TESTIS: IMPROVED OUTCOME WITH RITUXIMAB-CHOP WITH CNS AND CONTRALATERAL TESTIS PROPHYLAXIS. FINAL RESULTS OF IELSG 10 STUDY

U. Vitolo,[1] E. Zucca,[2] A. Chiappella,[1] M. Martelli,[1] M. Balzarotti,[1] G. Benevolo,[1] P. De Masi,[1] A. Filippi,[1] M.K. Gospodarowicz,[2] A. Lopez-Guillermo,[2] G. Martinelli,[2] F. Merli,[1] T. Perrone,[1] P. Pregno,[1] A.H. Sarris,[2] S. Storti,[1] F. Cavalli[2]

[1]On the behalf of IELSG and IIL, Hematology, San Giovanni Battista Hospital, TORINO, Italy; [2]On the behalf of IELSG, IOSI, BELLINZONA, Switzerland

Background. Diffuse large B-cell lymphoma (DLBCL) of the testis (PTL) is a rare presentation of extranodal lymphoma at poor prognosis with a 5-yr overall survival of 40-55%. Contralateral testis, CNS and extranodal sites relapses are the main cause of failures. *Aims.* the IELSG10 study is a prospective phase II international trial for stage I or II PTL. It aimed at defining a standard treatment for PTL with a combined treatment of R-CHOP, intrathecal methotrexate and prophylactic scrotal radiotherapy (RT) with the addition, in stage II, of loco-regional RT. The trial was conducted by International Extranodal Lymphoma Study Group (IELSG) and Intergruppo Italiano Linfomi (IIL). The present analysis provides the final results of the whole study. *Methods.* Between June 2001 and December 2006, 53 untreated patients with stage I-II PTL were enrolled from 31 centres. Treatment was: R-CHOP21 (Rituximab 375 mg/mq, Cyclophosphamide 750 mg/mq, Doxorubicine 50 mg/mq, Vincristine 1.4mg/mq d 1 and Prednisone 40 mg/mq dd 1-5) for 6 or 8 (in stage II patients with slow response) courses; intrathecal methotrexate (IT-MTX) 15mg for 4 doses in courses 1 and 2; after chemotherapy 30Gy scrotal RT to the contralateral testis was planned to all patients reserving 30-36 Gy on loco-regional nodes for stage II disease. *Results.* median age was 64 years (22-80); 40 stage I and 13 stage II; 4 had bilateral testicular involvement and 6 LDH > normal. All received R-chemotherapy as planned. Fifty patients received adequate CNS prophylaxis (at least 4 IT-MTX); 3 less than 4 IT because of toxicity. Scrotal RT was given to 49 patients; 4 did not perform it (2 refusals, 1 progressive disease and 1 bilateral orchiectomy). Eight of the 13 stage II patients received nodal RT as planned. Fifty-two patients (98%) achieved a CR and 1 progressed after 4 R-CHOP. With a median follow-up of 36 months, 3-yr OS and 3-yr PFS (including progressions and deaths from any causes) were: 86% (95% CI 70-93%) and 82% (95% CI 66-91%) respectively. Eight patients relapsed or progressed: 2 in nodal sites, 4 in extranodal +/– nodal sites and 2 in CNS (1 isolated meningeal and 1 meningeal + nodal relapse). The actuarial risk of CNS relapse at 3 years was only 2% (95% CI 0-6%). No contralateral testis relapses were observed. Eight patients died: three because of DLBCL, 1 of heart failure, 1 of colon carcinoma and one of acute myeloid leukemia 4 and 21 months off therapy while in CR. The most severe (grade 3-4) toxicities were: leukopenia 27% and neurologic 13%. Infections were recorded in only 2 patients, no other extrahematological toxicities were observed. No toxic deaths occurred during treatment. *Conclusions.* the results showed that this combined treatment with R-CHOP21 and complete CNS and scrotal prophylaxis improves the outcome of PTL compared with data reported into the literature. An effective systemic control of the disease with no contralateral testis relapses and a reduced incidence of CNS relapse was achieved in a prospective population of PTL, mainly elderly, with a treatment with a low toxic profile.

Chronic myeloid leukemia - Clinical I

0402

FIRST REPORT OF THE TOPS STUDY: A RANDOMIZED PHASE III TRIAL OF 400MG VS 800MG IMATINIB IN PATIENTS WITH NEWLY DIAGNOSED, PREVIOUSLY UNTREATED CML IN CHRONIC PHASE USING MOLECULAR ENDPOINTS

J.G. Cortes,[1] M. Baccarani,[2] F. Guilhot,[3] B.J. Druker,[4] R. Yu,[5] M. Rudoltz,[5] T. Krahnke,[6] T. Hughes[7]

[1]The University of Texas, HOUSTON, USA; [2]Institute of Hematology and Medical Oncology Seragnoli," University of Bologna, BOLOGNA, Italy; [3]Centre Hospitalier Universitaire, La Miletrie, POITIERS, France; [4]Oregon Health & Science University Cancer Institute, PORTLAND, USA; [5]Novartis Pharmaceuticals, EAST HANOVER, USA; [6]Novartis Pharma AG, BASEL, Switzerland; [7]Institute of Medical and Veterinary Science, ADELAIDE, Australia

Background. The IRIS study (imatinib vs interferon/Ara-C) established 400 mg imatinib (SD-IM) as standard of care for patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP). In the IRIS trial achievement of a MMR predicted for progression-free survival (PFS) benefit (Druker 2006). Phase II trials have suggested that high dose imatinib (HD-IM), 800 mg, increases the likelihood of achieving major molecular response (MMR) (Kantarjian 2004), and may improve long-term progression-free survival (PFS) (Aoki 2006). *Aims.* The Tyrosine Kinase Inhibitor Optimization and Selectivity Study (TOPS) aims to determine whether a higher initial dose of imatinib (800 mg *vs* 400 mg) improves the probability of achieving MMR among patients in early chronic phase. *Methods.* Adult patients with newly diagnosed, previously untreated Ph+ CML-CP were randomized to receive either HD-IM or SD-IM in a 2:1 ratio. Patients were stratified by Sokal score at diagnosis. The primary endpoint was MMR (BCR-ABL/control gene ratio of ≤ 0.1% according to the standardized international scale) at 12 months. The study was powered to demonstrate a 50% improvement in the rate of MMR at 12 months between the two arms (90% power assuming a response rate of 60% for the 800 mg and 40% for the 400 mg). Secondary evaluations included: CCyR rates at 12 months, dose intensity in each arm, evolution of hematologic, cytogenetic, and molecular response over time, and safety. Patients received regular clinical and laboratory evaluations, including Bcr-Abl transcript measurement at baseline and then every three months. *Results.* 476 patients were enrolled from 19 countries at 103 study sites from June 2005 to December 2006. First analysis of this study will be completed by the time of EHA congress. Data to be presented include MMR at 12 months, dose intensity, evolution of responses over time, relationship to Sokal score, and safety. *Conclusions.* This will be the first presentation of data from TOPS, the first controlled trial to evaluate HD-IM in newly diagnosed CML-CP patients of all Sokal risk categories. Achievement of MMR at an early time point will be correlated in future analyses with event-free, progression-free, and overall survival to determine if CML-CP patients may benefit long-term from treatment with an initial imatinib dose of 800 mg. Approximately 75% of 12 month molecular data used to determine the rate of MMR at 12 months have been analyzed in a blinded fashion at the time of this abstract submission. All 12 month molecular data will be unblinded and available in May 2008.

0403

BOSUTINIB (SKI-606) SHOWS HIGH TOLERABILITY AND CLINICAL ACTIVITY IN PATIENTS WITH PHILADELPHIA CHROMOSOME POSITIVE LEUKEMIAS

C. Gambacorti-Passerini,[1] J. Cortes,[2] H. Kantarjian,[2] D. Kim,[3] A. Turkina,[4] T. Fischer,[5] F. Cervantes,[6] S. Agarwal,[7] B. Hewes,[7] T.H. Brummendorf[8]

[1]University Milano-Bicocca, MONZA, Italy; [2]MD Anderson Cancer Center, HOUSTON, USA; [3]St Mary's Hospital, SEOUL, South-Korea; [4]Hematology Research Center, ST. PETERSBURG, Russian Federation; [5]University of Mainz, MAINZ, Germany; [6]Hospital Clinic Provincial, BARCELONA, Spain; [7]Wyeth Research, CAMBRIDGE, USA; [8]Universitätsklinikum Hamburg-Eppendorf, HAMBURG, Germany

Background. Bosutinib (SKI-606) is an orally available, dual Src/Abl kinase inhibitor. *Aims.* To assess safety and clinical activity of bosutinib (B) in patients (Pts) with Philadelphia chromosome positive (Ph+) chronic myelogenous leukemia (CML) or acute lymphocytic leukemia (ALL)

who are resistant/intolerant to Imatinib (I) or other TKIs, we conducted a phase 1/2 study. *Methods.* Pts received B at 400 to 600 mg/day, with a median age of 56 (CP), 53 (AP), 48 (BP), 60 (ALL) yrs. The planned duration of the study was 1 year, but it was subsequently extended in pts showing clinical response to B. *Results.* Pts evaluable for safety analysis are: 152 in CP-CML, 32 in AP-CML, 23 with BP-CML and 17 with ALL. The most frequent treatment emergent adverse events (AE) observed for CP were diarrhea (68%), nausea (43%), vomiting (28%), abdominal pain (27%), rash (24%) and for advanced phase were diarrhea (61%), nausea (43%) and vomiting (38%), pyrexia (29%), pain (29%); these effects usually subsided spontaneously within the first 4 weeks of treatment. Grade 3/4 AEs occurring in >5% of CP pts were diarrhea (7%) and rash (7%). G3/4 lab based abnormalities included [CP: thrombocytopenia (T, 14%), neutropenia (N, 9%), anemia (A, 1%) and increased ALT (7%) and advanced phase: T, 71%, A, 32%, N, 46%]. Only 3% of advanced phase pts observed G3/4 pleural effusion. Even pts with blast phase leukemia experienced moderate hem toxicity with G3/4 T in 78% (but 74% at baseline), G3/4 N in 61% (22% at baseline), and G3/4A in 30% (17% at baseline). In 115 I-resistant or intolerant CP-CML pts with no prior exposure to other TKIs, 89% had complete hematologic response (CHR, unconfirmed); 41% had major cytogenetic response (MCyR) and 33% achieved a Major Molecular response. CP pts with exposure to other TKIs had CHR and MCyR rates of 77% and 20%. AP-CML pts exposed to I only had CHR/MCyR rates of 50% and 44% while BP-CML and ALL pts had CHR/MCyR rates of 15% and 18%. Nineteen different Bcr-Abl mutations were detected in 37 CP pts and 13 in 27 advanced phase pts: activity was observed in all of them with the exception of T315I. *Conclusions.* Bosutinib was well tolerated in pts with Ph^+ leukemias, with primarily low-grade gastrointestinal and dermatologic AEs, and no related pleural effusion. Results updated to March 2008 and including response durations will be presented.

0404

NILOTINIB 800 MG DAILY AS FIRST LINE TREATMENT OF CHRONIC MYELOID LEUKEMIA IN EARLY CHRONIC PHASE: RESULTS OF A PHASE 2 TRIAL OF THE GIMEMA CML WORKING PARTY

G. Rosti,[1] F. Castagnetti,[2] F. Palandri,[2] M. Breccia,[3] L. Levato,[4] A. Capucci,[5] M. Tiribelli,[6] G. Alimena,[3] F. Stagno,[6] M. Rondoni,[5] D. Alberti,[7] G. Marzocchi,[2] S. Luatti,[2] M. Amabile,[2] A. Poerio,[2] G. Martinelli,[2] F. Pane,[8] G. Saglio,[9] M. Baccarani[2]

[1]Institute of Hematology "Seràgnoli", BOLOGNA; [2]Dpt. of Hematology-Oncology "L. and A. Seràgnoli", BOLOGNA; [3]Hematology Unit, UNIVERSITY OF ROMA "LA SAPIENZA" ROMA; [4]Division Of Hematology, CATANZARO; [5]Division of Hematology, BRESCIA; [6]Chair of Hematology, UDINE; [7]Novartis Farma, ORIGGIO (VA); [8]Onco-Hematology Unit, University Federico II, NAPOLI; [9]Dpt. of Clinical and Biological Sciences, University of Torino, TORINO, Italy

Background. Imatinib (IM) 400 mg daily is the standard treatment for chronic myeloid leukemia in early chronic phase (ECP): the results of the IRIS trial have shown a 72 months overall survival of 95%; the EFS and PFS were 83% and 93%, respectively. The complete cytogenetic response (CCgR) rate of the IM 400 mg arm was 25% at 3 months (at 6, 12, 18 and 60 months it was 51%, 69%, 76% and 87%, respectively). Nilotinib, a second generation TKI , has a higher binding affinity and selectivity for Abl with respect to IM, being 20 to 50 times more active in imatinib-sensitive cell lines and is highly effective in IM resistant patients, across every disease phase. Among 320 patients in late chronic phase, resistant or intolerant to IM, who received nilotinib 400 mg BID for at least 6 months, the rates of major and complete cytogenetic response were 56% and 40 %, respectively, with very few progressions. Based on these data, nilotinib is currently registered for the treatment of CML patients intolerant or resistant to IM, but it may compete with IM for the frontline treatments of ECP patients. *Aims*. To investigate the therapeutic efficacy and the safety of nilotinib 400 mg BID in ECP, Ph-pos CML patients. *Methods*. An open-label, single stage, multicentric, phase II study (ClinicalTrials.gov. NCT00481052), that was approved by the reference IEC in Bologna, S.Orsola-Malpighi University Hospital and by the IECs of each participating institution, was open to accrual in June, 2007; all patients provided written informed consent. The primary endpoint is the CCgR rate at 1 year; the kinetic of MR is studied by Q-PCR, baseline and after 1, 2, 3, 6, 9 and 12 months form starting the treatment. *Results*. Seventy-three patients have been enrolled from 20 Centres between June, 2007 and February, 2008. The median age was 51 years (range 18-83), 56% low, 32% intermediate and 12% high Sokal risk. Median follow-up is currently 75 days (range 1-210). Thirty-four patients completed 3 months on treatment: 33/34 achieved a CHR; 20/23 evaluable pts (11 pending) reached a MCgR (87%) with 18/23 in CCgR (78%). The MMR rate (BCR-ABL:ABL < 0.1% according to the International Scale) was 52% (13/25 evaluable patients - 9 pending). Adverse events (grade III/IV): hematologic toxicity was recorded in 2 pts (6% - only 1 epysode of grade IV neutropenia); non-hematologic AEs in 4 pts (12% - no grade IV) and biochemical abnormalities in 10 pts (29%, mainly transient increased bilirubin or transaminases - no grade IV). *Conclusions.* The results that have been achieved so far strongly support the hypothesis that in ECP, Ph-pos CML patients the response to nilotinib may be faster than the response to IM.

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0405

A PROSPECTIVE RANDOMIZED STUDY OF IMATINIB 400 MG VS 800 MG AS A FRONTLINE THERAPY IN SOKAL HIGH RISK (HR) PH-POS CHRONIC MYELOID LEUKEMIA (CML) PATIENTS

M. Baccarani,[1] I. Haznedaroglu,[2] K. Porkka,[3] F. Castagnetti,[1] D. Alberti,[4] G. Alimena,[5] M. Amabile,[1] H. Bostrom,[6] H. Hjorth-Hansen,[7] V. Kairisto,[6] G. Martinelli,[1] J. Nielsen,[8] F. Palandri,[1] F. Pane,[9] G. Rege-Cambrin,[10] D. Russo,[11] G. Saglio,[10] G. Specchia,[12] N. Testoni,[1] O. Weiss-Bjerrum,[13] G. Rosti,[1] B. Simonsson[14]

[1]Dpt of Hematology-Oncology Seragnoli, BOLOGNA, Italy; [2]Hematology Unit, Ankara University, ANKARA, Turkey; [3]Hematology Research Unit, Helsinki University Central Hospital, HELSINKI, Finland; [4]Novartis Oncology Italy, ORIGGIO (VA), Italy; [5]Hematology Unit, University of Roma La Sapienza, ROMA, Italy; [6]Dpt Clinical Genetics, UPPSALA UNIVERSITY HOSPITAL, Sweden; [7]Hematology Unit, Trondheim University, TRONDHEIM, Norway; [8]Hematology Unit, Aarhus University, AARHUS, Denmark; [9]Onco-Hematology Unit, University of Napoli Federico II, NAPOLI, Italy; [10]Dpt. of Clinical and Biological Sciences, University of Torino, TORINO, Italy; [11]Hematology Unit, University of Brescia, BRESCIA, Italy; [12]Hematology Unit, University of Bari, BARI, Italy; [13]Dpt Hematology, University Hospital, COPENHAGEN, Denmark; [14]Hematology Unit, Uppsala University, UPPSALA, Sweden

Background. Imatinib mesylate (IM), 400 mg daily, is the drug of choice for the frontline treatment of Ph pos CML. Several biologic findings and clinical observations suggest that increasing the dose of IM may result in improved therapeutic efficacy. The complete cytogenetic response (CCgR) rate to IM 400 mg is significantly affected by Sokal risk (49% in high risk patients at 1 year, IRIS study). Thus, newly diagnosed high risk patients, who account for 20 to 30% of all CML patients, may benefit from a dose increase front-line. *Aims.* To compare the effects of IM, 400 mg or 800 mg daily, in previously untreated, early chronic phase (ECP), Sokal high risk, Ph pos, CML patients. *Methods.* In an international, multicenter, prospective, randomized study (Italy, Scandinavian Countries and Turkey) (Clin. Trials Gov. NCT00514488), 215 patients were enrolled over a 3-year period and were randomized (1:1) to receive IM 400 or 800 mg daily. Cytogenetic response was assessed by chromosome banding analysis and FISH analysis of marrow cells, after 3, 6 and 12 months. Molecular response was assessed by RQ-PCR of blood cells, after 3, 6, and 12 months. The primary efficacy variable was the CCgR rate at 12 months, based on the intention-to-treat. *Results.* As of February 2008, 205/215 patients are evaluable for the primary efficacy variable, 103 in the 400 mg arm, and 102 in the 800 mg arm. The CCgR rates (400 mg *vs* 800 mg) were 20% *vs* 23% after 3 months, 53% *vs* 52% after 6 months, and 60% *vs* 63% after 12 months (primary efficacy variable). Treatment failures (non complete hematologic response or no CgR at 6 months, less than partial CgR at 12 months, or loss of hematologic response or of CgR at any time) were 19/103 (18%) in the 400 mg arm *vs* 16/102 (16%) in the 800 mg arm. In the 400 mg arm, the median administered dose was 400 mg, and there was no difference in outcome between the patients who received the full dose and those who received a dose ranging beteween 300 and 400 mg, with a CCgR rate of 67% and 76% respectively. In the 800 mg arm, the median administered dose was 700 mg, and the CCgR rate was 91% in those who received 600 to 800 mg *vs* 54% in those who received less than 600 mg. Treatment discontinuations for adverse events were 4/103 in the 400 mg arm *vs* 7/102 in the 800 mg arm. Molecular response data are under evaluation and will be presented at the meeting. *Conclusions.* In this international, prospective, randomized study of IM 400 vs 800 mg daily, in a selected, high risk population of patients with Ph pos CML, no difference could be detected overall, according to treatment arm, in the rate of CCgR, of failure, and of adverse events, during the first 12 months of treatment. A dose-

response relationship was observed in the 800 mg arm but not in the 400 mg arm. The patients will be followed for progression-free and overall survival. The data will be available for metanalyses.

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0406

HIGH DOSES OF IMATINIB MESYLATE (800MG/DAY) SIGNIFICANTLY IMPROVE RATES OF MAJOR AND COMPLETE CYTOGENETIC REMISSIONS (MCR, CCR) - RESULTS FROM THE FIRST PLANNED INTERIM ANALYSIS OF A MULTICENTER, RANDOMISED, 2-ARM - PHASE III STUDY COMPARING IMATINIB STANDARD DOSE (400 MG/DAY) WITH IMATINIB HIGH DO

A.P. Andreas,[1] D.W. Wolf,[2] D.F. Fong,[2] T. Lion,[3] I. Dyagil,[4] Z. Masliak,[5] D. Boskovic,[6] G. Laimonas,[7] S. Lejniece,[8] S. Goranov,[9] L. Gercheva,[10] A. Stojanovic,[11] D. Peytchev,[12] N. Tzvetkov,[13] R. Griniute,[14] R. Oucheva,[15] G. Fincato,[16] H. Ulmer,[2] G.G. Gastl[2]

[1]Central European Leukemia Study Group (CELSG), Innsbruck and Hospital BHS Linz, LINZ, Austria; [2]Central European Leukemia Study Group (CELSG), INNSBRUCK, Austria; [3]CCRI Children Cancer Research Institute, VIENNA, Austria; [4]Department of Hematology, RC Radiation Medicine, KIEV, Ukraine; [5]Institute of Blood Pathology and Transfusion Medicine, LVIV, Ukraine; [6]Clinical Center of Serbia, Institut za Hematologiju, BELGRAD, Serbia; [7]Vilnius University Hospital Santariskiu Clinic, VILNIUS, Lithuania; [8]National Center of Haematology, RIGA, Latvia; [9]University Hospital for Active Treatment "St. George", PLOVDIV, Bulgaria; [10]University Hospital for Active Treatment "St. Marina", VARNA, Bulgaria; [11]Clinic for Hematology, National Clinical Center Skopje, SKOPJE, Macedonia; [12]National Center of Hematology and Transfusiology, SOFIA, Bulgaria; [13]University Hospital for Active Treatment, PLEVEN, Bulgaria; [14]Kaunas University Hospital, KAUNA, Lithuania; [15]Alexandrovska University Hospital, SOFIA, Bulgaria; [16]Novartis, ORIGGIO VA, Italy

Background. Recent phase II studies revealed that high doses of Imatinib (800mg/day) are capable to induce higher and earlier rates of cytogenetic and molecular responses, both in second line after failure to interferon alfa and in newly diagnosed chronic phase (CP) CML patients. *Aims.* We wanted to validate these data in a larger cohort of patients performing an international 2 arm phase III trial comparing the standard dose of Imatinib (400mg/day, arm A) with high dose Imatinib (800mg/day) for 6 months, followed by 400mg maintenance (arm B). *Methods.* Between February 2004 and December 2006 a total of 227 pretreated Ph+/BCR-ABL positive CP CML patients were randomized into this 2 arm phase III trial. The first planned interim analysis was performed after 50% of the patients had been treated for 12 months since randomisation. *Results.* There were no significant differences between treatment groups regarding sex (44.5% male, 55.5% female), age (median: 46 years for both groups), and different pretreatments, which included hydroxyurea (96%), interferon (72%), busulfan (17%) and *others* (26%; mainly AraC±combinations). Rates of complete haematological responses did not differ significantly between both arms at 3, 6 and 12 months (mo), respectively (53% arm A vs 59% arm B at 3 mo, 92% *vs* 85% (mo 6), 82% *vs* 90% (mo 12)). After 3 and 6 months, however, during the time where high doses of Imatinib (800mg/day) were applied in arm B, significantly ($p<0.05$) more patients achieved a major cytogenetic response (MCR; 21% arm A *vs* 37% arm B (mo 3); 34% *vs* 54% (mo 6) and a complete cytogenetic response (CCR; 6% arm A *vs* 25% arm B at 3 mo; 20% *vs* 44% at 6 mo). Moreover, significantly ($p<0.05$) more patients achieved a major molecular response (MMR) at 6 months in the Imatinib HD arm B compared to arm A (20% *vs* 7%). At 12 months, following dose reduction of Imatinib to 400 mg/d for *maintenance* at month 6 in the HD arm B, the rates of MCR (the primary endpoint of the study) were comparabel (57% arm A, 59% arm B). Nevertheless, there was still a clear trend to higher rates of CCR (37% arm A *vs* 48% in arm B) and MMR (16% arm A *vs* 21% arm B) in the HD arm, but at the time of the interim analysis these values did not reach statistical significance. In contrast to non-hematological toxicities, grade 3/4 hematological toxicities were significantly more common in the HD arm B (anemia: 2% (arm A) *vs* 14% (arm B); leukopenia: 24% *vs* 46%; thrombocytopenia 15% *vs* 39%). In spite of high rates of grade 3/4 leukopenias in the experimental arm B grade 3/4 infections were low in both arms: 2% (arm A), 3% (arm B). The cumulative median doses of imatinib were 400 mg (arm A) and 767 mg (arm B), respectively. *Conclusions.* These phase III data support the concept of more rapid and higher rates of cytogenetic and molecular remissions when higher doses of imatinib are applied.

Chronic lymphocytic leukemia - Biology and Clinical

0407

SHORT TELOMERE LENGTH IS AN INDEPENDENT PREDICTOR OF SURVIVAL, PROGRESSION, RICHTER'S SYNDROME TRANSFORMATION AND RECURRENT INFECTIONS: AN ANALYSIS ON 421 CLL PATIENTS INCLUDING BLINDED VALIDATION ON 230 CASES

D. Rossi,[1] C. Lobetti Bodoni,[2] E. Genuardi,[2] L. Monitillo,[2] M. Cerri,[1] C. Deambrogi,[1] D. Drandi,[2] I. Ricca,[2] A. Rocci,[2] M. Boi,[2] S. Ferrero,[2] D. Capello,[1] L. De Paoli,[1] L. Bergui,[2] P. Omedè,[2] M. Massaia,[2] M. Boccadoro,[2] G. Gaidano,[1] M. Ladetto[2]

[1]Amedeo Avogadro University of Easten Piedmont, NOVARA; [2]University of Turin, TURIN, Italy

Background. In chronic lymphocytic leukemia (CLL) short telomeres have been associated to poor outcome. However validation on a large series is required to verify if TL is worth entering clinical practice. *Aims.* i) to definitely validate TL as an independent prognostic factor for time to first treatment (TTFT) and overall survival (OS) in a large CLL series (including a large blinded sample); ii) to assess TL impact on other relevant CLL endpoints, including Richter's syndrome (RS) transformation and infection risk. Methods. We used two consecutive series of 421 CLL (historical series n=191 from University of Turin; blinded validation series n=230 from Avogadro University). TL was assessed on PBMC collected at diagnosis by Southern blotting. Results. ROC analysis identified a cut-off point of 4650bp. Median TL was 6000bp (25th-75th:4614-7487bp). TL<4650bp was observed in 100/387 (25.8%) CLL. TL<4650bp associated with advanced Binet stage ($p=.003$), IGHV-homology>98% ($p<0.001$), del11q22-q23 ($p=.009$), CD38>30% ($p=.018$). Univariate log-rank analysis (UA) identified TL<4650bp as a risk factor (RF) of short TTFT (19.2 months *vs* 85.2 months; $p<0.001$), along with Binet B-C, IGHV-homology >98%, CD38>30%, ZAP70>20%, del11q22-q23, del17p13, +12, and absence of del13q14 ($p<.001$ in all instances). Multivariate analysis selected TL<4650bp as an independent TTFT predictor (HR:2.04; $p=.002$).

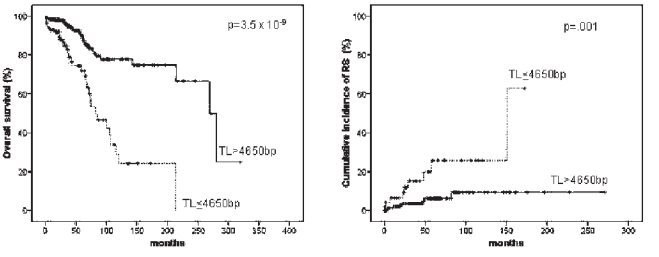

Figure 1.

UA identified TL<4650bp as a RF of short OS (82.2 *vs* 269.3 months; $p=3.5\times10^{-9}$ Figure 1A), along with age>65y, Binet B-C, IGHV-homology >98%, and del17p13 ($p<0.001$ in all instances). Multivariate analysis selected TL<4650bp as an independent OS predictor (HR:3.15; $p=<0.001$). TL<4650bp consistently identified a subset of patients with short TFS and OS, despite harboring favorable predictors as documented by bivariate log-rank test . Indeed, TL<4650bp segregated a CLL group displaying short TTFT despite being characterized by Binet A (1.7×10^{-8}), IGHV-homology<98% ($p=1.6\times10^{-10}$), CD38<30% ($p=1.4\times10^{-8}$), ZAP70<20 ($p=3.3\times10^{-11}$), and normal FISH or del13q14 only ($p=4.1\times10^{-11}$). Also, TL<4650bp segregated a CLL group displaying short OS despite having age<65y ($p=1.4\times10^{-7}$), Binet A ($p=0.001$), IGHV-homology <98% ($p=1.4\times10^{-6}$), and del17p13 absence ($p=6.2\times10^{-5}$). Results were superimposable in the historical and blinded validation series. The validation series (n=230) allowed the investigation of additional outcomes, namely RS transformation and infection risk. UA identified TL<4650bp as a RF for RS (5-year risk: TL<4650bp 35.8% *vs* TL>4650bp 6.3%; $p=0.001$. Multivariate analysis selected TL<4650 (HR:4.1, $p=0.007$) (Figure 1B) and lymphadenopathy >3cm (HR: 7.4, $p=2.1\times10^{-4}$) as independent predictors of RS. UA identified TL<4650bp as a RF of short time to recurrent infections (TL<4650bp 3.0 months vs TL>4650bp 26.3 months; $p=1.9\times10^{-4}$). Multivariate analysis identified TL<4650bp (HR:6.35; $p=0.002$) and IGHV-homology >98% (HR: 3.51;

p=0.007) as independent predictors of recurrent infections. Conclusions. This is the largest TL analysis so far in CLL and is provided with an independent and blinded validation cohort. The implications of our results are multifold. First, short TL is validated as an independent OS and TFS predictor. Second, short TL identifies a CLL subgroup with rapid disease progression and short survival despite the presence of favorable predictors. Third, short TL independently predicts RS and recurrent infections. Because short TL are the sole independent predictor of all CLL outcomes, we advise its more widespread clinical use.

0408

THE MOST FREQUENT T(14;19)(Q32;Q13)-POSITIVE B-CELL MALIGNANCY CORRESPONDS TO AN AGGRESSIVE SUBGROUP OF ATYPICAL CHRONIC LYMPHOCYTIC LEUKEMIA

F. Nguyen-Khac,[1] E. Chapiro,[1] I. Radford-Weiss,[2] C. Bastard,[3] I. Luquet,[4] C. Lefebvre,[5] E. Callet-Bauchu,[6] D. Leroux,[5] P. Talmant,[7] M.-J. Mozziconacci,[8] F. Mugneret,[9] S. Struski,[10] S. Raynaud,[11] J. Andrieux,[12] C. Barin,[4] M. Jotterand,[13] H. Mossafa,[14] S. Ramond,[15] C. Terré,[9] E. Lippert,[9] F. Berger,[16] P. Felman,[17] H. Merle-Béral,[1] O.A. Bernard,[18] F. Davi,[1] R. Berger[18]

[1]Pitie-Salpetriere, PARIS, France; [2]Necker-Enfants Malades, PARIS, France; [3]H Becquerel, ROUEN, France; [4]Genetique, REIMS, France; [5]Cytogenetique Onco-Hematologique, GRENOBLE, France; [6]Hematologie Biologique, LYON, France; [7]Oncohematologie, NANTES, France; [8]Paoli-Calmettes, MARSEILLE, France; [9]Cytogenetique, DIJON, France; [10]Hematologie, STRASBOURG, France; [11]Pasteur, NICE, France; [12]Genetique Medicale, LILLE, France; [13]Cytogenetique du Cancer, LAUSANNE, Switzerland; [14]Pasteur-Cerba, CERGY PONTOISE, France; [15]Hotel-Dieu, PARIS, France; [16]Anatomopathologie, LYON, France; [17]Hematologie biologique, LYON, France; [18]Inserm E210, PARIS, France,

Translocation t(14;19)(q32;q13) involving BCL3 and IGH was first reported in chronic lymphocytic leukemia (CLL) and then, together with variant BCL3-translocations, in other B-neoplasms. The Groupe Francophone de Cytogenetique Hematologique (GFCH) collected 43 chronic lymphoproliferative disorders with t(14;19) or variant in order to analyze to what extent this uncommon abnormality could define a subgroup among B-cell malignancies, and to document the consequences of the rearrangement. Clinical and biological patients' informations were gathered at diagnosis when available. The cytological, histopathological and immunological reviews were centralized and performed for 30/43 cases. All conventional cytogenetic data were reviewed by the GFCH. Detection of IG and BCL3 rearrangement, and recurrent abnormalities frequent in CLL were performed by hybridation *in situ* fluorescence. Genes expression was measured by RQ-PCR and IGHV mutation analysis were performed using published techniques. In our series the diagnosis felt more frequently in the CLL/SLL type (WHO classification) (n=30, 70%), with however atypical features when compared to common CLL: heterogeneous mixture of cells (70%) and signs of disease progression (80%), Matutes score < 4 (78%), high frequency of trisomy 12q and 6q deletion (67% *vs* 13.6-16%, p<10-11 and 19% *vs* 4.6-6%, p<0,02 respectively), low incidence of 13q deletion (14% *vs* 55-57.4%, p<10-5), high frequency of unmutated status of IGHV (90% *vs* 46%, p<0,001) and over-representation of the V4-39/D6-13/JH5 repertoire (25% *vs* 0.9-1.2%, p<0,001). BCL3 expression was also increased in all studied cases (median: 11.5% *vs* 2.9%, p=0.002). There were 64% Binet stage A and 36% stages B/C. The median time from diagnosis to initial therapy was 10 months for A stages, 1.3 months for all CLL stages, 2.3 months regarding CLL with trisomy 12, which was very short compared to the median treatment-free intervals for common CLL groups with 17p deletion (9 months), 11q deletion (13 months), 12q trisomy (33 months) and 13q deletion as the sole abnormality (92 months) reported in the literature. Finally, while t(14;19)(q32;q13) was more frequent in CLL, it may be observed in various types of B-cell lymphomas, mostly in marginal zone lymphomas (n=6, 14%). *Conclusions*. t(14;19)-positive CLL deserves to acquire the status of particular agressive subgroup defined by cell polymorphism and signs of disease progression, $CD5^+$ immunophenotype and Matutes score < 4, trisomy 12 and 6q deletion associated with t(14;19), unmutated IGVH status with an over-representation of the V4-39/D6-13/JH5 repertoire, and high BCL3 expression. These observations indicate the deregulation of the NFkB pathway in mature B-cell malignancies which could represent a therapeutic target.

0409

CD49D EXPRESSION IS AN INDEPENDENT RISK FACTOR OF PROGRESSIVE DISEASE IN EARLY STAGE CHRONIC LYMPHOCYTIC LEUKEMIA

D. Rossi,[1] A. Zucchetto,[2] F.M. Rossi,[2] D. Capello,[1] M. Cerri,[1] C. Deambrogi,[1] S. Cresta,[1] S. Rasi,[1] L. De Paoli,[1] C. Lobetti Bodoni,[3] P. Bulian,[2] G. Del Poeta,[4] M. Ladetto,[3] V. Gattei,[2] G. Gaidano[1]

[1]Division of Hematology, Amedeo Avogadro University, NOVARA; [2]Clinical and Experimental Onco-Hematology Unit, CRO, AVIANO; [3]Division of Hematology, University of Turin, TURIN; [4]Department of Hematology, University of Tor Vergata, ROMA, Italy

Background. Chronic lymphocytic leukemia (CLL) is a markedly heterogeneous disease. This notion is best exemplified by the variability in time to progression and in survival of Binet A CLL. The identification of prognostic subgroups within Binet A CLL is currently a major challenge. CD49d represents a novel CLL prognosticator, whose value in Binet A patients is unknown. *Aims*. This study aimed at verifying whether CD49d expression may further refine the prognostic stratification of Binet A CLL. Methods. The study was based on a consecutive series of 140 Binet A CLL representative of this disease stage and provided with a large and homogeneous dataset of biological and clinical variables. CD49d expression was assessed on PBMC collected at CLL diagnosis by three-color flow cytometry. A cut-off point of 30% was utilized to define positivity. *Results*. At diagnosis, CD49d>30% was observed in 54/140 (38.6%) Binet A CLL. CD49d>30% associated with markers of proliferating CLL, namely CD38>30% (p=3.9×10^{-6}), short telomere (p=0.021), high LDH (p=0.007) and β-2-microglobulin (p=.020). CD49d expression was not associated ($p>0.05$ in all cases) with IGHV homology, del11q22-q23 or del17p13, number of FISH lesions,TP53 inactivation by deletion and/or mutation, or ZAP70 expression. Also, CD49d expression was not associated ($p>0.05$ in all cases) with clinical markers of tumor burden. Univariate log-rank analysis identified CD49d>30% as a risk factor of progressive disease, in terms of time to progression (CD49d>30% 38.0 months *vs* CD49d<30% 66.7 months; p=4.7×10^{-4}), treatment free survival (TFS) (CD49d>30% 50.2 months *vs* CD49d<30% not reached; p=8.3×10^{-5}), and time to lymphocyte doubling (CD49d>30% 28.2 months *vs* CD49d<30% 53.0 months; p=.009). Multivariate analysis selected CD49d>30% as an independent TFS predictor after adjustment for biological (HR 2.39, p=0.019), clinical (HR 4.34, p=6.4×10^{-5}), and both biological and clinical variables analysed together (HR 2.59, p=0.032). Within Binet A subgroups harboring favorable predictors, CD49d>30% consistently identified a subset of patients with short TFS, as documented by bivariate log-rank test. Indeed, among patients harboring favorable biological predictors, CD49d>30% segregated a group of CLL displaying short TFS despite being characterized by IGHV homology <98% (p=.007), number of FISH lesions <2 (p=1.3 ×10^{-4}), normal FISH or del13q14 only (p=0.005), wild type TP53 (p=1.6 ×10^{-4}), telomere length >4250 bp (p=0.004), CD38<30% (p=0.005), or ZAP70<20% (p=0.003). Also, among patients harboring favorable clinical predictors, CD49d>30% identified a group of CLL displaying short TFS despite being characterized by Rai 0 (p=0.001), absence of splenomegaly (p=2.2×10^{-4}), lymphocytes <20 ×10^9/l (p=0.002), Hb >13 g/dl (p=2.7×10^{-5}), platelets >150×10^9/l (p=2.1×10^{-4}), BM lymphocytes <50% (p=0.009), non-diffuse BM pattern (p=0.001), β-2-microglobulin <2.5 mg/l (p=0.003), and LDH <1 x ULN (p=0.007). Conclusions. CD49d>30% is a marker of disease proliferation and dissemination in Binet A CLL. Our observations, along with the easiness of CD49d assessment, indicate CD49d>30% as a new marker for the initial prognostic assessment of Binet A CLL. In particular, CD49d>30% is useful for the identification a subgroup of Binet A CLL that displays rapid disease progression and need of treatment, despite being characterized at diagnosis by favorable predictors.

0410

TP53 MUTATIONS AND DEL17P13 PREDICT SIMILAR OUTCOME AND CHEMOREFRACTORINESS IN CHRONIC LYMPHOCYTIC LEUKEMIA

M. Cerri,[1] C. Deambrogi,[1] D. Capello,[1] S. Cresta,[1] S. Rasi,[1] E. Sozzi,[2] L. De Paoli,[1] V. Gattei,[3] F. Forconi,[2] G. Gaidano,[1] D. Rossi[1]

[1]Division of Hematology, Amedeo Avogadro University, NOVARA; [2]Division Of Hematology and Bone Marrow Transplantation, University of Siena, SIENA; [3]Clinical and Experimental Onco-Hematology Unit, C.R.O., AVIANO, Italy

Background. Chronic lymphocytic leukemia (CLL) harboring del17p13 displays a dismal prognosis and a high risk of chemorefractoriness. The tumor suppressor TP53 is located on chromosome band 17p13 and loss

of TP53 is thought to be responsible for the poor prognosis of del17p13 CLL. Aims. This study aimed at verifying whether TP53 inactivation through mutation harbors the same prognostic role as del17p13. Methods. The study was based on a consecutive series of 224 CLL provided with a large and homogeneous dataset of biological and clinical variables. TP53 mutation status was assessed on PBMC collected at CLL diagnosis by direct sequencing of TP53 exons 2 to 10. *Results.* TP53 mutations were observed in 23/224 (10.3%) CLL, and included missense mutations in 20/23 (86.9%) cases, and insertions, short deletions and splicing site mutations in 1/23 (4.3%) cases each. TP53 mutations affected exon 4 in 1/23 (4.3%) cases, exon 5 in 4/23 (17.3%), exon 6 in 4/23 (17.3%), exon 7 in 5/23 (21.7%), and exon 8 in 9/23 (39.1%). No mutations were found in exons 2, 3, 9 or 10. Mutations targeted the TP53 DNA-binding domain in 21/22 cases. Functional codons targeted by mutations were TP53 DNA-binding codons (5/22) and zinc-ligand codons (2/22). All mutations predicted a reduction of trans-activation activity of the mutated p53 protein (median residual activity: 6.44% by http:\\p53.free.fr/Database/p53_recomendations.html). FISH karyotype was available in 213/224 cases. Overall, 29/213 (13.5%) CLL harbored TP53 inactivation through deletion and/or mutation: 11/213 (5.1%) CLL were TP53-mutated/del17p13, 10/213 (4.6%) were TP53-wt/del17p13, and 8/213 (3.7%) were TP53-mutated/no del17p13. Median residual TP53 trans-activation activity was similar between the TP53-mutated/no del17p13 and the TP53-mutated/del17p13 subgroups (9.54% *vs* 10.30% respectively; p=0.224). Univariate log-rank analysis identified TP53 mutations as a risk factor of short treatment free survival (TFS) (TP53-mutated: 17.2 months *vs* TP53-wt: 87.5 months; p=0.006), overall survival (OS) (TP53-mutated: 91.7 months *vs* TP53-wt: not reached; $p=1.1\times10^{-5}$), time to chemorefractoriness (TP53-mutated: 6.3 months *vs* TP53-wt: 75.4 months; $p=4.4\times10^{-5}$), time to alkylator refractoriness (TP53-mutated: 9.3 months *vs* TP53-wt: 68.0 months; p=0.002) and time to fludarabine refractoriness (TP53-mutated: 6.3 months *vs*TP53-wt: 82.1 months; $p=4.0\times10^{-4}$). TFS, OS and time to chemorefractoriness did not differ between CLL harboring TP53 mutations in the absence of del17p13 and CLL harboring del17p13 (TFS: TP53-mutated/no del17p13: 16.1 months, TP53-wt/del17p13: 1.0 months, TP53-mutated/del17p13: 18.3 months; OS: TP53-mutated/no del17p13: 94.7 months, TP53-wt/del17p13: 56.0 months, TP53-mutated/del17p13: 91.7 months; time to chemorefractoriness: TP53-mutated/no del17p13: 5.7 months, TP53-wt/del17p13: 55.0 months, TP53-mutated/del17p13: 5.5 months) (p>0.05 for all comparison). Multivariate analysis selected TP53 mutation as an independent predictor of TFS (HR:2.48; p=0.21), OS (HR:3.48; p=0.002), and chemorefractoriness (HR:4.20; p=0.001) after adjustment for IGHV gene homology, CD38 expression, FISH karyotype, and exposure to fludarabine. *Conclusions.* Mutation in the absence of del17p13 is the sole mechanism of TP53 inactivation in ~4% CLL. TP53 mutations carry the same prognostic relevance as del17p13 in terms of CLL progression, survival and risk of chemorefractoriness. Because of the practical implications for choice of therapy, screening for TP53 mutations, in addition to del17p13 assessment, should be included in the initial prognostic assessment of CLL.

0411

CLINICAL AND BIOLOGICAL CHARACTERIZATION OF CIRCULATING ENDOTHELIAL CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA

G.M. Rigolin,[1] R. Maffei,[2] L. Rizzotto,[1] M. Ciccone,[1] O. Sofritti,[1] G. Daghia,[1] F. Cibien,[1] F. Cavazzini,[1] R. Marasca,[2] G. Castoldi,[1] A. Cuneo[1]

[1]Hematology - University of Ferrara, FERRARA; [2]Hematology Section - COM - University of Modena, MODENA, Italy

Background. Several studies have shown that bone marrow-derived endothelial cells (EC) may contribute to tumor angiogenesis and that in the peripheral blood of cancer patients there is an increased amount of circulating ECs (CECs) that may participate to new vessel formation. Recent data also showed that in B-cell neoplasms ECs are in part tumor-related reflecting a novel aspect of tumor angiogenesis. All together these observations suggest that tumors can elicit the sprouting of new vessels from existing capillaries through the secretion of angiogenic factors and that, in some cases, cancer cells can also mimic the activities of ECs by participating in the formation of vascular-like networks. *Aims*. To characterize the clinical and biological role of CECs in a series of 85 chronic lymphocytic leukemia (CLL) patients. *Methods*. CEC levels were evaluated by multiparameter flow cytometry and correlated with known clinical and biological parameters. For biological studies, CECs were first isolated by immunomagnetic sorting and then characterized by phenotypic studies with antibodies recognizing endothelial and CLL antigens, by FISH analyses with specific probes and by gene expression profiling comparing CLL CECs with CECs from normal subjects, and with monocytes and lymphocytes from the same CLL patient. *Results*. CEC levels were significantly higher in CLL patients in comparison to normal healthy subjects (p=0.037). Higher CEC levels were associated with advanced disease stage (p=0.012) and with lack of response to treatment or progressive disease (p=0.005). No association was demonstrated with CD38/ZAP70 expression and FISH/cytogenetic abnormalities. In all experiments more than 95% of immunomagnetically sorted cells were of EC origin as demonstrated by phenotypic analyses (VEGFR2$^+$, vWF$^+$, CD144$^+$, UEA1 lectin$^+$, CD45$^-$, CD14$^-$, CD5$^-$, CD19$^-$). FISH analysis showed that a significant proportion of sorted CECs was tumor-derived because they harbored the same genetic lesion as observed in neoplastic CLL cells. The fraction of CECs showing cytogenetic aberrations averaged 40.7% (range, 20-78%). More then 85% of CECs presented features of EPCs because they expressed CD133, a marker gradually lost during EC differentiation and absent in mature ECs. CLL CEC had a similar gene expression pattern for several genes characterizing CEC function such as CD144, CD34, CD133, CD146, CD31, VEGFR2, VEGFR3, VWF, and TIE2. Moreover, CLLCEC showed a strongly different gene expression pattern compared to normal CEC characterised by increased cell survival and proliferation including activation of Wnt and inhibition of Notch signalling pathways, reduction of cell adhesion to extracellular matrix and enhanced pro-angiogenic function. Gene expression profiling analysis also suggested that similarities exist with clonal CLL lymphocytes. *Conclusions*. These findings suggest that in CLL CECs are in part tumor related and with a gene expression profile that may indicate their contribution to tumor neovasculogenesis and possibly to the spreading and progression of the disease.

Stem cell transplantation in multiple myeloma

0412

NON-MYELOABLATIVE ALLOGRAFTING FOR NEWLY DIAGNOSED MYELOMA: FINAL RESULTS OF A PROSPECTIVE PHASE II STUDY BY GRUPPO ITALIANO TRAPIANTO MIDOLLO OSSEO

L. Giaccone,[1] R. Sorasio,[1] F. Patriarca,[2] D. Mattei,[3] B. Allione,[4] F. Carnevale Schianca,[5] A. Rambaldi,[6] M. Casini,[7] V. Montefusco,[8] M. Parma,[9] P. Bavaro,[10] F. Onida,[11] A. Busca,[12] L. Castagna,[13] A.P. Iori,[14] E. Benedetti,[15] N. Mordini,[3] M. Rotta,[1] F. Fiore,[1] A. Filippi,[16] A.P. Palumbo,[1] I. Resta,[1] M. Festuccia,[1] M. Aglietta,[5] A. Levis,[4] R. Foà,[14] P. Di Bartolomeo,[10] E. Pogliani,[9] G. Lambertenghi-Deliliers,[11] M. Falda,[12] M. Petrini[15]

[1]Divisione di Ematologia dell'Università di Torino, AOU San Giovanni Battista, TORINO; [2]Divisione di Ematologia, Università di Udine, UDINE; [3]Divisione di Ematologia, Azienda Ospedaliera Santa Croce e Carle, CUNEO; [4]Divisione di Ematologia, Azienda Ospedaliera SS Antonio e Biagio e Arrigo, ALESSANDRIA; [5]Divisione di Ematologia IRCC, CANDIOLO, TORINO; [6]Divisione di Ematologia, Ospedali Riuniti, BERGAMO; [7]Divisione di Ematologia, Ospedale Regionale, BOLZANO; [8]Divisione di Ematologia, Istituto Nazionale Tumori, MILANO; [9]Divisione di Ematologia, Azienda Ospedaliera San Gerardo, MONZA; [10]Divisione di Ematologia, Ospedale di Pescara, PESCARA; [11]Divisione di Ematologia, Ospedale Maggiore, MILANO; [12]Divisione Ospedaliera di Ematologia, AOU San Giovanni Battista, TORINO; [13]Clinical Humanitas, ROZZANO; [14]Divisione di Ematologia, Università La Sapienza, ROMA; [15]Divisione di Ematologia, Università di Pisa, PISA; [16]Divisione di Radioterapia, AOU S. Giovanni Battista, TORINO; [17]Università di Torino, TORINO; [18]Divisione di Ematologia dell'Università di Torino, AOU San Giovanni Battista, TORINO, Italy,

Background. The dramatic reduction of transplant-related mortality (TRM) with nonmyeloablative conditionings has extended the eligible age for transplantation up to 65-70 years. Therefore the role of allografting in the treatment of multiple myeloma has to be redefined. *Aims.* At 15 Italian Centers, patients younger than 65 years with newly diagnosed multiple myeloma were enrolled in a prospective phase II study to evaluate the effect of non-myeloablative allografting on overall (OS) and event-free (EFS) survivals. From January 2000 to June 2005, 106 patients entered the study. Induction chemotherapy consisted of VAD-based regimens, followed by cyclophosphamide and growth factor mobilized peripheral blood stem cell harvest. To improve disease control an autograft with melphalan 200 mg/m^2 was planned 2-4 months before a low-dose (2 Gy) TBI-based allograft from HLA-identical siblings. Graft-*vs*-host disease (GVHD) prophylaxis included cyclosporin and mycophenolate mofetil. *Results.* One-hundred-two (96%) patients, median age 54 (30-65), completed the program whereas 4 withdrew their consent. Incidence of acute grade II-IV GHVD was 40% (4% grade IV), while chronic GVHD occurred in 52%. Cox models for the development of GVHD showed that the infusion of higher doses of donor CD34$^+$ cells correlates with higher incidence of acute, but not chronic, GVHD (HR 1.11, CI 1.03-1.19, p=0.004). Overall TRM was 14%. Fifteen % of patients died from disease progression, 3 from secondary tumor (3%). After a median follow-up of 5 (2.3-8.4) years, OS was not reached and median EFS was 37 (31-54) months post-transplant. Overall response, complete (CR) plus partial remission, was 91% (93/102), with 54 patients achieving CR. Disease relapse occurred in 45/102 patients, including 14/54 of those who reached CR post-transplant. By multivariate-analysis the achievement of at least a very good partial remission prior to allografting was significantly associated with longer OS (HR 0.24,CI 0.08-0.72, p=0.01) and longer EFS (HR 0.34,CI 0.18-0.65, p<0.01). Interestingly, chronic GVHD was not correlated with either the achievement of post-transplant CR (HR 0.88,CI 0.47-1.65, p=0.7) or its duration (HR 0.94,CI 0.55-1.6, p=0.8). Presence of del(13) was evaluated only in a subset of 39 patients: 13 carried del(13) and 26 did not. OS was not reached in the patients without del(13) and was 4.3 years in patients with del(13) (p=0.18). Summary and *Conclusions.* After 5 years of follow-up, this study shows that a tandem auto-allo approach is a treatment option for newly diagnosed multiple myeloma. Given the significant importance of pre-transplant disease reduction to enhance graft-versus-myeloma, a new multicenter trail that incorporates new drugs in the induction phase is currently in progress.

0413

REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION (RIC ALLO-SCT) FOR PATIENTS WITH HIGH-RISK MULTIPLE MYELOMA (MM): A SURVEY FROM THE SOCIETE FRANCAISE DE GREFFE DE MOELLE ET DE THERAPIE CELLULAIRE

M. Mohty,[1] L. Quoc-Hung,[2] F. Nicolini,[2] F. Garban,[3] H. Esperou,[4] M. Attal,[5] N. Milpied,[6] B. Lioure,[7] P. Bordigoni,[8] I . Yakoub-Agha,[9] J.H. Bourhis,[10] B. Rio,[11] E. Deconinck,[12] M. Renaud,[13] J.L. Harousseau,[1] D. Blaise,[14] M. Michallet[2]

[1]CHU de Nantes, NANTES; [2]CHU de Lyon, LYON; [3]CHU de Grenoble, GRENOBLE; [4]Agence de Biomedecine, PARIS; [5]CHU de Toulouse, TOULOUSE; [6]CHU de Bordeaux, BORDEAUX; [7]CHU de Strasbourg, STRASBOURG; [8]CHU de Nancy, NANCY; [9]CHU de Lille, LILLE; [10]Institut Gustave Roussy, PARIS; [11]CHU Hotel-Dieu, PARIS; [12]CHU de Besancon, BESANCON; [13]CHU de Poitiers, POITIERS; [14]Institut Paoli-Calmettes, MARSEILLE, France

The results of RIC allo-SCT for MM are still under considerable debate. While EBMT data did not support the universal use of RIC for MM allografts, the Italian randomized multicenter study suggested that in newly diagnosed myeloma, survival in recipients of a hematopoietic stem-cell autograft followed by RIC allo-SCT from an HLA-identical sibling is superior to that in recipients of tandem stem-cell autografts. The aim of this multicenter retrospective national study was to identify prognostic factors for outcome of high-risk patients with MM after allo-SCT prepared by RIC. Data from 219 patients (median age 52 years, range 27-66), who received grafts from a sibling (n=197) or unrelated donor (n=22) were analyzed. At time of transplant, only 37 patients (17%) received RIC allo-SCT in CR or VGPR, while 134 patients (61%) were transplanted in PR. 48 patients were transplanted either in stable disease (n=15) or were in refractory/progressive disease (n=33). All patients have received at least one autologous transplant prior to RIC allo-SCT. The graft source was PBSCs in the majority of patients (n=183). 21% of the patients received the Seattle Fludarabine and low dose TBI RIC regimen, while 53% of patients received Fludarabine, Busulfan and ATG. 32 patients (15%) died of transplant-related complications. The incidences of grade 2-4 acute GVHD and extensive chronic GVHD were 37% and 20% respectively. At 3 years, overall and progression free survivals (OS, PFS) were 41% (95%CI, 34-49) and 19% (95%CI, 14-27) respectively. Disease status (CR, PR, SD *vs* progressive) was significantly associated with overall survival (p=0.0002; Figure 1). In multivariate analysis, disease status at time of RIC allo-SCT, was the strongest parameter associated with an improved OS and PFS (p=0.005 and p=0.004 respectively). Despite its obvious caveats, the relatively low TRM observed in this series, suggest that there is still space to investigate RIC allo-SCT for MM. However, RIC allo-SCT appears to result in a durable response only if it is applied early in the disease history, especially when patients are still chemosensitive. Since the latter results are also expected to be further improved with the systematic and early use of maintenance therapies (Bortezomib and/or Lenalidomide) after RIC allo-SCT, randomized or quasirandomized prospective studies are still warranted.

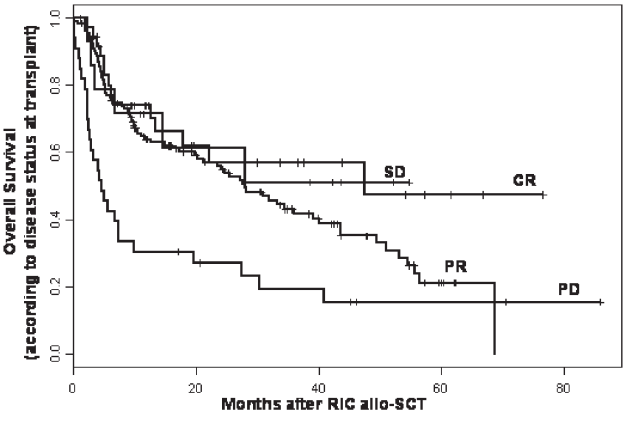

Figure 1.

0414

UPDATED ANALYSIS OF ALLOGENEIC HSCT AFTER RIC FOR HEMATOLOGICAL MALIGNANCIES FROM THE SOCIÉTÉ FRANÇAISE DE GREFFE DE MOELLE OSSEUSE ET DE THÉRAPIE CELLULAIRE (SFGM-TC) REGISTRY

M. Michallet,[1] M. Michallet,[2] Q. Le,[2] M. Mohty,[3] M. Sobh,[2] F.E. Nicolini,[2] J.-M. Boiron,[3] H. Espérou,[3] M. Attal,[3] N. Milpied,[3] B. Lioure,[3] P. Bordigoni,[3] I. Yackoub-Agha,[3] J. Bourhis,[3] B. Rio,[3] E. Deconninck,[3] M. Renaud,[3] N. Raus,[2] D. Blaise[3]

[1]Hôpital Edouard Herriot, LYON; [2]Edouard Herriot Hospital, LYON; [3]Société Française de Greffe de Moelle et de Thérapie Cellulaire, PARIS, France

This report updates a retrospective study from SFGM-TC registry concerning 1108 patients who underwent allogeneic hematopoeitic stem cell transplantation (HSCT) after reduced intensity conditioning (RIC) from HLA identical siblings (84%) and unrelated donors (16%) for hematological malignancies. At time of conditioning, 442 patients were in CR, 337 in PR, 107 in stable disease (SD) and 222 in progressive disease (PD). As conditioning, 255 patients received fludarabine and TBI (2 grays), 465 patients fludarabine, busulfan and ATG and 388 patients an other regimen. After transplant, 336 patients (30%) developed an acute GVHD grade II (grade II: 178, III: 80 and IV: 78). A chronic GVHD was present in 388 patients (35%) (185 limited and 203 extensive). With a median follow-up of 30 months, the 3 and 5-year probability of overall survival (OS) were 43.5% (40-47) and 32%(29-35) respectively and the 3 and 5-year probability of event-free survival (EFS) were 35%(31-39) and 28% (24.5-31) respectively. The TRM at 1 year, 2 years and 3 years was 15% (13-17), 18% (15.5-21) and 20% (17-23).

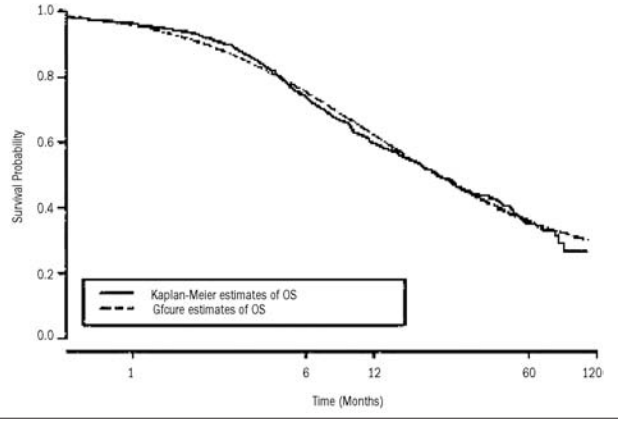

Figure 1.

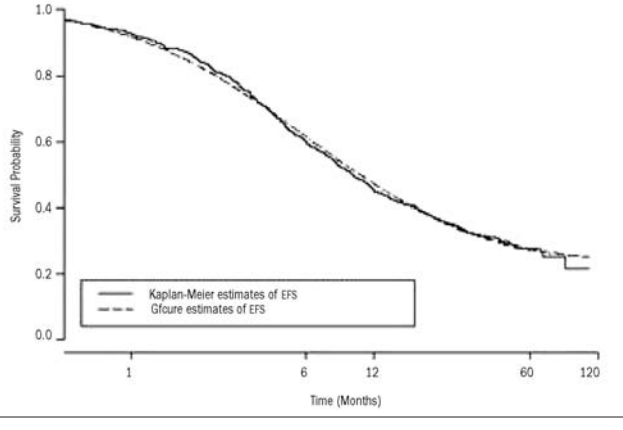

Figure 2.

A mixture model, gfcure with Splus statistical package determined the percentages of long-term survivors and its adequacy was verified graphically. The probability to be a long-survivor was 24% (17.5-32.5) (Figure 1) and to be a long event-free survivor was 23% (19-28) (Figure 2). The multivariate analysis has tested recipient and donor age, disease status pre-transplant, number of transplants before RICT, HSC source, sex matching, HLA matching, CMV status and ABO compatibility. The only factor which had a significant impact on long-term survival after RICT was the disease status just prior conditioning: PR *vs* CR: HR: 3.63 [1.14-9.18] $p<0.001$ and PD *vs* CR: HR: 4.35 [2.22-8.51] $p<0.0001$. In conclusion, these updated data demonstrate that allogeneic HSCT after RIC was able to possibly cure 23% of patients with haematological malignancies and the most important factor to take into account remains to be in CR pre-transplant.

0415

A PROSPECTIVE PETHEMA STUDY OF TANDEM AUTOLOGOUS TRANSPLANTATION *vs* AUTOGRAFT FOLLOWED BY REDUCED-INTENSITY CONDITIONING ALLOGENEIC TRANSPLANTATION IN NEWLY DIAGNOSED MULTIPLE MYELOMA

J. Blade,[1] L. Rosiñol,[1] J.A. Pérez-Simón,[2] A. Sureda,[3] J. De la Rubia,[4] F. De Arriba,[5] J.J. Lahuerta,[6] J.D. González,[7] R. Martínez-Martínez,[8] B. Hernández-Ruíz,[9] J. García-Frade,[10] D. Carrera,[11] A. León,[12] M. Hernández,[13] P. Fernández-Abellán,[14] J.M. Bergua,[15] J. San Miguel[16]

[1]Hospital Clinic, BARCELONA; [2]H.Clinico, SALAMANCA; [3]H. Sant Pau, BARCELONA; [4]H. La Fe, VALENCIA; [5]H. Morales Messeguer, MURCIA; [6]H. 12 de Octubre, MADRID; [7]H. Materno-Insular, LAS PALMAS GRAN CANARIA; [8]H. Clinico San Carlos, MADRID; [9]H. Gneral Ciudad Real, CIUDAD REAL; [10]H. Rio Hortega, VALLADOLID; [11]H. Asturias, OVIEDO; [12]H. Jerez de la Frontera, JEREZ DE LA FRONTERA; [13]H. Universitario Canarias, STA. CRUZ TENERIFE; [14]H.Alicante, ALICANTE; [15]H. San Pedro Alcántara, SAN PEDRO DE ALCÁNTARA; [16]H. Clinico Universitario, SALAMANCA, Spain

Background. Two randomized trials showed that tandem autologous transplant (ASCT) results in a significantly longer EFS and OS in patients failing to achieve CR or near-CR with a single transplant, but there was no survival plateau. Promising results have been reported using dose-reduced intensity conditioning (Allo-RIC), especially after debulky with an autologous transplant. *Aims.* To investigate the efficacy in terms of response and survival from a second transplant intensification (2nd ASCT *vs* Allo-RIC) in patients with chemosensitive disease who failed to achieve CR or near-CR with a first ASCT. Patients and *Methods.* Patients diagnosed with MM from Oct 1999 to Dec 2004 younger than 70 years received 6 courses of VBMCP/VBAD and responding patients were intensified with busulphan/melphalan or MEL-200 followed by stem cell support. Patients not achieving CR or near-CR were planned to receive a second transplant (2nd ASCT with CVB - cyclophosphamide, etoposide and BCNU - or MEL-200 intensification or an Allo-RIC with fludarabine/MEL-140 conditioning, if sibling donor available). *Results.* Eighty-five patients received a 2nd ASCT while 25 underwent an Allo-RIC. The CR rate was significantly higher with allo-RIC (40% *vs* 11%, $p= 0.001$). There was a trend towards a higher TRM with the allogeneic procedure (5% *vs* 16%, $p=0.07$). The incidence of grade II-IV aGVHD was 32% and 14 of 21 (66%) patients at risk developed cGVHD. After a median follow-up from the second transplant of 5.2 years, there was a trend towards a longer progression-free survival (median 31 mos *vs* not reached, $p=0.08$) in favour of Allo-RIC with a plateau for allografting and none of the 10 patients who achieved CR after Allo-RIC ha relapsed. The overall survival was not significantly different (median 58 mos. Vs not reached, $p=0.9$), although there was a plateau for the Allo-RIC group not observed with 2nd ASCT. *Conclusions.* In patients with MM failing to achieve at least nCR with a first ASCT, the Allo-RIC offers more long-term benefit than a second ASCT.

0416

THE HEMATOPOIETIC CELL TRANSPLANTATION-SPECIFIC COMORBIDITY INDEX (HCT-CI) PREDICTS SURVIVAL AND NON-RELAPSE MORTALITY IN LYMPHOMA AND MYELOMA PATIENTS RECEIVING RIC ALLOGRAFT

L. Farina,[1] B. Bruno,[2] F. Patriarca,[3] F. Spina,[1] R. Sorasio,[2] M. Morelli,[1] R. Fanin,[3] M. Boccadoro,[2] P. Corradini[1]

[1]Istituto Nazionale Tumori, University of Milano, MILANO; [2]Ospedale San Giovanni Battista, University of Torino, TORINO; [3]Azienda Ospedaliera Universitaria, UDINE, Italy

Background. The allogeneic hematopoietic cell transplantation-specific comorbidity index (HCT-CI) has been recently developed to identify patients at high risk of morbidity and mortality after an allogeneic stem cell transplant (alloSCT). Reduced-intensity conditioning (RIC) regimens have decreased non-relapse mortality (NRM) in heavily pre-treated patients. Aims. We performed a retrospective study to assess whether comorbidities, according to HCT-CI, may influence the outcome of lymphoma and multiple myeloma patients undergoing a RIC alloSCT. Methods. Between 2000 and 2007, 197 patients received a RIC alloSCT from a HLA identical sibling (n= 123) or unrelated (n=74) donor in three Italian Transplant Units. Median age at transplant was 52 years (range, 17-69) and 41% of the patients were 55 or older. Diseases included non Hodgkin's lymphoma (n=103), multiple myeloma (n=68) and Hodgkin's lymphoma (n= 26). Median number of previous treatments was 3 (range, 0-8). Disease risk according to Kahl *et al.* (Blood 2007) was low, intermediate and high in 29%, 42% and 29% of patients, respectively. Thirty-three of 68 myeloma patients received a non-myeloablative conditioning regimen, whereas the others received a RIC regimen. The incidence of grade ≥2 acute graft-versus-host disease (aGVHD) was 35%. Patients with HCT-CI of 0, 1-2 and ≥3 were 64 (32%), 61 (31%) and 72 (37%), respectively. Variables included in multivariate analysis were age (<55 or ≥55), disease risk (low, intermediate and high), number of previous lines of therapy (≤ and >2), HCT-CI (0, 1-2 and ≥3), Karnofsky Perfomance Status (PS, >80% and ≤80%). Results. One-year OS and PFS were 87%, 60%, 60% and 91%, 75%, 70% in patients with HCT-CI of 0, 1-2 and ≥3, respectively. Cumulative incidence of NRM was 6%, 22%, 25% at 1 year and 6%, 24% and 27% at 2 years, whereas relapse mortality was 5%, 20%, 17% at 1 year and 7%, 27% and 23% at 2 years. By multivariate analysis only Karnofsky PS (p=0.00051) and HCT-CI (p=0.0038) were correlated with OS, whereas PFS was influenced by disease risk category (p=0.013), number of previous therapies (p=0.038), and both Karnofsky PS (p=0.031) and HCT-CI (p=0.0009). Interestingly HCT-CI was the only significant factor that could predict NRM (p=0.034) while Karnofsky PS failed to show a significant correlation (p=0.1). Of note, age (≥55) was not statistically significant either in OS, or PFS or NRM. The cumulative incidence of grade ≥2 aGVHD in patients with HCT-CI 0, 1-2, ≥3 was 26%, 35% and 41%, respectively (p=0.25). Myeloma patients with HCT-CI 0, 1-2 or ≥3 showed a similar OS whether they were transplanted with a non-myeloablative or RIC regimen (HCT-CI 0: p= 0.951, HCT-CI 1-2: p= 0.946; HCT-CI ≥3: p=0.063). Conclusions. Although the data need to be confirmed in prospective trials, these results showed that HCT-CI may be a useful tool to predict OS, NRM and also PFS after RIC alloSCT in lymphoma and myeloma patients. In the clinical setting HCT-CI and not age should be used for patient risk assessment.

Cytogenetics and molecular diagnostics

0417

DIFFERENCES IN CYTO- AND MOLECULAR GENETIC ABERRATIONS BETWEEN YOUNG (<2YR) AND OLDER CHILDREN WITH ACUTE MYELOID LEUKEMIA

B.V. Balgobind,[1] I.H.I.M. Hollink,[1] D. Reinhardt,[2] J. Bradtke,[3] A. Teigler-Schlegel,[3] A.R. Von Bergh,[1] J. Cloos,[4] G.J.L. Kaspers,[4] E. van Wering,[5] Z. Zemanova,[6] J. Stary,[7] J. Cayuela,[8] A. Baruchel,[8] M.M. van den Heuvel-Eibrink,[1] C.M. Zwaan[1]

[1]Erasmus MC - Sophia's Children Hospital, ROTTERDAM, Netherlands; [2]AML-BFM Study Group, HANNOVER, Germany; [3]Children's University Hospital, GIESSEN, Germany; [4]VU Medical Center, AMSTERDAM, Netherlands; [5]DCOG, THE HAGUE, Netherlands; [6]General Teaching Hospital, PRAGUE, Czech Republic; [7]2nd Medical School, Charles University, PRAGUE, Czech Republic; [8]St. Louis Hospital, PARIS, France

Background. Young children (defined as <2 years old) with acute myeloid leukemia (AML) do not differ in outcome when compared with older children with AML. Previously, distinct cytogenetic aberrations specific for AML in young children have been reported, such as t(7;12), and t(1;22), which is found exclusively in FAB M7. Moreover, young children with AML are characterized by a high frequency of 11q23-rearrangements. However, so far, no information is available on differences in the molecular genetic background of these two age groups. *Aims*. We performed a retrospective analysis of the distribution of different cytogenetic and molecular aberrations in young (<2yr) and older children (≥ 2yr) with AML. *Methods*. 421 pediatric AML cases (age 0-19 yr) were screened for the recurrent cytogenetic aberrations (i.e. 11q23-rearrangement, inv(16), t(8;21) and (t15;17)) using FISH and PCR, next to standard karyotyping, which was done by the collaborative groups. Also molecular screening of the mutational hotspots was performed , including c-KIT (n=229), N- and K-RAS (n=187), FLT3/ITD (n=162), FLT3-TKD (n=230), PTPN11 (n=216), MLL-PTD (n=240), CEBPalpha (n=251) and NPM1 (n=291). *Results*. The predominant cytogenetic aberration in the young children with AML consisted of 11q23-rearrangements, which occurred in 44% of the young children *vs* 17% in the older children (p=<0.005). We also found significant differences in other cytogenetic subgroups of AML between young and older children, i.e. normal karyotype, 5% *vs* 18%, respectively (p=0.008) and complex karyotype, 12% *vs* 5% (p=0.03). t(7;12) (n=3) and t(8;16) (n=3) were only detected in young children, in contrast to t(15;17) (n=16) and t(8;21) (n=44), which were only seen in older children. For the molecular aberrations a significantly different age distribution was found for NPM1 mutations (0% young *vs* 9% in older children; p=0.05) and FLT3-ITD (0% *vs* 21%, respectively; p=0.005). Mutations in the other genes showed no clear correlation with age. Several non-random associations between molecular and cytogenetic aberrations were detected. 89% of c-KIT mutations were associated with core-binding factor AML in children ≥2 years old. In young children, 2/4 c-KIT-mutated cases were associated with an MLL-rearrangement. NPM1, CEBPalpha and FLT3-ITD mutations in older children were significantly correlated with normal karyotype AML (respectively 57%, 37% and 75%; p=<0.05). In young children, 71% of RAS mutations were associated with an 11q23-rearrangement *vs* 48% in older children (p=0.02). In older children however, 41% of the RAS mutations were associated with a normal karyotype. *Conclusions*. These data suggest that young children with AML are characterized by differences in the type and frequency of cytogenetic and molecular genetic abnormalities when compared with older children with AML, possibly reflecting differences in underlying biology between these age groups. These differences may become clinically relevant in the era of molecularly targeted therapy.

0418

COMPREHENSIVE MOLECULAR ANALYSES OF TP53 GENE ALTERATIONS IN ACUTE MYELOID LEUKEMIA WITH COMPLEX KARYOTYPE

F.G. Rücker,[1] L. Bullinger,[1] C.-M. Kugler,[1] S. Miller,[1] H.A. Kestler,[1] P. Lichter,[2] H. Döhner,[1] K. Döhner[1]

[1]University of Ulm, ULM; [2]DKFZ, HEIDELBERG, Germany

Approximately 10 to 15% of acute myeloid leukemia (AML) cases exhibit a complex karyotype, defined by three or more chromosome abnormalities without presence of a specific fusion transcript. In order

to identify novel genomic regions of interest in this AML subgroup we applied comparative genomic hybridization to microarrays (array-CGH) allowing high-resolution genome-wide screening of genomic imbalances. Using this approach, we identified deletions of 17p13, affecting a genomic region that harbours TP53, in 48 of 100 unselected cases. Since TP53 is frequently involved as a tumor suppressor gene in numerous malignancies, we aimed to investigate its role in this AML subgroup. In 46 of the 48 patients 17p13 deletions were validated by FISH (fluorescence in-situ hybridization) using a TP53 locus specific DNA-probe. In a first series, 83 of the 100 cases were evaluated for TP53 mutations by direct sequencing of exons 4 to 9. In total, 47 mutations occurring in 34 cases were identified with a maximum of four mutations per case. Of these 47 mutations 14 were known polymorphisms [exon 6 (silent mutation) n=3, intron 9 n=4, intron 4 n=4, intron 7 n=3]; five cases exhibited known mutations in intronic splice sites (intron 4 and intron 8, n=2 each, and intron 6 n=1). In addition to 24 known mutations (missense n=23, nonsense n=1) four novel mutations were identified, one in intron 4 and three missense-mutations in exon 5. Combining the array-CGH findings with the mutation status, TP53 gene alterations were detected in 60% of the patients. Interestingly 19% of these cases showed biallelic mutations with deletion of one allele and a mutation in the remaining allele. In addition, TP53 gene alterations were associated with distinct genomic amplifications. All cases exhibiting amplifications in 8q24 or 12p13 (n=3 each) showed altered TP53, respectively, as well as three out of four cases with amplifications in 9p24 or 13q12, respectively. Correlation of these findings with global gene expression (GEP) data, which were available in a subset of cases (n=43), revealed specific expression patterns that were highly associated with TP53 alterations. For example the signature for biallelic TP53 mutations was enriched for genes involved in JAK-STAT signaling as gene set enrichment analysis (GSEA) provided significant associations with the BioCarta pathways *STAT3-signaling* and *IL22 soluble receptor signaling*. Furthermore, we also observed a significant enrichment for genes belonging to the *ARF-*, *VEGF-* and *Hypoxia/p53*-pathways, thereby suggesting that altered p53 function might indeed be of functional relevance in the affected AML cases. In conclusion, these data suggest that loss of normal p53 function plays an important role in the subgroup of AML with complex karyotype. Further analyses will facilitate to disclose the underlying pathways.

0419

DIAGNOSTIC AND PROGNOSTIC IMPACT OF GENE EXPRESSION PROFILING BASED MOLECULAR MARKER PREDICTION IN CYTOGENETICALLY NORMAL AML

L. Bullinger,[1] T. Hielscher,[2] C. Stirner,[1] R. Kranz,[1] Y.H. Kim,[3] S. Fröhling,[1] R.F. Schlenk,[1] A. Benner,[2] K. Döhner,[1] J.R. Pollack,[3] H. Döhner[3]

[1]University of Ulm, ULM, Germany; [2]DKFZ, HEIDELBERG, Germany; [3]Stanford University, STANFORD, USA

Background. In acute myeloid leukemia (AML), cyotgenetics is one of the most powerful prognostic markers. The largest subset of patients, cytogenetically normal AML (CN-AML), comprises a heterogeneous group with intermediate prognosis. However, in the past few years, mutations in genes like FLT3, CEBPA and NPM1 have been identified in CN-AML, and the presence of such mutations carries important prognostic information. Furthermore, DNA microarray-based gene expression profiling (GEP) has been shown to powerfully capture the molecular heterogeneity of cancers, and has been applied to build classifiers and clinical-outcome predictors in AML. *Aims.* While prior studies have defined gene-expression patterns associated with NPM1, CEBPA, and FLT3, none has assessed the clinical-relevance of identified signatures. *Methods.* Here, we profiled a large set of clinically-annotated CN-AML specimens (n=142) entered within a multicenter trial for patients < 60 years (AMLSG HD98A trial) using 40k cDNA microarrays. In this data set we applied supervised analysis (LASSO penalized logistic regression) to define gene-expression patterns characterizing FLT3 internal tandem duplication (ITD), CEPBA and NPM1 mutations. Next, we evaluated the utility of the respective signatures with regard to CN-AML classification and prognostication in an independent data set of CN-AML cases (n=83, entered within our ongoing multicenter trial AMLSG 0704 for patients <60 years) analyzed by Affymetrix microarrays (Human Genome U133 Plus 2.0 Arrays). *Results.* While in the cDNA data we were able to define distinct signatures associated with NPM1, CEBPA and FLT3 consisting of 39, 27, and 47 genes, respectively, only the NPM1 signature revealed a high prediction accuracy of >95% in leave-one-out cross validated classification. Prediction of FLT3-ITD or CEBPA mutation performed less well with accuracies of 80% and 73%, respectively. However, for both CEBPA and FLT3-ITD the predicted mutation class labels performed slightly better than the marker itself with regard to the prognostic impact on overall survival (CEPBA: p=0.00635 *vs* p=0.00683, FLT3-ITD p=9.57e-06 *vs* p=5.11e-05; logrank test). For class prediction the signatures derived from our cDNA data performed equally well in our independent test set analyzed by Affymetrix microarrays taking into account the smaller cohort. Furthermore, in the independent data our classifier signatures also provided prognostic information in addition to the molecular markers. *Summary and Conclusions.* With regard to the GEP based *misclassifications* we speculate that the signatures identified cases with alternative genetic or epigenetic changes that either phenocopy or block the effects of the respective aberrations. Therefore, the signature genes provide a starting point to dissect these *mutations* pathways, and our findings underscore the potential clinical utility of a gene-expression based measure of clinically-relevant *mutation pathway activation*.

0420

IMPROVED DETECTION AND MOLECULAR MONITORING OF FIP1L1-PDGFRA BY ANALYSIS OF PATIENT-SPECIFIC GENOMIC DNA FUSION JUNCTIONS

C. Score,[1] C. Walz,[2] J.V. Jovanovic,[3] A.V. Jones,[1] K. Waghorn,[1] C.E. Hidalgo-Curtis,[1] D. Grimwade,[3] F.H. Grand,[1] A. Reiter,[2] N.C.P. Cross[1]

[1]Leukaemia Research, SALISBURY, UK; [2]III. Medizinisch Klinik, MANHEIM, Germany; [3]Cancer Genetics Laboratory, Guy's Hosptial, LONDON, UK

FIP1L1-PDGFRA is a cytogenetically cryptic fusion seen in roughly 10% of patients with a provisional diagnosis of idiopathic hypereosinophilic syndrome (IHES). Previous studies have highlighted the importance of detecting FIP1L1-PDGFRA since positive patients are usually excellent responders to imatinib, however unambiguous detection of this fusion by FISH or RT-PCR is complicated by several factors. Heterozygous deletion of CHIC2 detected by FISH, a surrogate marker for FIP1L1-PDGFRA, is not always reliable since the proportion of cells carrying the abnormality may be low and overlap with the background false positive rate. In many cases the fusion mRNA is only detectable by nested RT-PCR, probably due to a combination of the heterogeneity of breakpoints within FIP1L1, the high degree of alternative splicing as well as highly variable levels of expression. We aimed to develop a more reliable method by which FIP1L1-PDGFRA could be detected, exploiting the fact that genomic breakpoints within PDGFRA are tightly clustered. We designed a multiplex Long Range PCR (LR PCR) assay with 15 forward primers spanning the breakpoint region within FIP1L1 (intron 5 to exon 16) and one reverse primer in PDGFRA intron 13. To date we have screened 202 samples with IHES or persistent unexplained eosinophilia and detected the genomic junction sequence in all RT-PCR positive samples (n=43). Genomic fusions were amplified by single step PCR in all cases whereas only 22 (51%) of these samples were single step RT-PCR positive. Importantly, FIP1L1-PDGFRA was detected in two cases that had been scored as RT-PCR negative. The ability to detect minimal residual disease (MRD) with high sensitivity is also important and therefore we designed patient-specific primer/probe combinations to quantify residual FIP1L1-PDGFRA positive cells from genomic DNA (gDNA). Absolute quantitation of the fusion at presentation (n=11) by gDNA RQ-PCR revealed a 40 fold variation between patients (range 0.027-1.1 copies of FIP1L1-PDGFRA per haploid genome). Comparison of gDNA RQ-PCR and nested RT-PCR for detection of MRD in follow up samples revealed large discrepancies with 47% (28/60) positive by gDNA RQ-PCR but only 29% (17/58) positive by nested RT-PCR, again highlighting the inaccuracies of the standard RT-PCR technique. Analysis of gDNA also gave a 1-2 log greater sensitivity than the analysis of cDNA by RQ-PCR. MRD assessment using gDNA showed that 11 of 12 patients achieved complete molecular response to imatinib within a median of 9 months (range 3-21) of starting treatment, with a sensitivity of detection of between 1 in 104 and 1 in 105 as assessed by quantitation of the ALB gene. We conclude that detection and quantitation of FIP1L1-PDGFRA from gDNA is more sensitive at both diagnosis and follow up compared to analysis of cDNA. Improved detection of this fusion is likely to lead to improved patient management.

0421

EARLY ASSESSMENT OF MINIMAL RESIDUAL DISEASE (MRD) BY OPTIMIZED REAL-TIME QUANTITATIVE PCR (RQ-PCR) ASSAY FOR DETECTION OF WT1 TRANSCRIPTS PROVIDES AN INDEPENDENT PREDICTOR OF DISEASE RELAPSE IN ACUTE MYELOID LEUKEMIA: A EUROPEAN LEUKEMIANET (ELN) STUDY

D. Cilloni,[1] A. Renneville,[2] F. Hermitte,[3] R.K. Hills,[4] S. Daly,[5] J. Jovanovic,[6] E. Gottardi,[1] M. Fava,[1] S. Schnittger,[7] T. Weiss,[7] B. Izzo,[8] J. Nomdedeu,[9] A. van der Heijden,[10] B. van der Reijden,[10] V. van der Velden,[11] P. Rohon,[12] H. Ommen,[13] J. Polak,[14] J. Jansen,[10] C. Preudhomme,[2] G. Saglio,[1] D. Grimwade[6]

[1]University of Turin, TURIN, Italy; [2]Dept Hematol Hopital Calmette, LILLE, France; [3]R&D Ipsogen, MARSEILLE, France; [4]Dept Haematol, Univeristy of Wales, CARDIFF, UK; [5]Dept. Haematol MRI, MANCHESTER, UK; [6]Dept Genetics KCL, LONDON, UK; [7]Molec Diag MLL, MUNICH, Germany; [8]Dept Hematol, University of Naples, NAPLES, Italy; [9]Dept Hematol Hosp De St Pau, BARCELONA, Spain; [10]Dept Hematol UMC St Radboud, NIJMEGEN, Netherlands; [11]Dept Immunol Erasmus MC, ROTTERDAM, Netherlands; [12]Dept Hematol Faculty Hosp, OLOMOUC, Czech Republic;[13]Dept Hematol Amtssygehus, AARHUS, Denmark; [14]DNA diag Inst Hematol, PRAGUE, Czech Republic

Early assessment of minimal residual disease (MRD) by optimized real-time quantitative PCR (RQ-PCR) assay for detection of WT1 transcripts provides an independent predictor of disease relapse in acute myeloid leukemia: A European LeukemiaNet (ELN) study. *Background.* Quantitative RQ-PCR assays to detect leukemic fusion transcripts have been shown to identify reliably the acute leukemia patients at highest risk of relapse, allowing development of a more individualized treatment approach. *Aims.* To establish and evaluate an optimized RQ-PCR assay for quantification of Wilms' Tumor gene (WT1) expression which could provide a more precise measurement of disease response and quality of remission in patients lacking a leukemia-specific MRD target. *Methods.* Nine published and in house WT1 assays were systematically evaluated across a network of 11 European laboratories. Assays were excluded on the basis of lack of RNA specificity, inferior sensitivity or involvement of regions which are hotspots for mutation. An assay located within the 5 region affording the greatest sensitivity was ultimately selected and evaluated in 729 diagnostic samples from 588 patients, together with follow-up samples from 106 patients (median age 45 years) treated with intensive combination chemotherapy. *Results.* WT1 was over-expressed in the majority of AML cases, with comparable levels in PB and BM (PB - median 4637 WT1 copies/104 ABL copies, range 0-1132709; BM - median 7212, 0-750571), as compared to normal BM (median 19.8, 0-213), PB (median 0.01, 0.01-47.6) and PBSCs (median 6.1, 0-39). There was no correlation between WT1 expression at diagnosis and age, presenting WBC, NPM1 or FLT3 mutation status or cytogenetic risk group, although significantly higher expression was found in acute promyelocytic leukemia with t(15;17) (median 19195 range 280-177500, p=0.01). Cases with low WT1 expression were screened for mutations of the 5' region and in 40% mutations were detected. While Cox regression analysis showed no association between baseline WT1 level and relapse (p=0.3), a greater response in terms of WT1 transcript reduction following induction chemotherapy was associated with a significantly reduced risk of relapse (hazard ratio 0.54 per log reduction (95% CI 0.36-0.83), p=0.004), which remained significant when adjusting individually for age, presenting WBC and cytogenetic risk group. Failure to normalize WT1 transcript levels by the post-induction and post-consolidation timepoints was associated with a higher risk of relapse (100% and 67% respectively at 5 years, as compared to 44% and 42% for patients with WT1 levels within the normal range at these stages p=0.05, p=0.004). Analyses of sequential PB and BM samples from 15 AML cases with low WT1 expression (<250 copies) were undertaken, which showed no significant modulation in transcript level on regeneration after chemotherapy, indicating that in $WT1^+$ AML, transcript levels detected in follow-up samples reliably reflect disease status. *Conclusions.* This study suggests that normalization of WT1 transcripts as determined by an optimized RQ-PCR assay provides an independent predictor of disease relapse in AML Moreover, it lends support to the incorporation of early assessment of MRD to develop more robust risk scores, to enhance risk stratification and identify patients who may benefit from allogeneic transplant.

Gene therapy, cellular immunotherapy and vaccination

0422

LENTIVIRAL GENE THERAPY OF HEMATOPOIETIC STEM CELLS RESULTS IN PHENOTYPE CORRECTION IN A MOUSE MODEL OF POMPE DISEASE

M. Stok,[1] N. van Til,[2] F.S.F. Aerts Kaya,[2] T.P. Visser,[2] M. Kroos,[3] M. de Waard,[4] E. Jacobs,[5] M. Willart,[6] P. van der Wegen,[5] B. Scholte,[5] B. Lambrecht,[6] D. Duncker,[4] A. van der Ploeg,[7] A. Reuser,[3] M. Verstegen,[2] G. Wagemaker[2]

[1]Erasmus MC, ROTTERDAM; [2]Hematology, Erasmus MC, ROTTERDAM; [3]Clinical Genetics, Erasmus MC, ROTTERDAM; [4]Experimental Cardiology, Erasmus MC, ROTTERDAM; [5]Cell Biology & Genetics, Erasmus MC, ROTTERDAM; [6]Pulmonary Medicine, Erasmus MC, ROTTERDAM; [7]Pediatrics, Erasmus MC, ROTTERDAM, Netherlands

Background. Pompe disease is a storage disorder characterized by progressive muscle weakness, caused by deposition of glycogen due to a reduced function of acid alpha-glucosidase (GAA). In the early onset form, death occurs within the first year of life by cardiac and respiratory failure. Available enzyme replacement therapy (ERT) does not provide a lasting cure and requires life-long administration. *Aims.* To develop a more efficient alternative for ERT we have explored the potential of lentivirus (LV) mediated gene transfer of hematopoietic stem cells (HSC) to correct glycogen storage in a Pompe (Gaa-/-) mouse model. *Methods.* Lineage negative HSC from Gaa-/- donor mice were transduced *ex vivo* with third generation self-inactivating (SIN) LV-SF-GAA or LV-SF-GFP (green fluorescent protein) as control, both driven by the promoter of the spleen focus forming virus (SF), and transplanted into sublethally irradiated Gaa-/- mice, 8-12 week of age. *Results.* Transplantation of SIN-LV-SF-GAA resulted in sustained (up to 1.5 year) high level expression of alpha-glucosidase in peripheral blood cells (PBC). Over-expression of the enzyme, as observed in PBC, resulted in reconstitution of alpha-glucosidase expression and clearance of glycogen in heart, diaphragm, stomach, uterus, liver and spleen. Skeletal muscle displayed a significant reduction of glycogen, although not as prominent as the other tissues. The large activity increase of alpha-glucosidase in heart tissue resulted in a near normalization of heart geometry and function, as visualized by echography. In addition, locomotor function and skeletal muscle strength, as well as respiratory function were significantly improved and stablized, proportional to the level of glycogen clearance. Adverse effects on the hematopoietic/immune system, measured at the progenitor and the end cell levels, were not observed. *Conclusions. Ex vivo* hematopoietic system mediated gene therapy corrects defects in the Pompe mouse model, indicating that the approach may provide a valid alternative for ERT in the treatment of Pompe disease. Codon optimization of the transgene is anticipated to provide a further elevation of enzyme levels. Current experiments aim at establishing the minimum cell dose and conditioning regimen required for optimal efficacy and long-term monitoring for potential adverse effects.

0423

AN ORIGINAL GENE THERAPY STRATEGY OF HEMOPHILIA B USING LENTIVIRALLY MODIFIED HEMATOPOIETIC STEM CELLS

M. Cambot,[1] C. Lavenu-Bombled,[1] J. Cossonière,[1] J.P. Rosa,[2] A. Dubart-Kupperschmitt[1]

[1]INSERM, LE KREMLIN-BICÊTRE CEDEX; [2]INSERM U689, PARIS, France

Gene therapy approaches involving Hematopoietic Stem Cell (HSC) could be used to cure genetic and infectious diseases affecting hematopoietic or non hematopoietic tissues. New developments have improved safety in using integrative viral vectors while they retain ability to stably and efficiently modify HSC. We chose hemophilia B, a monogenic bleeding disorder due to defects in the FIX gene, as a model to study gene therapy using modified HSC. Although the existing substitutive treatment is effective, high cost and side effects require the development of alternative treatments to improve patient's lives. Therefore, hemophilia B is the target of numerous gene therapy strategies. Despite encouraging therapeutic effects in animal models, clinical trials were very disappointing due to low and transient transgene expression. The strategy we develop should overcome several problems identified in former studies, such as the development of antibodies against FIX or

immune response against transduced cells, and should allow a bleeding correction, even in presence of pre-existing inhibitory antibodies. The permanence of the therapeutic effect will be ensured by engraftment of *in vitro* transduced HSC using an integrative lentivector encoding FIX. FIX expression, driven by a megakaryocytic specific promoter, will be restricted to differentiated megakaryocytes (Mk). Fusion to a peptide derived from Platelet Factor 4 (PF4) will target FIX into α-granules of Mk and platelets. Thus, the rFIX stored in platelet α granules will be locally delivered on the site of vascular injury, in response to platelet activation. The global efficacy of this strategy is studied in three different models. Human HSC *in vitro* differentiated into Mk are used to assess the biological activity of the therapeutic FIX synthesized by Mk and stored in α-granules, a non-physiological situation. FIXko mice are used to study the therapeutic efficacy of the strategy. And a preclinical model in the macaque is developed to study gene therapy approaches using HSC in the long term. In these three models, we showed that human GPIbα promoter restricts the expression of a transgene to differentiated Mk and that a 4 amino-acid peptide derived from PF4 is sufficient to target transgenic FIX to α-granules of Mk. For macaque HSC transplantation, we focus on improving the conditions of HSC transduction and on pre-conditioning the animals for an optimal and permanent engraftment. We reproducibly obtain efficacies of transduction at a mean of 50% using an HIV-derived vector encoding GFP. For *in vivo* studies, the sub-myeloablative protocol tested is not fatal to animals and induces a bone marrow aplasia and an immuno-suppressive effect necessary for HSC engraftment. After 600 days, GFP^+ cell engraftment is low (1%) but stable for one year in bone marrow and peripheral blood. The follow-up of engrafted animals is ongoing to study lentiviral integration sites, evolution of the clonality of hematopoietic reconstitution. In future experiments comparing modified HSC engraftment after myelo-ablative or sub-myelo-ablative conditioning, we will study efficacies and kinetics of hematopoietic reconstitution, persistence of transgene expression, immune response and safety concerns.

0424

REDIRECTING T-CELLS AGAINST CANCER CELLS BY TRANSFER OF BROADLY TUMOR REACTIVE GAMMA-DELTA-T-CELL RECEPTORS

J.H.E. Kuball, V. Marcu-Malina, M. Theobald

UMC Utrecht, UTRECHT, Netherlands

Background. Adoptive transfer of alpha-beta-T-lymphocytes is a promising treatment for a variety of malignancies, but often not feasible due to difficulties in generating T-cells reactive with the targeted antigen from patients. To facilitate rapid generation of cells for therapy, T-cells can be programmed with genes encoding for an antigen-specific alpha-beta-T-cell receptor (TCR). However, this concept is limited by the low affinity of most tumor-reactive alpha-beta-TCR chains. One attractive alternative to mediate a selective anti-tumor-reactivity with a high-affinity TCR arises from the ability of gamma-delta-T-cells to mediate anti-tumor-reactivity while ignoring healthy-environment. The gamma-delta-T-cell-receptor (TCR) itself is not effected by rigors of negative selection and is supposed to mediate this intriguing property. Gamma2delta2-TCRs recognize non-classical antigens including metabolites of the mevalonate pathway. *Aims and Methods.* To test the ability of gamma2delta2-TCRs to redirect alpha-beta-T cells selectively against tumor cells, the gamma2delta2-TCR was retrovirally transduced into human alpha-beta-T cells. *Results.* Strong surface-expression of introduced gamma2delta2-TCRs was observed while endogenous alpha-beta-TCR chains were down-modulated. Functional analysis revealed that a gamma2delta2-TCR efficiently reprograms both, $CD4^+$ and $CD8^+$ T-cells against a broad panel of cancer cells while ignoring normal cells. Moreover, tumor-specific T-cell proliferation, cytokine secretion and killing were significantly enhanced by additional application of biphosphonates. *Summary*. In summary, gamma2delta2-TCRs are an attractive alternative to redirect alpha-beta-T cells against cancer cells with both, an improved efficacy and safety profile as compared to currently used alpha-beta-TCRs.

0425

REGULATORY T CELLS ARE RESISTANT TO PROTEOSOME INHIBITOR MEDIATED APOPTOSIS AND CAN BE EXPANDED *in vitro* IN UPON CULTURE WITH BORTEZOMIB

J.A. Pérez-Simón, B. Blanco, L.I. Sánchez-Abarca, M. Díez-Campelo, S. Tabera, P. Hernández-Campo, S. Gutierrez-Cosio, C. Rodriguez-Serrano, F. Sanchez-Guijo, C. Cañizo, J.F. San Miguel

Hospital Universitario, SALAMANCA, Spain

Background. We have previously shown that bortezomib selectively induces apoptosis among alloreactive T-cells after mixed lymphocyte reaction (MLR) (Blanco, Blood 06). Using this approach, it could be possible to induce a selective ablation of immune response against host antigens. On the other hand, regulatory T cells (Treg) play a crucial role in keeping an appropriate homeostasis of the immune response in steady state and also contribute to decrease the risk of GVHD after allogeneic transplantation. Some studies have been reported describing alternative methods to induce *in vitro* allodepletion although, those which have been evaluated into the clinical setting , have failed to obtain a clear benefit in terms of lowering the risk of GVHD, at least in part due to the fact that Tregs may also be affected by *in vitro* depletion. *Aims.* In the current study we have evaluated the effect of bortezomib on Treg viability. *Results.* Firstly, we analyzed the effect of bortezomib on Treg viability after MLR using anexin/7-AAD staining. While viability significantly decreased among activated (alloreactive) T-cells, both resting and regulatory T cells remained unaffected (Figure 1A).

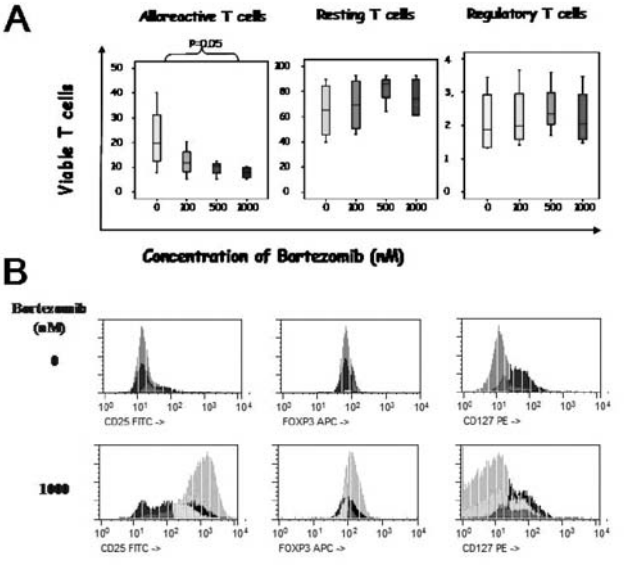

Figure 1.

Since activated T-cells were sensitive and Treg were resistant to the pro-apoptotic effect of bortezomib, we asked whether we could obtain a purified Treg population after *in vitro* expansion with antiCD3-CD28 plus IL2 (50 U/mL) in the presence of the drug. Interestingly, after 4 weeks of culture, T cells had a significantly higher expression of FoxP3 and CD25 and lower expression of CD127 upon culture with bortezomib (Figure 1B). We next evaluated the effect of *in vitro* expanded T-cells on the proliferation of effector T-cells (Teff) stimulated with antiCD3-CD28. Functional assays confirmed that T-cells expanded in the presence of bortezomib (Tr) inhibited Teff proliferation while this effect was not observed when we used bortezomib-untreated *in vitro*-expanded T-cells, with a mean (SD) of 27.3% (7.3) *vs* 68.7% (9.6) of non-proliferating Teff after co-culture with bortezomib-untreated *vs* treated (Tr) *in vitro*-expanded T-cells ($p<0.001$). Furthermore, Tr significantly decreased the production of IFN among Teff as assessed by intracytoplasmic-IFN staining (16 *vs* 3% IFN^+ Teff after stimulation with antiCD3-CD28 and co-culture with bortezomib-untreated *vs* treated T-cells). According to these data, the presence of bortezomib allowed to obtain T-cells with immunoregulatory properties (Tr). Finally we asked whether bortezomib allowed to expand the Treg already present in the initial culture or did modify the functional characteristics of T-cells allowing to obtain a new *in vitro*-generated Tr population. For this purpose, we selected CD25 negative T-cells and expanded these cells as previously described in the presence of bortezomib. Interestingly, Tr

were also obtained using this approach, which showed similar characteristics in terms of phenotype and functionality as compared to Tr obtained from unpurified T-cells. *Conclusions.*Treg are resistant to the pro-apoptotic effect of bortezomib. Furthermore, *in vitro* expansion in presence of bortezomib allowed to obtain Tr which showed immunoregulatory properties. This effect may be useful in the allogeneic transplant setting

0426

VACCINATION OF ACUTE MYELOID LEUKEMIA PATIENTS WITH DENDRITIC CELLS ELECTROPORATED WITH MRNA ENCODING THE WILMS TUMOR PROTEIN WT1: A PHASE I/II TRIAL

L.R. Van de Velde,[1] A. Van Driessche,[2] N. Cools,[3] G. Nijs,[2] B. Stein,[2] K. Vermeulen,[4] K. Pieters,[4] I. Vrelust,[5] A.P. Gadisseur,[5] W.A. Schroyens,[5] I.J. De Vries,[6] D.A. Price,[7] Z.N. Berneman,[8] V.F. Van Tendeloo[2]

[1]Antwerp University Hospital, EDEGEM, Belgium; [2]Center for Cellular Therapy and Regenerative Medicine, EDEGEM (ANTWERP), Belgium; [3]Lab of Exp. Hematology, Univ. of Antwerp, WILRIJK (ANTWERP), Belgium; [4]Lab. of Molecular Diagnostics in Hematology, EDEGEM (ANTWERP), Belgium; [5]Division of Hematology, Antwerp University Hospital, EDEGEM (ANTWERP), Belgium; [6]Radboud Univ. Nijmegen, Dep. of Tumor Immunology, Centre for Mol. Life Sciences, NIJMEGEN, Netherlands; [7]Weatherall Inst. of Mol. Med., Univ. Oxford, J. Radcliff Hosp., OXFORD, UK; [8]Division of Hematology and CCRM, Antwerp University Hospital, EDEGEM (ANTWERP), Belgium

Background. To date, Wilms tumor protein (WT1) is acknowledged as a valuable target for active specific immunotherapy in several solid and hematological malignancies, such as leukemia. Preclinical data from our laboratory and that of Hans Stauss have shown that WT1 RNA-electroporated dendritic cells (DC) stimulate WT1-specific T cells *in vitro* (Van Driessche A *et al.* Leukemia 2005;19:1863-1871). *Aims.* We started a phase I/II dose-escalation trial in which patients with acute myeloid leukemia (AML) in remission received intradermal injections with WT1 RNA-loaded DC. Feasibility, safety and immunogenicity of the vaccine were investigated. *Methods.* Eight patients received four biweekly DC vaccines. A delayed-type hypersensitivity (DTH) test was performed 2 weeks following the last vaccination. Patients underwent an apheresis and monocytes were isolated using CD14-labeled magnetic beads by CliniMACS. DC were generated in 6-day cultures in clinical-grade medium supplemented with serum, GM-CSF and IL-4 and maturated with PGE2 and TNF-a. Keyhole limpet hemocyanin (KLH) was added during maturation as a $CD4^+$ helper antigen. Mature DC were harvested, electroporated with WT1 mRNA and used as vaccines. Patients were monitored for minimal residual disease (MRD) by analyzing WT1 RNA expression in peripheral blood by qRT-PCR. When the patient was HLA-A2+, tetramer staining was performed to detect WT1-specific CD8+ T cells. Before and after the vaccination cycle, peripheral blood was collected for immunomonitoring purposes. *Results.* There was successful DC generation and vaccine production in all patients selected. No serious adverse events or toxicity was seen. A decrease in WT1 RNA expression was observed during the course of the vaccination in 4/6 patients who had an increased WT1 mRNA level in peripheral blood at the start of DC vaccination. A vaccine-specific immune response was demonstrated in 8/8 patients by an *in vivo* DTH reaction both to KLH as well as to WT1. By tetramer analysis, detectable levels of WT1-specific CD8+ T cells could be demonstrated during the course of the vaccination both in the peripheral blood as well as in the expanded DTH-infiltrating T cells from the skin biopsies. Preliminary data from immunomonitoring in pre- and post-vaccination T cell samples from 4 patients show a mixed T helper (Th)1/Th2 response towards the KLH and the WT1 protein following vaccination. *Conclusions.* We conclude that vaccination of AML remission patients with WT1 RNA-loaded DC is feasible and safe. Furthermore, the vaccine elicits anti-vaccine T-cell responses *in vivo* and a decrease in WT1 RNA expression levels was observed during MRD monitoring in some vaccinated patients.

A. Van de Velde (Ann.van.de.velde@uza.be) is the holder of a Clinical Research Grant (2005/31) awarded by the European Hematology Association.

Vascular biology, bleeding disorders and transfusion medicine

0427

ANGIOGENESIS AND VEGF-/RECEPTOR EXPRESSION IN MYELOPROLIFERATIVE DISEASES: CORRELATION WITH CLINICAL PARAMETERS AND JAK2-V617F MUTATIONAL STATUS

M. Medinger, A. Gratwohl, A. Theocharides, A. Buser, D. Heim, R. Skoda, S. Dirnhofer, A. Tichelli, A. Tzankov

University Hospital, BASEL, Switzerland

Angiogenesis is essential for malignant tumor growth. This has been documented for solid tumors and there is emerging evidence that progression of haematological malignancies also depends on the induction of new blood vessels. The most important pro-angiogenic factor is vascular endothelial growth factor (VEGF), activating the VEGF receptor 1 (VEGFR-1) and receptor 2 (VEGFR-2). Therefore VEGF and its receptors may provide a target for therapy in hematological malignancies. In Bcr-Abl negative myeloproliferative diseases (MPD) there are only few data about the role of angiogenesis. We studied 100 patients with MPD, including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) by immunohistochemical assessment of microvessel density (MVD) and the expression of VEGF and its receptors VEGFR-1, VEGFR-2 and VEGFR-3 in bone marrow (BM) sections (Table 1). The normal controls (NC) consisted of 10 BM which were free of abnormalities. MVD, as assessed by CD34 staining, was calculated as the mean number of stained vessels in 5 fields at 200 magnification. Expression of VEGF/-receptors was assessed as mean of the number of positively staining cells in 5 fields. Our study showed a significantly higher degree of angiogenesis in PMF, PV and ET compared with NC (Table 1; $p<0.001$, $p<0.0007$ and $p<0.001$, respectively). Angiogenesis was higher in PMF than PV and ET ($p<0.001$ and $p<0.001$). Increased angiogenesis correlated with higher VEGF expression compared to NC ($p<0.009$). VEGFR-2 expression was significantly higher in MPD than in NC ($p<0.001$) and, in particular, in PMF ($p<0.001$) and PV ($p<0.001$). There were no significant differences between VEGFR-1 and VEGFR-3 expression compared to NC. MVD and VEGF expression was significantly higher in JAK2-V617F mutation positive MPD [30 (12-39) *vs* 22 (9-31) mean vessels per 1 mm^2 field (range); $p<0.001$ and 49% (20-65) *vs* 32% (19-42) mean %VEGF positive (+) cells (range); $p<0.001$] than in negative MPD, but there was no difference regarding VEGF receptors expression. In conclusion, this study showed increased angiogeneis in MPD patients as expressed by increased VEGF and VEGFR-2 expression. Patients with the JAK2-V617F mutation had a higher MVD and VEGF expression than patients without the JAK2-V617F mutation. Our data support the hypothesis of an autocrine VEGF/VEGFR-2 loop in the pathogenesis of MPD. Inhibition of angiogenesis could constitute a novel strategy for the treatment of MPD.

Table 1.

	PMF (n=45)	PV (n=30)	ET (n=25)	NC (n=10)
Age years (range)	59 (37-68)	64 (48-70)	60 (24-72)	62 (45-68)
Sex (M/F)	25/20	18/12	10/15	5/5
MVD Mean vessels per mm^2 field (range)	32 (13-79)	19 (11-59)	17.5 (7-23)	8 (1-15)
VEGF Mean %VEGF(+) cells (range)	51 % (10-75)	46% (15-70)	50% (10-75)	5% (0-9)
VEGFR-1 Mean %VEGFR-1 (+) cells (range)	22 % (5-42)	30% (7-52)	28% (6-49)	20% (2-39)
VEGFR-2 Mean %VEGFR-2 (+) cells (range)	49 % (8-69)	52% (12-79)	46% (15-63)	22% (0-9)
VEGFR-3 Mean %VEGFR-3 (+) cells (range)	20 % (3-42)	18% (0-39)	24% (10-75)	15% (0-23)

0428

MANAGEMENT OF REPRODUCTIVE EVENTS IN FEMALES WITH GAUCHER DISEASE

A. Zimran,[1] N. Belmatoug,[2] S. Granovosky-Grisaru,[1] R. Heitner,[3] D.A. Hughes,[4] P. Kaplan,[5] V. Malinova,[6] E. Mengel,[7] E. Morris,[8] M. Mrsic,[9] S. vom Dahl[10]

[1]Shaare Zedek Medical Centre, JERUSALEM, Israel; [2]Nadia, Beaujon Hospital, Assistance Publique-Hôpitaux de Paris, CLICHY, France; [3]Johannesburg Hospital, JOHANNESBURG, South Africa; [4]Royal Free & University College Medical School, LONDON, UK; [5]Children's Hospital of Philadelphia, PHILADELPHIA, USA; [6]Charles University & General Teaching Hospital, PRAGUE, Czech Republic; [7]Univeristy Clinic, MAINZ, Germany; [8]Addenbrookes Hospital, CAMBRIDGE, UK; [9]Clinical Hospital Centar Rebro, ZAGREB, Croatia; [10]St. Franziskus Hospital, COLOGNE, Germany

Background. The principal manifestations of type 1 (non neuronopathic) Gaucher disease include increased risk of bleeding, anaemia, splenomegaly, hepatomegaly and bone disease. An effective disease-specific treatment for type 1 disease has been available since 1991 in the form of alglucerase (enzyme replacement therapy) and later its recombinant form, imiglucerase. Treatment goals and monitoring guidelines for imiglucerase provide the basis for a disease management strategy (Andersson *et al.*, 2005; Pastores *et al.*, 2004; Weinreb *et al.*, 2004). These goals and guidelines, however, do not make specific reference to females for whom the manifestations of Gaucher disease could have significant impact during reproductive events such as menarche and menstruation; fertility; pregnancy, parity, delivery and lactation; and menopause. *Aims.* To examine the reciprocal effects of reproductive events and Gaucher disease in untreated and enzyme-treated females to identify an optimal management approach. *Methods.* A panel of international clinicians experienced in the management of Gaucher disease convened to review and present evidence from the peer-reviewed literature, a pharmacovigilance database on alglucerase and imiglucerase, and their own clinical experience to support discussions and recommendations. Data from further recent surveys were also reviewed. *Results.* Menarche may be delayed in symptomatic girls with Gaucher disease who are not treated with enzyme replacement therapy. Menorrhagia seems to be more common than in the non-Gaucher population. There is no evidence of decreased fertility in Gaucher disease. Pregnancy and delivery are not trivial in Gaucher disease. Women with Gaucher disease may be at increased risk of complications because of peripartum bleeding and worsening bone involvement. Therapy with alglucerase or imiglucerase before and during pregnancy may help reduce the rate of spontaneous abortion and risk of complications in the peripartum period. There is no evidence of any teratogenic effect of alglucerase and imiglucerase on the foetus, or of any adverse effect on infants breast fed by mothers receiving imiglucerase. Breast feeding may present a physiological challenge to mothers with Gaucher disease. There is a lack of information on menopause in women with Gaucher disease. *Conclusions.* Menarche and pregnancy are the main issues to be considered in women with Gaucher disease. Neither is a contraindication to enzyme replacement therapy. Planned conception, and a multidisciplinary approach to the management of pregnancy, is the most desirable route to parenthood for women with Gaucher disease. Ideally, for symptomatic patients who require enzyme replacement therapy, therapeutic goals should be achieved before conception to help ensure women are in an optimal clinical state to withstand the physiological demands of pregnancy and the post partum period. Patients treated with imiglucerase prior to pregnancy should be allowed (or encouraged) to continue imiglucerase treatment throughout pregnancy and breast feeding (despite the package insert warning). Disease monitoring during reproductive events should be vigilant to the risk of exacerbated bone disease, especially osteoporosis, and peripartum bleeding associated with Gaucher disease. The impact of Gaucher disease on menopause is poorly understood and requires further study especially in relation to bone mineral density and appropriate treatments.

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0429

HEALTHY BLOOD DONORS WITH A POSITIVE COOMBS TEST: NATURAL ANTI-C3 AND FRAMEWORK-SPECIFIC ANTIBODIES TOGETHER INHIBIT COMPLEMENT DEPENDENT PHAGOCYTOSIS OF SENESCENT RED BLOOD CELLS

H.U. Lutz,[1] B.M. Frey,[2] P. Stammler,[1] A. Siderow,[2] M. Kradolfer,[1] V. Alaia[1]

[1]ETH Zurich, ZURICH; [2]Regional Blood Transfusion Service SRK, SCHLIEREN, Switzerland

Background. Coombs-positive red blood cells (RBC) or RBC with a positive direct agglutination test (DAT-positive RBC) from healthy blood donors carry elevated numbers of IgG molecules as is known for many years (for a review see: Garratty. Gerontology. 1991;37:68-94). In almost all cases this phenomenon is not an early sign of an autoimmune hemolytic anemia. The reason for this opsonization has remained elusive. Aims and *Methods.* By studying the specificity of IgG molecules eluted from density separated RBC (Lutz *et al.* Biochim.Biophys. Acta 1992;1116:1-10) and by performing phagocytosis experiments we tried to identify the specificities of bound IgG molecules. We compared the specificities of eluted IgG molecules with those of a pair of naturally occurring antibodies (NAbs), which we have characterized earlier (Jelezarova and Lutz, Mol. Immunol. 2005;42:1393-1403) and studied the concentration of the two types of NAbs in plasma from these donors by ELISA. *Results.* The majority of RBC-associated IgG molecules was on dense RBC from Coombs-positive healthy blood donors. These blood donors had increased numbers of dense RBC as compared to aged-matched controls and some of their RBC were even denser than the densest cells of controls. Their densest cells were in fact older than the oldest cells of controls based on the band 4.1a/b ratio. Phagocytosis of senescent RBC from these donors was 1.5 to 2 fold higher than that of senescent control cells, implying that Coombs-positive old RBC carried more opsonins than those of controls. In contrast to this, their old RBC were less efficiently phagocytosed than senescent control cells in an *in vitro* assay in which FcR-mediated phagocytosis was suppressed by physiological concentrations of whole human IgG. The additional IgG molecules on Coombs-positive RBC from healthy blood donors comprised primarily anti-C3 and framework-specific anti-idiotypic NAbs. This was evident from immunoblots with IgG molecules eluted from senescent RBC after dissociation of IgG complexes. The increased binding of the pair of these NAbs to their senescent RBC was most likely due to the fact that healthy blood donors with a positive Coombs test had increased concentrations of the two types of NAbs in their plasma at the same ratio as in controls. Suppression of complement-dependent phagocytosis occurred by binding of this pair of NAbs to senescent RBC via two possible modes: 1) by binding to senescent cell-associated anti-band 3 NAbs (Lutz *et al.* Blood Cells 1988;14:175-195), which otherwise would have preferentially captured C3b (Lutz *et al.* J. Biol.Chem. 1993;268:17418-17426), and 2) by binding to C3 fragments on senescent RBC. *Conclusions.* Binding of anti-C3 NAbs together with framework-specific anti-idiotypic NAbs to senescent RBC may have suppressed their complement-dependent phagocytosis and thereby prolonged their *in vivo* life span.

0430

HOME BLOOD AND PLATELET TRANSFUSIONS AS PART OF A DOMICILIARY PROGRAM OF SUPPORTIVE CARE IN PATIENTS WITH BLOOD MALIGNANCIES: AN ITALIAN SINGLE-CENTRE EXPERIENCE

P. Alfieri

Division of Haematology, MODENA, Italy

Home care has achieved a relevant role in the global management of patients with blood malignancies improving quality of life and reducing health care costs. Transfusion therapy is of major impact in this clinical setting. In our department a home care service supported by AIL (Italian Association against Leukaemia-Lymphoma-Myeloma) is operating in order to assist haematologic patients that are fragile, unfit, not self-sufficient, in different stages of disease (terminal, advanced, chronic, in causal therapy). Here we share a single-centre experience in home transfusion assistance throughout a 9-yrs period (July 1999 - February 2008), by describing patients' characteristics, clinical indications, operating procedures and safety issues. All patients undergone home transfusions were eligible for domiciliary program of supportive care. Transfusions were requested to blood bank according to haemoglobin levels (Hb < 8 g/dL), platelet count (PLT <10.000/mcl) and main clinical indications. Informed consent was obtained before first home transfusion. Anti-

allergic premedication with hydrocortison and clorfenamine was routinely administered before any platelet transfusion so as to minimise risks of adverse reaction. Consultant haematologist was responsible for clinical surveillance during transfusion, by home-assisting patients in the first fifteen minutes of infusion and after then being telephonically available in case of emergency Among the 335 patients enrolled in the AIL home care program, 156 (46.5%) received at least one home transfusion. Patients (male=75, female=81, median age=75, in terminal phase=80, with advanced disease=43, chronically ill=24, in causal therapy=9) were affected by acute myeloid leukaemia (26%), myelodisplastic syndrome (15%), chronic myeloproliferative disorders (15%), multiple myeloma (14%), non-Hodgkin's lymphoma (13%), chronic lymphocytic leukaemia (7%), acute lymphoid leukaemia (3%), Hodgkin's lymphoma (3%), aplastic anaemia (2%), other chronic anaemias (3%). Total number of transfusion was 2012: packed red blood cells (RBC) were 1369 (68%) while platelet concentrates (PC) were 643 (32%). 65 patient were transfused both for RBC and PC; 83 patients received only RBC, 8 were patients transfused only with PC. The average number of transfusions per patient was 13 (min 1 - max 131). Patients with myelodisplasia, acute leukaemia and myeloproliferative disorders had higher transfusion requirement, accounting respectively for 18, 14 and 14 transfusions per patient throughout an entire period of home assistance. No serious adverse reaction was reported. The consultant haematologist was contacted on-call in 107 cases (about 5% of all transfusions) because of the onset of fever < 38°C (15 cases), nausea and/or vomiting (11 cases), skin rash (9 cases) and above all extravasation (72 cases). Home transfusions are a valid option in the effort to increase the accessibility and convenience of care in the setting of haematologic patients. The major concern about out-of-hospital transfusions is represented by the difficult emergency medical care in case of severe reactions. In our operating model these risks are severely diminished through a careful patients' selection and a clinical appropriateness, and obviously by a strict collaboration between the local Division of Haematology and Transfusion Centre. The role of fundraising organisations like AIL is nowadays essential to sustain these programs. Several issues, such as those regarding standard operating protocols and cost analysis, remain open.

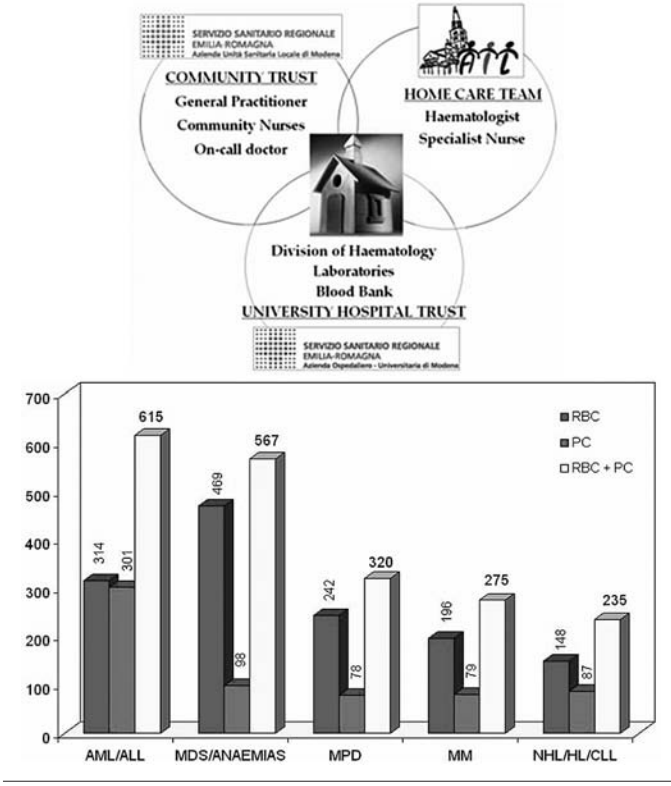

Figure 1.

0431

THE BURDEN OF CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP) IN ADULTS: RESULTS OF A DELPHI PANEL OF 32 EUROPEAN CLINICAL EXPERTS

F. Rodeghiero,[1] M. Michel,[2] J. Besalduch,[3] D. Provan,[4] M. Rummel,[5] M. Aivado,[6] K. Grotzinger,[6] N.L. Papo,[6] S. Stamenitis,[7] N. Höbel[7]

[1]Ospedale S Bartolo di Vicenza, VICENZA, Italy; [2]Henri Mondor Hospital, CRETEIL, France; [3]Hospital San Dureta, PALMA DE MALLORCA, Spain; [4]Queen Mary's School of Medicine and Dentistry, LONDON, UK; [5]Universitaetsklinikum Giessen und Marburg GmbH, GIESSEN, Germany; [6]Glaxo-SmithKline Research & Development, COLLEGEVILLE, USA; [7]Kendle GmbH, MUNICH, Germany

Background. The investigation and management of patients with chronic adult ITP varies widely and there is a lack of data on current treatment strategies in Europe. *Aims.* To provide insights into current treatment practices and preferred treatment strategies for chronic ITP in Europe (France, Germany, Italy, Spain and the UK). *Methods.* This is part of a comprehensive project investigating the burden of chronic ITP which involves both expert opinion and real patient data. Treatment centres with large ITP patient loads were identified in each country. A physician from each centre and experienced in treating ITP was invited to participate in a Delphi panel. The Delphi Method is a structured process for collecting and distilling knowledge from a group of experts by means of a series of questionnaires interspersed with controlled opinion feedback. Thirty-two haematologists/oncologists (France 8, Germany 8, Italy 7, Spain 7 and UK 2) were interviewed individually and remained anonymous to each other. The interviews consisted of 49 questions (43 of which were open-ended) about ITP treatment patterns, evaluation of current treatment options and rationale for ITP treatment choices. The questionnaire was developed by 5 ITP clinical experts in conjunction with a clinical research organisation (CRO) and study sponsor, and was administered by the CRO. *Results.* There was strong consensus for the use of corticosteroids as a first choice treatment (29/32, 91%) to prevent bleeding in non-splenectomised patients and to a lesser extent in splenectomised patients (66%). Physicians focused on increasing patients' platelet levels, but did not agree on a specific target level (from 10,000 - 30,000/μL, median 20,000/μL). The second choice treatments were splenectomy for 75% of experts, IVIG for 72% and rituximab for 25%. If patients were already splenectomised, the second choice treatments were immunosuppressants for 69% of experts, IVIG for 50%, rituximab for 44% and others (danazol, dapsone) for 19%. The physicians' choice of treatment was based on their prior clinical experience (91% of experts) and on official guidelines (American Society of Hematology, 72% of experts). The experts perceived patients' comorbidities (75% of experts) and refusal of splenectomy (75% of experts) as their main reasons for deviating from their usual patient management. Improved treatment tolerability was reported as the most important unmet medical need in chronic ITP, because of the poor side effect profile of current treatments (88% of experts). The greatest impact of chronic ITP on patients in terms of health-related quality of life (HRQoL) was perceived by the experts to be patients' fear of bleeding (66% of experts) and treatment side effects (60% of experts). The impact of ITP on HRQoL was compared to that of diabetes (53% of experts) and chronic heart disease (38% of experts). Conclusion: Corticosteroids were the most commonly used treatment for chronic ITP across all participating countries. The positioning of splenectomy and rituximab in treatment strategies was more controversial and differed among countries. Physicians highlighted the need for new ITP treatments with improved tolerability. This study provided some of the first insights into current ITP treatment practices and perceptions in Europe.

Non-Hodgkin's lymphoma - Indolent

0432

PHASE 3 STUDY OF PATIENTS WITH RELAPSED, REFRACTORY MANTLE CELL LYMPHOMA: COMPARISON OF TREATMENT WITH TEMSIROLIMUS *vs* INVESTIGATOR'S CHOICE THERAPY

E. G. Verhoef,[1] G. He,[2] J. Romaguera,[3] R. Herbrecht,[4] M. Crump,[5] A. Strahs,[6] O. Hanushevsky,[6] F. Binlich,[6] B. Hewes,[6] B. Coiffier[7]

[1]University Hospital Gasthuisberg, LEUVEN, Belgium; [2]Johannes Gutenberg-Universität, MAINZ, Germany; [3]MD Anderson Cancer Center, HOUSTON, TX, USA; [4]Hôpital de Hautepierre, STRASBOURG, France; [5]Princess Margaret Hospital, TORONTO, Canada; [6]Wyeth Research, CAMBRIDGE, MA, USA; [7]Hôpital Lyon Sud, PIERRE BÉNITE, France

Background. Mantle cell lymphoma (MCL) is characterized by an 11;14 translocation that results in overexpression of mRNA of cyclin D1, a key cell cycle regulatory protein. Translation of cyclin D1 mRNA is regulated by the mammalian target of rapamycin (mTOR). Temsirolimus, a specific inhibitor of mTOR, has produced tumor responses in patients with relapsed MCL (Witzig *et al.* J Clin Oncol 23:5347, 2005). *Aims.* In this phase 3, randomized, open-label study, we compared the antitumor activity of temsirolimus with investigator's choice therapy (IC) in patients with relapsed and/or refractory MCL. *Methods.* Patients with confirmed MCL must have received 2-7 prior lines of therapy and received an alkylating agent, an anthracycline, and rituximab. All patients provided written informed consent. Patients were randomly assigned (1:1:1) to 1 of 2 schedules of IV temsirolimus, 175 mg weekly 3 times followed by either 75 mg (175/75 mg, arm 1) or 25 mg (175/25 mg, arm 2) weekly, or to IC (arm 3), which included the single agents gemcitabine (42%); fludarabine (26%); chlorambucil, cladribine, etoposide (6% ea); cyclophosphamide, thalidomide, vinblastine (4% ea); or alemtuzumab, lenalinomide (2% ea). The primary endpoint was progression-free survival (PFS) based on central independent review of radiologic and clinical data. Accrual of 177 patients to achieve 105 PFS events was planned to test the hypothesis that temsirolimus would increase PFS compared with IC. Power was 80% and α=0.025 comparing temsirolimus with IC (assuming medians of 6.2 and 3.0 months, respectively). *Results.* We report final results for 162 patients (54 patients per arm): median age 67 years, 81% male, 50% >3 prior regimens, 32% prior stem cell transplantation, and a median of 2 prior rituximab and other immunotherapy regimens.

Table 1.

Parameter	Temsirolimus		IC
	175/75 mg Arm 1	175/25 mg Arm 2	Arm 3
Progression-free survival, independent assessment			
Median mo (97.5% CI)	4.8 (3.1, 8.1)	3.4 (1.9, 5.5)	1.9 (1.6, 2.5)
Increase in median *	153%	79%	
HR (97.5% CI)*	0.44 (0.25, 0.78)	0.65 (0.39, 1.10)	
p-value, log-rank test*	0.0009	0.0618	
Overall survival			
Median mo (95% CI)	10.9 (8.1, 14.1)	8.5 (5.8, 14.0)	5.8 (4.8, 12.4)
Increase in median*	88%	47%	
HR (95% CI)*	0.62 (0.37, 1.05)	0.80 (0.48, 1.33)	
p-value, log-rank test*	0.0714	0.3876	
Obj response rate (95% CI)	22% (11, 33)	6% (0, 12)	2% (0, 5)
p-value, Fisher's exact test*	0.0019	0.6179	

*Arm 1 or arm 2 : arm 3

After 105 PFS events, patients treated with temsirolimus 175/75 mg had significantly longer PFS than those treated with IC (p=0.0009, Table 1); patients treated with temsirolimus 175/25 mg did not have significantly longer PFS than those treated with IC (p=0.0618). Patients treated with temsirolimus 175/75 mg showed a trend toward longer overall survival compared with those treated with IC (p=0.0714). The objective response rate for patients treated with temsirolimus 175/75 mg and IC was 22% and 2%, respectively (p=0.0019). The most frequently occurring grade 3 or 4 adverse events were thrombocytopenia (arm 1: arm 2: arm 3, 59%: 52%: 36% patients), anemia (20%: 11%: 17%), neutropenia (15%: 22%: 26%), and asthenia (13%: 19%: 8%). The mean relative dose intensity (actual dose/assigned dose) for the 3 doses of temsirolimus 175 mg for arms 1 and 2 was 0.74 and 0.70, respectively, and for temsirolimus 75 mg and 25 mg was 0.69 and 0.86, respectively. *Conclusions.* Administration of IV temsirolimus, 175 mg per week for 3 weeks followed by weekly 75-mg doses, to patients with relapsed, refractory MCL resulted in an acceptable safety profile and significantly increased PFS and objective response rate compared with IC.

0433

RESULTS FROM THE RANDOMIZED PHASE 3 FIRST-LINE INDOLENT TRIAL (FIT) OF CONSOLIDATION OF FIRST REMISSION WITH 90Y-IBRITUMOMAB TIUXETAN IN ADVANCED FOLLICULAR NON-HODGKIN'S LYMPHOMA (FL)

J.A. Radford,[1] F. Morschhauser,[2] A. Van Hoof,[3] U. Vitolo,[4] P. Soubeyran,[5] H. Tilly,[6] P.C. Huijgens,[7] A. Kolstad,[8] M. Kunz,[9] A. Hagenbeek[10]

[1]Christie Hospital, MANCHESTER, UK; [2]Hôpital Huriez, LILLE, France; [3]General Hospital St-Jan, BRUGGE, Belgium; [4]Azienda Universitaria Ospedaliera S.Giovanni Battista, TORINO, Italy; [5]Institut Bergonié, BORDEAUX, France; [6]Centre Henri Becquerel, ROUEN, France; [7]Vrije Universiteit Medisch Centrum, AMSTERDAM, Netherlands; [8]The Norwegian Radium Hospital, OSLO, Norway; [9]Bayer Schering Pharma AG, BERLIN, Germany; [10]UMC Utrecht/HOVON, UTRECHT, Netherlands

Background. Radiolabeled monoclonal antibodies are highly effective in first-line treatment of FL and may improve the quality of response when used in consolidation therapy. *Aims.* We conducted an international, prospective, controlled, randomized phase 3 trial to evaluate the efficacy and safety of consolidation with 90Y-ibritumomab tiuxetan (Zevalin; Zev) radioimmunotherapy in patients with advanced FL in first remission after induction treatment. This study evaluated Zev for an off-label indication. *Methods.* Major patient eligibility criteria included: CD20+ grade 1 or 2 FL, stage III/IV at diagnosis, normal peripheral blood cell counts, <25% bone marrow involvement, CR/CRu or PR after first-line induction therapy, and informed consent. After induction, patients were randomized to receive either Zev (250 mg/m^2 rituximab on day -7 and day 0 followed on day 0 by Zev 14.8 MBq/kg; max 1184 MBq) or no further treatment (control). The primary end point was progression-free survival (PFS), calculated from the time of randomization (approximately 2 weeks prior to Zev administration). *Results.* 414 patients (Zev, n=208; control, n=206) were enrolled at 77 centers in 13 European countries and Canada. Patient demographics (Zev/control) were: median age 55/53 yrs; stage IV 64%/66%; B-symptoms 22%/20%; high-risk FLIPI 24%/21%. Induction therapies were also well balanced and included: chlorambucil n=39; CVP n=106; CHOP n=122; CHOP-like n=61; fludarabine combinations, n=22; rituximab combinations, n=59. After a median observation period of 3.5 yrs, the median PFS significantly increased from 13 mo (control) to 37 mo (Zev; p<0.0001; hazard ratio [HR] 0.465). For patient subgroups in PR or CR after induction, median PFS was 6 *vs* 29 mo (p<0.0001; HR 0.304) and 29.5 *vs* 54 mo (p=0.0154; HR 0.613), respectively. FLIPI scores were retrospectively determined for 71% of patients in this study. For the subgroup with low-risk FLIPI, median PFS was 24 mo in the control arm and not reached in the Zev consolidation arm (p=0.0502; HR 0.599). For patient subgroups with intermediate-risk and high-risk FLIPI scores, median PFS was 11 *vs* 54 mo (p<0.0001; HR 0.227) and 6.5 *vs* 24 mo (p=0.0789; HR 0.587), respectively. After Zev consolidation, 77% of patients in PR after induction converted to CR. As expected, toxicity associated with Zev was primarily hematologic, and grade 3 or 4 infections occurred in 8% of patients (2% in the control arm). *Conclusions.* In patients with advanced FL responsive to first-line induction treatment, consolidation with Zev resulted in high PR to CR conversion rates regardless of type of first-line induction treatment, prolonged PFS by 2 years overall, and extended PFS in all FLIPI subgroups.

0434

BRIEF CHEMOIMMUNOTHERAPY WITH RITUXIMAB (R)-FND ± R MAINTENANCE AS FIRST LINE TREATMENT IN ADVANCED FOLLICULAR LYMPHOMA (FL) IN ELDERLY: PRELIMINARY ANALYSIS OF A PROSPECTIVE RANDOMIZED TRIAL

U. Vitolo,[1] M. Ladetto,[1] C. Boccomini,[1] E. Gamba,[2] I. Alvarez,[1] L. Baldini,[1] M. Ceccarelli,[3] A. Chiappella,[1] P. Corradini,[1] A. De Renzo,[1] F. Di Raimondo,[1] A. Gallamini,[1] A. Guarini,[1] B. Mantoan,[1] M. Martelli,[1] V. Naso,[1] G. Parvis,[1] M. Petrini,[1] P. Pinto,[1] S. Pozzi,[1] A. Pulsoni,[1] L. Rigacci,[1] C. Tarella,[1] A. Tucci,[1] F. Zaja,[1] E. Gallo[1]

[1]Intergruppo Italiano linfomi (IIL), Hematology, S Giovanni Battista, HOSPITAL AND UNIVERSITY TORINO; [2]Roche, MONZA; [3]Unit of Cancer Epidemiology, CPO Piemonte, TORINO, Italy

Background. FL is common in elderly and the aim of treatment is usually palliation. *Aims.* in order to plan a treatment specifically devised for elderly patients with a reduced amount of chemotherapy, we investigated the efficacy and safety of a brief chemo-immunotherapy R-FND + Rituximab consolidation followed by randomization between Rituximab maintenance or observation. *Methods.* From January 2004 to December 2007, 235 patients (age 60-75) with untreated advanced stage FL were enrolled into 33 IIL centres. Patients gave their written informed consent. Treatment plan was: 4 courses of R-FND (Rituximab 375 mg/m^2 day 0, Fludarabine 25 mg/m^2 dd1-3, Mitoxantrone 10 mg/m^2 day 1, Dexamethasone 10 mg dd 1-3) every 28 days followed by 4 weekly Rituximab (375 mg/m^2); responding (CR+CRu+PR) patients were randomized to receive Rituximab maintenance (375 mg/m^2 every 2 months for 4 doses) or observation. PCR analysis for IgH/Bcl-2 rearrangement was performed on bone marrow (BM) samples at diagnosis, after R-FND, after Rituximab consolidation and during maintenance/observation phase. An interim analysis was planned to evaluate safety and response of chemo-immunotherapy before randomization, after the first 80 randomized pts (January 2004 to December 2005). This analysis included 95 patients recruited within this time frame. *Results.* Median age was 65 (60-75); advanced stage II 14%, stage III 16% and stage IV 70%; 63% had BM involvement and 20% B symptoms; FLIPI Low 10%, Intermediate 30%, High 60%. PCR analysis was done in 91 patients at diagnosis: 56% were Bcl-2+. Eighty patients were randomized between maintenance or observation; 15 patients were not due to: progressive disease 6, stable disease 2, adverse events 3, concomitant neoplasia 1, withdrawal of consent 2, lost 1. Overall response, CR rate and PCR negativity associated with CR after R-FND and Rituximab consolidation are shown in the following Table 1.

Table 1.

	N	ORR	CR	PCR negativity associated to CR (46 patients Bcl2+ evaluable)
Baseline	95	-	-	-
R-FND x 4	95	86 (90%)	51 (54%)	18 (39%)
Rituximab Consolidation	88	80 (84%)	68 (72%)	31 (67%)

Nineteen patients (54%) of 35 PRs after R-FND were converted to CR with Rituximab consolidation. PCR negativity increased from 39% to 67% with Rituximab consolidation after R-FND. A high CR rate was achieved also in poor prognosis patients (CR according to FLIPI: Low 75%, Intermediate 88%, High 81%). The most frequent CTC grade 3-4 toxicity was neutropenia in 25% of the courses, with only 6 serious infections. Pulmonary and cardiac toxicities were < 1%. No toxic deaths occurred. Any grade Rituximab related reactions were seen in 9% of the courses with Rituximab discontinuation in only 2 patients. So far 91 patients are alive and 4 died of lymphoma. *Conclusions.* A brief chemo-immunotherapy R-FND + Rituximab consolidation is safe and effective with a high CR rate and PCR negativity in elderly patients with untreated FL even in FLIPI high risk. Rituximab consolidation converted to CR 54% of PRs after R-FND and increase Bcl2 rearrangement negativity rate. The whole study will provide insights on the role of Rituximab maintenance after R-chemotherapy.

0435

ROMIDEPSIN INDUCES CLINICALLY SIGNIFICANT AND DURABLE RESPONSES IN RELAPSED OR REFRACTORY CTCL: A NCI INTERNATIONAL, MULTICENTER STUDY

S. Bates,[1] R. Piekarz,[1] R. Frye,[1] S. Allen,[2] M. Craig,[3] L. Geskin,[4] L. Hutchins,[5] D. Joske,[6] M. Kirschbaum,[7] J. Leonard,[8] M. Prince,[9] C. Reeder,[10] J. Nichols[11]

[1]National Cancer Institute, BETHESDA, USA; [2]North Shore Univ Hospital, MANHASSET, USA; [3]West Virginia University, MORGANTOWN, USA; [4]University of Pittsburgh, PITTSBURGH, USA; [5]Arkansas Cancer Research Center, LITTLE ROCK, USA; [6]Sir Charles Gairdner Hospital, PERTH, Australia; [7]City of Hope Cancer Center, DUARTE, USA; [8]Cornell University, NEW YORK, USA; [9]Peter MacCallum Cancer Centre, EAST MELBOURNE, Australia; [10]Mayo Clinic, SCOTTSDALE, USA; [11]Gloucester Pharmaceuticals, CAMBRIDGE, USA

Background. Romidepsin, an HDAC inhibitor, has previously demonstrated activity as a single agent in patients with T-cell lymphomas. *Aims.* To evaluate the efficacy and tolerability of romidepsin in the treatment of advanced CTCL. *Methods.* A Phase II, open-label, multi-arm multicenter study enrolled 71 CTCL patients from the NCI and 9 extramural sites. Patients with relapsed or refractory CTCL (Stages IA-IVB) with either < 2 prior systemic therapies (Arms 1, 5) or > 2 prior systemic therapies (Arm 3), received romidepsin at 14 mg/m^2 over 4 hrs on Days 1, 8 and 15 q 28 days. Responses were assessed by Investigators using a composite endpoint that evaluated changes in skin (via an overall skin assessment tool), lymph nodes, extranodal visceral lesions and abnormal circulating T-cells. Separately, Investigators assessed responses using the composite endpoint with skin changes based on target lesion measurements. To account for inter-observer variability, disease response was also assessed by an Independent Response Review Committee (IRRC) that was composed of 3 physicians who were experts in the field of CTCL. *Results*. 71 patients [48(67.6%) male and 23(32.4%) female; mean age of 56.0 + 13.0 yrs] were eligible. 63 patients were included in the evaluable population (defined as having received a minimum of two cycles of therapy). The mean number of prior therapies received was 3.4 (range 1-10), with patients receiving on average 2.4 (range 1-7) prior systemic and 1.8 (range 1-3) skin-directed therapies. Objective response rates (ORR) in the evaluable population are summarized in the Table 1 below by stage.

Table 1.

Clinical Stage	N	ORR (CR + PR)	CR	PR
ALL	63	25 (39.7%)	4 (6.3%)	21 (33.3%)
IA-IIA	8	5 (62.5%)	0 (0.0%)	5 (62.5%)
IIB-IIIB	21	10 (47.6%)	1 (4.8%)	9 (42.9%)
IVA-IVB	34	10 (29.4%)	3 (8.8%)	7 (20.6%)

IRRC determined ORR was 29% (18/63). ORR using the composite endpoint with skin changes based on target lesion measurement was 35% (23/65). The median duration of response was 336 days (11 months) and the maximum duration of response as of data cut-off was 5.5+ years. Treatment emergent study drug-related AEs were reported in 70 patients, with Grade 3 and Grade 4 events reported in 58 (81.6% and 15 (21.1%) patients, respectively. Two (2) deaths were considered possibly related to treatment. The most frequent drug-related AEs (all grades) were nausea (81.7%), fatigue (73.2%), electrocardiogram T-wave amplitude decreased (69.0%), hemoglobin decreased (59.2%), platelet count decreased (59.2%), anorexia (53.5%) and hypocalcaemia (53.5%). Serious AEs considered possibly, probably or likely related to treatment were reported in 26 (36.6%) patients. *Conclusions.* This study demon-

strates durable clinical benefit (ORR of 39.7% and median duration of response of 11 months) and tolerability of romidepsin in patients with recurrent or refractory CTCL. Significant responses were observed at all stages of disease, including an ORR of 29.4% in Stage IV patients.

0436

SUMMARY OF THE CLINICAL BENEFIT OF VORINOSTAT IN RELAPSED/REFRACTORY CTCL

E.A. Olsen,[1] S. Rizvi,[2] J. Garcia-Vargas,[2] C. Sanz-Rodriguez,[2] J. Viscusi,[2] M. Gates,[2] M. Duvic[3]

[1]Duke University Medical Center, DURHAM; [2]Merck Research Laboratories, UPPER GWYNEDD; [3]MD Anderson Cancer Center, HOUSTON, USA

Background. Vorinostat is an orally active, potent inhibitor of histone deacetylase. Two completed Phase II studies have evaluated the clinical benefit of vorinostat in patients with cutaneous T-cell lymphoma (CTCL). *Aims*. Here we report corroborative conclusions on the efficacy and safety of vorinostat in CTCL based on the results from two Phase II studies. *Methods*. An initial study exploring several vorinostat dosing regimens and a second pivotal study using a 400 mg qd dose, enrolled patients with advanced CTCL who were refractory to and/or intolerant of other therapies. Patients in the initial study must have received ≥1 prior systemic therapy for CTCL compared with ≥2 in the pivotal study. Only those patients in the initial study that were treated with vorinostat 400 mg qd (the FDA-approved dose of vorinostat in CTCL) are included in the comparison. The primary endpoint in both studies was objective response rate (ORR). *Results.* The two studies were similar with respect to baseline characteristics. A total of 87 patients were included in the combined analysis of the initial and pivotal studies (13 and 74 patients, respectively). At baseline, 72 patients had CTCL Stage IIB or higher. The median number of prior systemic therapies at baseline was five in the initial study *vs* three in the pivotal study. ORRs were comparable between the patients taken from the initial study and the pivotal study overall (30.8% and 29.7%, respectively) and in patients with Stage IIB or higher (36.4% and 29.5%, respectively). In addition, several patients who did not meet the objective response criteria achieved pruritus relief and/or prolonged stable disease. Median time to objective response ranged from approximately 2 to 3 months; 88 days and 55 days in the initial and pivotal studies, respectively. Median duration of response (DOR) was 113 days in the initial study *vs* in excess of 4 months (upper range: 322 days) in the pivotal study (DOR not reached, study ongoing for duration). The median time to progression was clinically meaningful in the overall patient population for both studies (initial study: 85 days; pivotal study: not reached, but estimated to exceed 5 months). Of particular interest was the clinically meaningful reduction in pruritus in patients who were symptomatic at baseline in the initial and pivotal studies (72.7% and 31.9%, respectively reported pruritus relief). Furthermore, complete resolution of pruritus was observed in 9.1% and 11.1% of patients, respectively. Three drug-related grade 3/4 AEs were observed in the initial study: thrombocytopenia, anemia and dehydration (all 8%). Twenty-one patients (28%) in the pivotal study had drug-related grade 3/4 AEs. The most common were fatigue (5%), pulmonary embolism (5%), thrombocytopenia (5%), and nausea (4%). One patient in the initial study and 9 in the pivotal study discontinued due to AEs. *Conclusions.* Even though required prior treatment and the method for evaluating response differed between the two studies, response rates were similar and clinically meaningful in a substantial proportion of patients with CTCL (Stage IB and above), who had progressive, persistent, or recurrent disease subsequent to prior therapies.

Multiple myeloma: improving standard therapy for elderly

0437

A PHASE IB, MULTI-CENTER, OPEN-LABEL, DOSE-ESCALATION STUDY OF ORAL PANOBINOSTAT (LBH589) AND I.V. BORTEZOMIB IN ADULT PATIENTS WITH MULTIPLE MYELOMA (MM)

O. Sezer,[1] M. Kaiser,[1] D. Siegel,[2] I. Prosser,[3] M.V. Mateos,[4] M. Kangas,[5] M. Jalaluddin,[6] P. Bourquelot,[5] J. Bladé,[7] J. San Miguel,[4] K. Anderson,[8] J. Bradner[8]

[1]Charité, BERLIN, Germany; [2]Hackensack University Medical center, HACKENSACK, NJ, USA; [3]The Canberra Hospital, CANBERRA, Australia; [4]Hospital Universitario de Salamanca, SALAMANCA, Spain; [5]Novartis Pharma AG, BASEL, Switzerland; [6]Novartis Pharmaceuticals Corp, FLORHAM PARK, NJ, USA; [7]Hospital Clinic I Provincial de Barcelona, BARCELONA, Spain; [8]Dana Farber Cancer Institute, BOSTON, MA, USA

Background. Panobinostat (LBH589) is a pan-deacetylase inhibitor (pan-DACi) targeting epigenetic and multiple oncogenic pathways. Pan-DACi activity has been associated with a decrease in oncoprotein stability (BCR-ABL, HER-2, AR, ER) via HSP-90 inhibition, tumor cell motility and invasion (α tubulin), and angiogenesis (HIF-1α). Cell death is also promoted by induction of apoptosis (P53) and cell cycle arrest mediated by histone hyperacetylation. Currently the agent is under clinical investigation as a single agent and in combination for a range of solid and hematologic malignancies. *In vitro* studies have shown panobinostat to induce cytotoxicity at low nanomolar concentrations in multiple myeloma (MM) cell lines resistant to dexamethasone, melphalan and doxorubicin. Additional *in vitro* and *in vivo* experiments have demonstrated potent synergy of panobinostat with bortezomib via cytotoxicity and MM tumor growth delay. Collectively, these data provide a strong rationale for the first clinical trial of this combination in MM patients. *Aims.* The primary objective of this phase I study is to establish the maximum tolerated dose (MTD) of panobinostat with bortezomib in a second-line setting. Safety, tolerability, pharmacokinetic pharmacodynamic profiles and preliminary efficacy of the combination will be assessed as secondary objectives. *Methods.* Up to 40 adult patients with relapsed MM (IMWG criteria) who have received at least one prior line of therapy, will be enrolled. Key exclusion criteria include prior exposure to an HDACi, primary refractory MM, peripheral neuropathy (greater than Grade 1) and impaired cardiac function. Informed consent is required of all patients. The study consists of dose-escalation and dose-expansion components. Escalation commences with 10mg of panobinostat administered 3 times weekly in combination with 1.0mg/m^2 of bortezomib on Days 1, 4, 8 and 11 over a 21 day cycle. A six-parameter, adaptive Bayesian logistic regression model guides the escalation to MTD with each dose-combination being studied in cohorts of six patients. Dose-limiting toxicities (DLT) are defined as adverse events or abnormal laboratory values assessed as clinically relevant, occurring during Cycle 1, and meeting pre-specified criteria. At the MTD, additional patients will be enrolled into a dose-expansion cohort to obtain further safety and tolerability information. For patients with worsening disease after Cycle 1, dexamethasone can be added. Responses are assessed using the International Uniform Response Criteria for MM. *Results.* As of 11 February 2008, Cohort 1 (10mg LBH589 + 1.0mg/m^2 bortezomib) has been successfully completed with none of six patients enrolled experiencing DLT. Hematological AEs included CTCAE Gr 2 anemia, Gr 2-4 neutropenia and thrombocytopenia (1 patient required a single platelet transfusion). Non-hematological AEs included fatigue (1; CTCAE Gr 3) and infection (1); no grade 4 events were recorded. A total of five SAEs have occurred during cycle 1, with one event (hospitalization due to pyrexia and pain) considered to be study treatment-related (bortezomib only). Given the observed safety data, panobinostat dose has been escalated to 20 mg in the combination treatment with bortezomib in cohort 2. Updated information on this and any subsequent cohorts will be presented. *Conclusions.* 10 mg panobinostat with 1.0mg/m^2 bortezomib (first dose level) was well tolerated.

0438

BORTEZOMIB, PEGYLATED-LYPOSOMAL-DOXORUBICIN AND DEXAMETHASONE AS INDUCTION PRIOR TO AUTOLOGOUS TRANSPLANT, FOLLOWED BY LENALIDOMIDE AS MAINTENANCE IN ELDERLY NEWLY DIAGNOSED MYELOMA PATIENTS

A. Palumbo,[1] P. Falco,[1] C. Crippa,[2] V. Montefusco,[2] F. Patriarca,[2] M. Ruggeri,[1] A. Larocca,[1] F. Cavallo,[1] A.M. Liberati,[2] F. Rossini,[2] N. Giuliani,[3] T. Guglielmelli,[2] G. Benevolo,[2] C. Cangialosi,[2] T. Caravita,[2] G. Rossi,[2] P. Corradini,[4] M. Boccadoro[1]

[1]A.O.U. San Giovanni Battista, TORINO; [2]Italian Multiple Myeloma Network, GIMEMA, ITALY; [3]Cattedra e UO Ematologia e Trapianto Midollo, Università degli Studi di Parma, PARMA; [4]Divisione Ematologia, Istituto Nazionale Tumori, MILANO, Italy

Background. New agents have been introduced as induction treatment prior to autologous stem cell transplant (ASCT) and as consolidation or maintenance thereafter to improve complete response (CR) rates. Melphalan at 100 mg/m^2 has been suggested as reduced-intensity conditioning regimen for elderly patients. *Aims.* In this prospective multicenter phase II study, we evaluate Bortezomib in combination with Pegylated-Lyposomal-Doxorubicin and Dexamethasone (PAD) as induction before reduced-intensity ASCT, followed by Lenalidomide and Prednisone (LP) as consolidation and Lenalidomide alone as maintenance in elderly multiple myeloma (MM) patients. Primary endpoints were safety (any Grade-3 non-hematologic toxicity < 30%) and efficacy (near complete response rate, nCR > 35%). *Methods.* Newly diagnosed MM patients aged 65-75 years were eligible. The induction included four 21-day PAD cycles (Bortezomib 1.3 mg/m^2 on days 1, 4, 8, 11, Pegylated-Liposomal-Doxorubicin 30 mg/m^2 on day 4 and Dexamethasone 40 mg/day on days 1-4, 8-11, 15-18 for cycle 1 and on days 1-4 for cycles 2-4). Cyclophosphamide (3 g/m^2) plus G-CSF was used to harvest stem-cells. Patients were then conditioned with tandem Melphalan 100 mg/m^2 and stem-cell support (MEL100). After ASCT patients received four 28-day LP cycles (Lenalidomide 25 mg/day on days 1-21 plus Prednisone 50 mg every other day) followed by Lenalidomide alone (10 mg/day on days 1-21 every 28 days) as maintenance. *Results.* One-hundred and one patients (median age 67) were enrolled. After the 4 PAD cycles 95% of patients achieved at least partial response (PR), 60% at least very good partial response (VGPR), 23% at least nCR, and 13% CR. After tandem MEL100, 95% of patients showed PR, 80% at least VGPR, 60% at least nCR, and 33% CR. After LP consolidation regimen all patients achieved PR, 89% at least VGPR, 78% at least nCR, and 56% CR. During induction therapy, 25% of patients experienced grade 3-4 hematologic toxicity therapy, while more frequent grade 3-4 non-hematologic toxicities were peripheral neuropathy (17%) and infections (11%). During LP consolidation one DVT and one discontinuation due to prolonged anemia and thrombocytopenia were recorded. *Conclusions*. PAD as induction pre ASCT followed by LP as consolidation induced a high response rate with a 56% CR rate recorded at the end of a reduced intensity ASCT regimen for elderly patients. Updated results will be presented at the meeting.

0439

RECOVERY OF RENAL IMPAIRMENT BY BORTEZOMIB-DOXORUBICIN-DEXAMETHASONE (BDD) IN MULTIPLE MYELOMA (MM) PATIENTS WITH ACUTE RENAL FAILURE. RESULTS FROM AN ONGOING PHASE II STUDY

P. Ludwig,[1] Z. Adam,[2] R. Greil,[3] F. Keil,[4] N. Zojer,[5] J. Thaler,[6] H. Gisslinger,[7] A. Lang[8]

[1]Wilhelminenspital, VIENNA, Austria; [2]Department of Hematooncology, University Hospital,, BRNO, Czech Republic; [3]Department of Internal Medicine III, Hospital Salzburg, SALZBURG, Austria; [4]Department of Internal Medicine and Intensive Care, Hospital Leoben, LEOBEN, Austria; [5]Department of Medicine I, Wilhelminenspital, VIENNA, Austria; [6]Department of Internal Medicine IV, Hospital of Mercy Sisters, WELS, Austria; [7]Department of Internal Medicine I, University Hospital Vienna, VIENNA, Austria; [8]Department of Internal Medicine, Hospital Feldkirch, FELDKIRCH, Austria

Background. Acute light chain induced renal failure (ARF) is a severe complication of progressive MM, often leading to permanent renal dysfunction and dependence on chronic hemodialysis in a substantial proportion of patients. Reversal of kidney failure can only be achieved by fast and substantial suppression of pathogenic light-chains by effective anti-MM therapy. *Aims.* To evaluate the efficacy of the BDD regimen in restoring renal function and in achieving tumor control. *Methods.* Up to now 57 patients have been enrolled. Documentation is available for 40 patients (age: median 64 years, range 41-82 years, DS stage I: 4%, II: 10%, III: 76%. 60% of pts presented with *de novo* MM, and 40% with progressive disease. ARF was defined in newly diagnosed patients as reduction of GFR to <50mL/min due to MM nephropathy and in previously treated pts with signs of tumor progression and a GFR of >60 mL/min within the last 4 weeks as a reduction of GFR by >25% to <60 mL/min. Treatment regimen: Bortezomib (1.3 mg/m^2, d 1, 4, 8, 11 until the first safety analysis and thereafter of 1.0 mg/m^2 d 1, 4, 8, 11), doxorubicin (9 mg/m^2, d 1, 4, 8, 11 until first safety analysis and thereafter of 9 mg^2, d 1, and 4) and dexamethasone 40 mg (d 1, 4, 8, 11). Cycles were repeated every 21 days. *Results.* 32 patients have completed at least 2 cycles and are evaluable for response as yet. Nine patients achieved CR/nCR, 9 VGPR, 4 PR and 2 MR (CR-PR: 69%, CR-MR: 75%): Median GFR at baseline was 16.8 mL/min (range: 4 to 48mL/min) and improved to 154mL/min (range: 19 - >180mL/min). Improvement of GFR correlated weakly with tumor response. Median GFR was 59 mL/min (19->180 mL/min) in 18 pts with CR/nCR and VGPR, 35 mL/min (20->180 mL/min) in 4 pts with PR, and 46/min (39-43mL/min) in 2 pts with MR. Toxicity was assessed in 29 pts. Three pts died during the first treatment cycle; 2 from pneumonia (including 1 with sepsis) and 1 pt (age 81 years) from myocardial infarction. Grade 3-4 toxicities: infections (16%), neutropenia (16%), cardiovascular (10%) and weakness (10%) and thrombopenia and acute hearing loss in 5%. Grade 1-2 toxicities: infections 21%, neutropenia 16% and weakness, diarrhoea, GI bleeding in 5%. Of note, 4 of the 7 infectious complications were due to herpes virus infections/reactivations. This led to a protocol amendment with reduction of doxorubicin treatment to days 1 and 4 (instead of d 1, 4, 8, 11) and of bortezomib dose reduction to 1.0mg/m^2 and to mandatory antibacterial and antiviral prophylaxis. *Summary*. Overall response rate was 75% (CR-MR), with 28% of the 32 evaluable pts achieving CR/nCR and 28% VGPR. A significant renal response (GFR >50mL/) was obtained in 14 (43%) and an >200% improvement in GFR to levels between 30-50 mLmin was noted in additional 6 (19%) patients. Renal response correlated weakly with myeloma response. Dose reduction of the regimen after initial untoward toxicity led to favourable tolerance in the subsequently treated patients.

0440

MELPHALAN + PREDNISONE *vs* MELPHALAN + PREDNISONE + THALIDOMIDE IN INDUCTION THERAPY FOR MULTIPLE MYELOMA IN ELDERLY PATIENTS: FIRST INTERIM RESULTS OF THE DUTCH COOPERATIVE GROUP HOVON

P. Wijermans,[1] M. Schaafsma,[2] Y. Van Norden,[3] R. Ammerlaan,4 P. Sonneveld,[5] S. Wittebol,[6] H. Sinnige,[7] P. Huijgens,[8] M. van Marwijk Kooy,[9] R. van der Griend[10]

[1]Haga Hospital, THE HAGUE; [2]Mediasch Specrum Twente, ENSCHEDE; [3]HOVON datacentre, ROTTERDAM; [4]Hovon datacenter, ROTTERDAM; [5]Erasmus MC, ROTTERDAM; [6]Meander MC, AMERSFOORT; [7]Jeroen Bosch Hospital, DEN BOSCH; [8]Free University MC, AMSTERDAM; [9]Sophia Hospital, ZWOLLE; [10]Diakonessen Hospital, UTRECHT, Netherlands

Background. The Dutch cooperative group HOVON started a randomised phase III study in elderly myeloma patients in September 2002 comparing the standaard Melphalan and Prednisone treatment with the combination Melphalan, Prednison and Thalidomide (HOVON 49 study). Patients with a multiple myeloma > 65 years of age with a stage IB or higher were candidates for this study. *Methods*. Melphalan was given in a dose of 0.25 mg/kg and prednisone 1 mg/kg for 5 days every 4 weeks. Thalidomide was given daily with ina dose of 200 mg. A maximum of 8 cycles was planned. If there was still a response further therapy was allowed till a plateau phase was reached. When a good response and a plateauphase was reached the patients who were randomised for Thalidomide received maintenance therapy with Thalidomide 50 mg/day till progression of their disease. It was planned to enter 420 patients in the study. However accrual decreased significantly due to positive outcome of other studies with Thalidomide. Therefor the study was stopped after the inclusion of 344 patients. This is the first analysis based upon the data of the first 320 patients. *Results*. 344 patients were entered. The first 320 patients were analysed for this report. 7 patients were non-eligible due to either being stage IA or not having a measurable tumor parameter. From one patient there was not a signed informed consent available. Eleven patients were excluded from this analysis because of insufficient data available at the time of evaluation. Thus data are presented of 301 patients; 149 in the M+P arm and 152 in the M+P+T arm. The median age was 72 years in both groups. The arms were well matched for age, sex, stage of the disease, performance status and type of M-protein. The best response on protocol was as folows M+P response rate 47% (with a CR 1%, VGPR 8% and PR 38% respectively) and for M+P+T arm a response rate of 63% (with a CR 1%, VGPR 28% and PR 34%) which was significantly better (p<0.001). There was a significant difference in the Event Free Survival in favour of the M+P+T arm (p< 0.001) but no difference was observed for the Progression Free Survival (p=0.08) and Overall Survival (p=0.28). *Toxicity*. Only one third of all the patients received cycle 3 of Melphalan and Prednison according the planned protocol. In all the other patients the doses had to be reduced and or delayed. Grade 2,3 and 4 toxicity of any type was seen in 59% of the patients in the M+P arm and in 87% of the M+P+T patients. This difference was mainly due to grade 2 and grade 3 neurotoxicity. After three cycles only 36% of the patients used the full Thalidomide dose and after 6 cycles this was only 28%. No differences between the two arms were seen for other toxicities. *Conclusions*. In this randomised phase III study we did observe a significant improvement in the Resonse Rate, the quality of the responses and time to response in favour of the M+P+T arm. This did translate in an improvement of the Event Free Survival but not in the Overall Survival. The toxicity of the five days schedule with 0.25 mg/kg/day Melphalan led to a substantial number of patients who did not received the planned therapy. Thalidomide added significantly to the toxicity (mainly neurotoxicity) of the treatment. We were unable to confirm the positive effect with Thalidomide as part of front-line therapy on Overall Survival as it was seen in other studies

0441

OVERALL SURVIVAL WITH DEXAMETHASONE IN PHASE III MULTIPLE MYELOMA TRIALS AFTER ADJUSTMENT FOR CROSS-OVER TO LENALIDOMIDE

J. Morgan,[1] K. Ishak,[2] B. Deniz,[2] T. Drayson,[3] M. Dimopoulos,[4] M. Weber,[5] M. Augustson,[6] J. Child,[7] R. Knight,[8] G. Begum,[9] A. Dunn,[9] A. Shearer,[10] J. Caro[2]

[1]The Royal Marsden NHS Foundation Trust & The Institute of Cancer Research, SURREY, UK; [2]United BioSource Corporation, MONTREAL, Canada; [3]University of Birmingham, BIRMINGHAM, UK; [4]University of Athens School of Medicine, ATHENS, Greece; [5]Anderson Cancer Center, HOUSTON, TX, USA; [6]Sir Charles Gairdner Hospital, NEDLANDS, Australia; [7]University of Leeds, LEEDS, UK; [8]Celgene, SUMMIT, NJ, USA; [9]Warwick Medical School, COVENTRY, UK; [10]Celgene UK, LONDON, UK

Background. In pivotal trials (MM-009/010) evaluating lenalidomide plus high dose dexamethasone (Len+Dex) *vs* Dex alone, 47% of the latter switched to Len±Dex at disease progression or following ethical study unblinding. Given the significantly better efficacy of Len+Dex, survival with Dex alone is overestimated. *Aims.* Use external data on survival to adjust Dex survival for the cross-over to Len±Dex. *Methods.* Pooled data from the UK Medical Research Council (MRC) MM IV, V, VI, and VIII trials enrolled between 1980 and 1997 were used to derive an equation predicting survival with second-line conventional therapies (including VAD, ABCM, Melphalan and cyclophosphamide), according to patient and disease characteristics. Applying this equation to MM-009/010 Dex patients yielded their expected survival without cross-over to Len±Dex. This was used to estimate survival for this subgroup in a lifetime simulation by adjusting the scale parameter of the post-progression survival equation estimated from MM-009/010. Since survival patterns change with additional lines of treatment, further calibration was necessary for multiple prior therapies. As patient-level data for this subgroup were not available from MRC, published Mayo clinic data on median survival with conventional therapies for patients with two prior therapies (12.6 months) was used to adjust the predicted median from the trial-based, MRC-calibrated equation. The simulation model was then used to compare long-term survival with Len+Dex *vs* Dex alone without cross-over. *Results.* Of 873 MRC patients who initiated second-line conventional treatment, 826 had died, with 17.6 months median survival from starting second-line treatment. Survival did not differ significantly between Dex- and non-Dex-containing regimens (p-value=0.79). Exponential survival with age, performance status, M-protein, B2M and time to progression as predictors provided best fit to the data. Application using this equation to predict survival for MM-009/010 Dex patients with one prior therapy yielded a median survival of 16.2 months (95%CI: 13.1-20.1) compared to 33.6 months (95%CI: 27.1-NE) observed with cross-over to Len±Dex. The median survival for patients with multiple prior therapies was 12.6 months (95%CI: 10.2 - 15.6), compared to 27.3 months (95%CI: 23.3-33.3) with cross-over to Len±Dex. The calibrated lifetime simulation yielded estimated mean survival of 2.2 life-years with Dex alone compared with 5.6 life-years with Len+Dex for patients with one prior therapy, and 1.5 life-years for Dex alone compared with 4.2 life-years for Len+Dex for patients with multiple prior therapies. *Conclusions*. Lenalidomide delivers significantly larger survival gains in this life-limiting orphan disease when appropriate adjustment has been made for the cross-over that occurred in the trials.

Myeloproliferative disorders

0442

FAMILIAL ERYTHROCYTOSIS ASSOCIATED WITH A MUTATIONAL HOTSPOT IN THE HIF2A GENE

M.J. Percy,[1] P.A. Beer,[2] G. Campbell,[3] A.W. Dekker,[4] A.R. Green,[2] D. Oscier,[5] M.G. Rainey,[6] R. van Wijk,[4] M. Wood,[3] T.R.J. Lappin,[7] M.F. McMullin,[7] F.S. Lee[8]

[1]Belfast City Hospital, BELFAST, UK; [2]University of Cambridge, CAMBRIDGE, UK; [3]Essex Rivers Healthcare NHS Trust, COLCHESTER, UK; [4]University Medical Center Utrecht, UTRECHT, Netherlands; [5]Royal Bournemouth Hospital, BOURNEMOUTH, UK; [6]Vale of Leven District General Hospital, ALEXANDRIA, UK; [7]Queen's University of Belfast, BELFAST, UK; [8]University of Pennsylvania School of Medicine, PHILADELPHIA, USA

Background. Erythrocytosis is characterized by an increased hematocrit, a raised hemoglobin and variable serum erythropoietin (Epo) levels. It can arise from defects in the oxygen sensing pathway, which result in elevated Epo synthesis. Mutations have been previously described in both the von Hippel Lindau (VHL) and the prolyl hydroxylase domain 2 (PHD2) proteins in individuals with erythrocytosis. Epo synthesis is regulated by a negative feedback mechanism involving the kidneys and the hypoxia inducible factor (HIF). Although the alpha subunit of HIF is constitutively expressed it is degraded by the proteasome in the presence of oxygen. The prolyl hydroxylase domain family of proteins (of which PHD2 is a member) modify the proline in a LXXLAP (where underlining indicates site of hydroxylation) motif in HIF-α. This allows the VHL protein to bind and to ubiquitinate HIF-α for proteasonal degradation. There are three isoforms of HIF-alpha and mouse studies have indicated that HIF-2α is the critical regulator of Epo synthesis. However in man it is unresolved which isoform controls Epo production. Recently, we identified a heterozygous HIF-2alpha mutation, Gly537Trp, in three generations of one family with erythrocytosis. Functional studies confirmed this particular mutation impacted significantly on the ability of HIF-2alpha to be targeted to the proteasome, thereby stabilizing HIF-2α. *Aims.* To analyse the HIF2A gene for mutations in a cohort of erythrocytosis individuals with serum Epo levels above the normal range and establish whether HIF2A is a significant cause of erythrocytosis. *Methods.* DNA was isolated from peripheral blood and PCR-direct sequencing of exon 12 of the HIF2A gene was performed. *Results.* Sequencing the HIF2A gene identified four individuals with two novel heterozygous mutations (Met535Val and Gly537Arg). All patients presented with erythocytosis at a young age with raised serum Epo levels, similar to the first case of HIF-2alpha mutation. There is no evidence of a von Hippel Lindau like syndrome in these patients. Furthermore two individuals had a clinical history of thrombosis as seen with the first family who possessed the Gly537Trp HIF-2α mutation. *Summary*. To date four of the five independent HIF2A mutations are located at Gly-537 suggesting a mutational hotspot associated with erythrocytosis. Both Met-535 and Gly-537 amino acids are located close to the hydroxylacceptor Pro-531 in HIF-2alpha. Pro-531 forms part of the conserved LXXLAP motif but interestingly none of the mutations detected thus far directly involve this motif. Examination of the three-dimensional structure of VHL bound to hydroxylated HIF-1alpha suggests that these mutations may impact on the association of HIF-2alpha with VHL. Furthermore, these results support a major role for HIF-2alpha in the regulation of Epo production in man and identify HIF2A mutations as a significant cause of erythrocytosis.

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0443

THE ETV6-PDGFRβ AND FIP1L1-PDGFRα FUSION PROTEINS ESCAPE UBIQUITINATION AND DEGRADATION

F. Toffalini,[1] A. Kallin,[1] L. Michaux,[1] P. Vandenberghe,[1] P. Pierre,[2] J. Cools,[1] J.B. Demoulin[1]

[1]Université Catholique de Louvain, BRUSSELS; [2]Cliniques du Sud-Luxembourg, ARLON, Belgium

Chimeric receptor tyrosine kinase genes are created by chromosomal rearrangements associated with chronic leukemia. These genes encode fusion proteins in which the intracellular kinase domain is fused to the N-terminal part of a partner protein that is replacing the receptor ligand binding domain. The constitutive activation of the kinase domain in the hybrid leads to constitutive signaling and uncontrolled cell proliferation. The TEL-PDGFRB (TPβ) hybrid protein is produced by the t(5;12) translocation between TEL/ETV6 and the platelet-derived growth factor receptor beta (PDGFRB) genes, and it is found in a sub-set of patients with chronic myelomonocytic leukemia (CMML). FIP1L1-PDGFRA (FPα) results from a deletion on chromosome 4q12 and it is the hallmark of chronic eosinophilic leukemia (CEL). In contrast to wild-type PDGFRα and β, which are quickly degraded upon activation, we observed that TPβ and FPα escaped down-regulation resulting in stabilization of the proteins in Ba/F3 cells. High stability of FPα hybrid was confirmed in peripheral blood mononuclear cells derived from a patient with eosinophilic leukemia. Similar data were obtained in cells expressing ZNF198-FGFR1, another fusion protein associated with the 8p11 myeloproliferative syndrome. CBL-mediated ubiquitination of receptor lysines targets them for lysosomal degradation. Ubiquitination of TPβ and FPα was much reduced compared to wild-type receptors despite marked CBL phosphorylation in cells expressing hybrid receptors. Treatment with proteasome inhibitors slightly increased TPβ stability and revealed a modest TPβ polyubiquitination. Deletion of the pointed (PNT) domain in TPβ, which is required for its polymerization and activation, strongly destabilized the protein, indicating that TPβ clustering also prevents its degradation. Stability assays with TPβ and FPα kinase inactive forms revealed that signaling is not responsible for the hybrid increased stability. In conclusion, chimeric receptor tyrosine kinases escape efficient down-regulation through lysosomes and proteasomes, by a mechanism that may involve altered ubiquitination and protein clustering.

0444

THE SELECTIVE JANUS KINASE (JAK) INHIBITOR, INCB018424, SHOWS EFFICACY IN A PHASE I/II TRIAL IN PATIENTS WITH PRIMARY MYELOFIBROSIS (PMF) AND POST POLYCYTHEMIA VERA/ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS (POST-PV/ET MF)

S. Verstovsek,[1] H. Kantarjian,[1] A. Pardanani,[2] D. Thomas,[1] J. Cortes,[1] R. Mesa,[2] W. Hogan,[2] J. Redman,[3] R. Levy,[3] K. Vaddi,[3] J. Fridman,[3] A. Tefferi[2]

[1]M.D. Anderson Cancer Center, HOUSTON, TEXAS; [2]Mayo Clinic, ROCHESTER, MINNESOTA; [3]Incyte Corporation, WILMINGTON, DELAWARE, USA

Background. Mutations in JAK2 and MPL have been recognized in Philadelphia chromosome-negative myeloproliferative disorders, including PV, ET and PMF, making JAK a therapeutic target for these diseases. INCB018424 is a potent, selective and orally bioavailable JAK inhibitor with an excellent safety profile supporting its clinical evaluation. *Aims.* The purpose of this study was to evaluate the safety and tolerability of INCB018424 and determine the MTD and preliminary efficacy in patients with PMF and Post-PV/ET MF. *Methods.* A phase I/II dose-escalation trial of INCB018424 was conducted with a PO BID continuous schedule. Responses are defined by the International Working Group (IWG) consensus criteria for treatment response in myelofibrosis with myeloid metaplasia (IWG-MRT). *Results.* Overall characteristics of the 32 patients enrolled include median age of 65 yrs; 67% males; 47% PMF, 38% post-PV and 15% post ET; and 87% with JAK2V617F mutation. The starting dose of 25 mg PO BID was demonstrated to be the maximum tolerated dose (MTD) (N = 6 pts). Two patients had grade 4 thrombocytopenia during the first cycle in the 50 mg PO BID cohort (N = 5 pts) which defined the dose limiting toxicity. Myelosuppression has been the only toxicity assessed to be related to drug thus far and this is an on-target effect. An expanded cohort of 21 additional patients has also been enrolled at the MTD, all of whom completed at least 3 months of therapy. A rapid and significant reduction in splenomegaly was observed in the group of 28 patients with palpable spleens at baseline. Sixteen patients have met the IWG criteria for splenic clinical improvement (CI) with a median time to response of <1 month. No patients have progressed after an initial response and based on the current follow up, the median duration of response is greater than 3 months. In addition, the majority of patients had improvement in their ECOG PS to 0 and their constitutional symptoms resolved or were significantly reduced. Laboratory correlates with treatment have included a marked reduction in proinflammatory and angiogenic cytokines, significant increases in hematopoietic growth factors and normalization of systemic JAK/STAT signaling. Follow up is too short to make valid conclusions regarding effects on anemia, V617F allele burden, bone marrow histology and cytogenetics. *Conclusions.* Treatment with INCB018424 results in marked spleen size reduction and symptom improvement without significant toxicity. Clinical improvements occurred regardless of mutation status or prior MPD diagnosis. Updated results for all 32 patients followed for at least 6 months will be available by the time of the meeting. Further dose modifications and schedules are being explored.

0445

THE CLINICAL PHENOTYPE OF PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA HARBORING MPL 515W>L/K MUTATION

E. Antonioli,[1] A. Pancrazzi,[2] P. Guglielmelli,[2] A. Bosi,[2] G. Barosi,[3] M. Ruggeri,[4] G. Specchia,[2] F. Lo Coco,[5] T. Barbui,[6] A.M. Vannucchi[2]

[1]University of Florence, FIRENZE; [2]Dept of Hematology, FLORENCE; [3]IRCCS Policlinico S. Matteo, PAVIA; [4]Ospedale San Bortolo, VICENZA; [5]Policlinico universitario Tor Vergata, Roma, ROMA; [6]Ematologia, Ospedali riuniti, BERGAMO, Italy

Background. A 515W>L/K mutation in MPL (MPLmut) has been described in 5-10% of patients (pts) with myelofibrosis, in some cases also associated with JAK2617V>F allele. We previously reported that among 217 subjects with myelofibrosis, the 18 MPLmut pts were more severely anemic than MPL wild-type (MPLWT) pts (Guglielmelli *et al.*, BJH 2007). MPL 515W>L mutation has been reported also in 1% of pts with essential thrombocythemia (ET). Aim and Methods. Aim of the study was to determine the frequency of MPL W515L/K mutation in 995 patients with ET and to correlate clinical and laboratory characteristics with MPL mutation. We have collected 31 MPLmut pts (3.1% of total) in an unselected series of 995 ET pts according to PVSG and WHO criteria. A novel quantitative real-time PCR assay for 515W>L and 515W>K allele in granulocyte DNA has been designed; detection limit was 0.01% for W>L allele and 0.1% for W>K allele. Results. 18 pts were 515W>L and 12 were 515W>K; 8 pt with W>L and 6 pts with W>K allele were homozygous for mutant allele. Mean mutant allele burden was 53(±26)% and 37(±23)% for W>K and W>L, respectively. In one patient a novel MPL mutation was identified that results in the substitution of tryptophan for arginine (515W>A). Eight MPLmut pts (26%) also harbored JAK2617V>F allele, as compared to 527/964 of MPLWT (57%; p=0.001); mean 617V>F allele burden was significantly lower in MPLmut (11±8%) than in MPLWT pts (27±15%; p=0.002). At diagnosis, hemoglobin level was lower in mutated pts (p=0.02) while platelet count, MCV and LDH level were significantly higher in MPLmut pts. There was no difference in splenomegaly, systemic symptoms and major thrombosis between MPLmut and MPLWT pts; however, pts with microvessel disease were significantly more frequent among MPLmut (61% *vs* 30%; p=0.0005). Among mutated pts, those with W>L were older (p=0.004) than W>K. We observed that W>L mutation was preferentially associated with lower haemoglobin level, while a significantly higher platelet count was found in patients with W>K. Bone marrow (BM) biopsy at diagnosis of MPLmut pts was reviewed blinded; total BM cellularity was significantly lower in MPL mutant pts, while there was no difference in the mean reticulin grade. On the other hand, presence of MPL mutation significantly affected the megakaryocytic lineage: the number of both megakaryocytes and megakaryocyte clusters per mm^2 of bone marrow area were significantly increased in MPL mutant pts (p= 0.006 and p=0.0002. respectively), while the mean number of megakaryocytes per cluster did not differ compared to MPL-wt pts. There was also an increase in the number of small megakaryocytes/mm^2 (p<0.0006), that represented 28.1±14.4% of all megakaryocytes in MPL mutant patients compared to 18.6±12.2 in MPL-wt patients. *Conclusions.* Frequency of MPL mutation in this large series of ET is higher than originally reported, and although some intriguing correlation with clinical and hematologic parameters could be evinced, MPL mutant patients did not constitute a defined sub-group within the spectrum of ET. MPL mutational screening is relevant in the diagnostic work-up of suspected ET as a tool to demonstrate occurence of clonal myeloproliferation, since a sizable proportion (≈8%) of JAK2 wild-type patients actually present a mutation at codon 515 of MPL.

0446

PEGYLATED INTERFERON-α-2A INDUCES COMPLETE HEMATOLOGICAL AND MOLECULAR RESPONSES WITH LOW TOXICITY IN POLYCYTHEMIA VERA: RESULTS OF THE PV-NORD PVN1 STUDY

J.J. Kiladjian,[1] B. Cassinat,[2] P. Turlure,[3] N. Cambier,[4] M. Roussel,[5] S. Bellucci,[5] S. Chevret,[2] B. Grandchamp,[6] C. Chomienne,[2] P. Fenaux[1]

[1]AP-HP, Hôpital Avicenne, BOBIGNY; [2]APHP, Hopital Saint-Louis, PARIS; [3]CHU Dupuytren, LIMOGES; [4]CHRU de Lille, Hopital Huriez, LILLE; [5]AP-HP,Hôpital Lariboisiere, PARIS; [6]AP-HP, Hopital Bichat; INSERM U656, PARIS, France

Background. Interferon-α (IFNα) is a non-leukemogenic treatment of polycythemia vera (PV), also able to induce cytogenetic remissions. Its use is nevertheless limited by toxicity, leading to treatment discontinuation in about 25% of patients within the first year. *Aims and Study design*. We completed a phase 2 multicenter study of pegylated-IFNα-2a in 40 PV patients. Objectives included evaluation of efficacy, safety, and monitoring of residual disease using JAK2V617F quantification in circulating granulocytes (%V617F). The PVN1 study was approved by the IRB of Paris 13 University, and by the French Health Products Safety Agency. Informed consent was provided according to the Declaration of Helsinki. *Results*. Median follow-up was 31.4 months. After 12 months of treatment, all the 37 evaluable patients had hematological response, including 94.6% complete responses (CR), and only 3 (8%) patients had stopped treatment. After the first year, 29 patients remained in hematological CR, including 24 still on pegylated-IFNα-2a, and 5 who had stopped treatment for 3 to 18 months (and without any other cytoreductive treatment). Sequential samples for %V617F monitoring, available in 29 mutated patients, showed %V617F decrease in 26 (89.6%). Median %V617F decreased from 45% before pegylated-IFNα-2a to 22.5%, 17.5%, 5%, and 3% after 12, 18, 24, and 36 months, respectively (p<0.0001). Molecular CR (JAK2V617F undetectable) was achieved in 7 patients, lasting from 6 to 18 months, and persisting after pegylated-IFNα-2a discontinuation in 5. Kinetics of decrease and final %V617F level were independent of baseline JAK2V617F level, and of presence of 9p LOH. Finally, no vascular event was recorded in the whole cohort after 31.4 months of median follow-up. *Conclusion*. Those results show that pegylated-IFNα-2a yields high rates of hematological and molecular CR in PV with limited toxicity, and could even eliminate the JAK2 mutated clone in selected cases.

Infectious diseases, supportive care

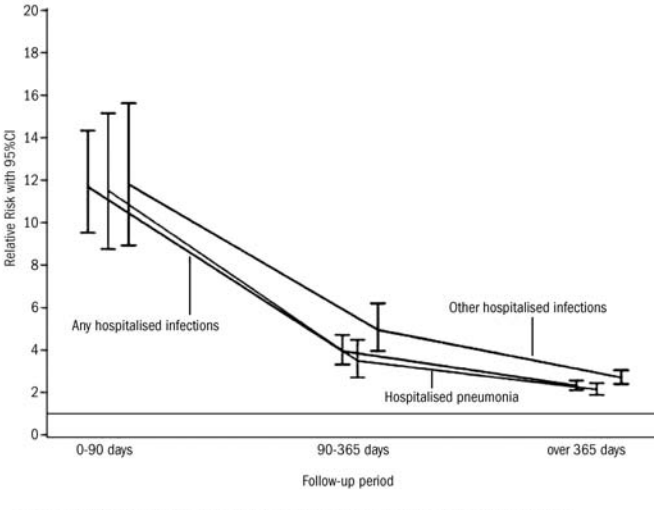
0447

INCIDENCE OF INFECTIONS IN SPLENECTOMISED PATIENTS AND GENERAL POPULATION COMPARISONS: NATIONWIDE COHORT STUDY IN DENMARK

W.M. Schoonen,[1] R.W. Thomsen,[2] D. Körmendiné Farkas,[2] A. Riis,[2] J.P. Fryzek,[3] H.T. Sørensen[2]

[1]Amgen, UXBRIDGE, MIDDLESEX, UK; [2]Aarhus University Hospital, AARHUS, Denmark; [3]Amgen Inc., THOUSAND OAKS, CA, USA

Background. Splenectomy is indicated for a variety of medical conditions. Two surgical procedures are available; open splenectomy (OS) or laparoscopic splenectomy (LS). The spleen is important in filtering and removal of blood-borne pathogens such as Streptococcus pneumoniae. Splenectomy has been associated with a high incidence of subsequent infections, but the risk compared to the general population has not been quantified. Further, LS may confer a lower risk of infection, but this has yet to be investigated. *Aims.* To estimate the risk of infection by time since surgical procedure for all splenectomised patients and LS patients separately, compared to the general population in Denmark. *Methods.* All individuals who underwent splenectomy from 1996 to 2005 were identified in nationwide Danish medical databases. For each splenectomised person we identified 10 population comparisons, frequency-matched on age, sex, and index date of the splenectomy. The outcomes of interest were hospitalised infections overall and hospitalised pneumonia in specific. For a subset of data from Northern Denmark we had additional data on microbiologically confirmed bacteraemia episodes. We compared rates of infection in splenectomised *vs* un-splenectomised individuals within 90 days, 91-365 days, and more than 365 days after the index date, using multivariable techniques to adjust for age, sex and comorbid diseases present at the index date. *Results*. We identified 3812 individuals with splenectomy (median age 60 (interquartile range 41-72) years, 43% females) and 38120 population comparisons. Overall, 21.9% of splenectomised individuals had a hospitalised infection at any point in time after splenectomy (median follow-up time 2.6 years), *vs* 10.8% of the population comparisons. The relative risk (RR) of any hospitalised infection from splenectomy was highest within 90 days when 6.7% had an event (RR=11.7, 95% confidence interval (CI) 9.5-14.3). The relative risk remained four-fold elevated during 91-365 days, and more than two-fold one year or longer after the splenectomy (Figure 1).



*Relative risk during follow-up in patients with splenectomy compared with population comparisons.
Risk estimates are shown both for infections overall and stratified by pneumonia and other infections.

Figure 1. Adjusted relative risk of hospitalised infections*

A similar pattern was observed for hospitalised pneumonia, with 12.3% having an event in the splenectomised group *vs* 6.5% of the comparisons. More than one year after splenectomy, risk of hospitalised infections other than pneumonia appeared to be relatively higher than risk of pneumonia (Figure 1). In our regional subcohort, bacteraemia was observed in 13.1% of 418 splenectomised subjects and in 1.2% of the 4180 comparisons The adjusted relative risk of bacteraemia was 177-fold (95% CI 50-624) increased within 90 days, 7.9 times (2.7-22.9) and 3.8 times (1.8-8.1) increased after 91-365 days and more than 365 days, respectively. Laparoscopic splenectomy was performed on 33 patients, two of whom had a hospitalised infection within 90 days (*vs* zero population comparisons) and a further five had a hospitalised infection one year or more after the splenectomy (*vs* 13 population comparisons). *Summary and conclusions.* Splenectomised individuals are at increased risk of hospitalised infection, in particular bacteraemia, compared to the general population. Relative risk of infection was highest in the 90 days following splenectomy and remained more than two-fold elevated more than a year after splenectomy. Data on laparoscopic splenectomy were limited but we did observe infections in those patients.

0448

IMPACT OF PRIOR INVASIVE PULMONARY ASPERGILLOSIS (IPA) ON OUTCOME IN PATIENTS RECEIVING REDUCED INTENSITY CONDITIONING (RIC) ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLO-SCT)

J. El cheikh, L. Wang, C. Faucher, S. Furst, P. Berger, D. Blaise

[1]Department of Haematology, Unité de Transplantation et de Thérapie Cellulaire (UTTC), Institut Paoli-Calmettes, Marseille, France

Background. Invasive Pulmonary Aspergillosis (IPA) is a major cause of morbidity in patients with hematological malignancies. The aim of this single centre retrospective study was to determine the impact of prior IPA on outcome after RIC Allo-SCT. *Methods.* All cases of proven or probable IPA diagnosed prior to performance of RIC Allo-SCT at the Paoli-Calmettes Institute Cancer Centre from 1 January 2000 through 31 December 2007 were included. 28 patients were identified among 434 patients undergoing Allo-SCT. Patients' data were collected from a maintained database and by retrospective clinical chart review. *Results.* Twenty eight cases were identified. Gender: M/F (16/12); median age at diagnosis was 53 years (18-65). 23 patients (82%) had acute myeloid leukemia. In all patients, IPA was diagnosed according to standard procedures. IPA therapy included: 20 patients (71%) were receiving Voriconazole; 4 patients Itraconazole, and 4 patients with an association of different antifungal drugs including Caspofungine, Liposomal Amphoteracinre-B, or Posaconazole. The median duration of treatment prior to RIC was 8 months range (1-16). The median time between the diagnosis of IPA and transplantation was 6 months (1-27); 20 patients (71%) received a graft from a family donor, 4 patients had a MUD, and 4 patients received cord blood cells donors. 21 patients (75%) received a conditioning regimen with Fludarabine Busulfan and ATG, and 4 patient (14%) received a conditioning with Fludarabine Cyclophosphamide and TBI, 3 patients (11%) Fludarabine and TBI, Most patients (n=25; 89%) received or continued a secondary prophylaxis against aspergillosis at time of Allo-SCT: 17 (68%) had a prophylaxis with Voriconazole; 3 patients Itraconazole, 2 patients Posaconazole. After RIC transplantation only 4 patients (14%) had reactivation of their IPA. The latter 4 patients were experiencing severe acute GVHD treated with high dose corticosteroids. None of these patients died of IPA. Actually 18 patients (64%) are still alive with a median follow up of 23.5 months (12.6-48.5). Overall survival at 2 years was 59% [95%CI, 43-83]. In all, 3 patients died from other causes not directly related to IPA, 2 patients because of relapse or disease progression, and one due extensive chronic GVHD. *Conclusions.* In comparison to literature in standard myéloablative allo-SCT setting, these data suggest that adequately treated and controlled IPA prior to RIC Allo-SCT do not worsen outcome. The latter is likely due to multiple changes in transplantation practice, including the RIC nature of the conditioning regimens, the infusion of peripheral blood stem cells, rapid diagnosis of IPA, and use of modern efficient antifungal drugs.

0449

HBV REVERSE SEROCONVERSION AND INTERVENTION WITH VACCINATION AFTER ALLOGENEIC HSCT IN PATIENTS WITH PREVIOUS HBV INFECTION

M. Onozawa, S. Hashino, R. Morita, K. Kahata, T. Kondo, J. Tanaka, M. Imamura, M. Asaka

Hokkaido University Hospital, SAPPORO, Japan

Background. Appearance of anti-hepatitis B surface antigen antibody (anti-HBs) and clearance of hepatitis B virus (HBV) from serum usually indicate resolution of hepatitis in patients infected with HBV. However, in most patients in whom HBV has been eliminated from serum, HBV DNA is still detectable in the liver using polymerase chain reaction. Reactivation of this dormant HBV in the liver is known as reverse seroconversion (RS). Previously, we reported that HBV-RS after allogeneic hematopoietic stem cell transplantation (allo-HSCT) was a frequent late-onset complication that can be predicted by careful monitoring of progressive disappearance of anti-HBs (Onozawa M *et al.* Transplantation. 2005.79(5):616-9). RS hepatitis after allo-HSCT is thought to be a phenomenon caused by naive donor immunity after loss of recipient-oriented immunity against HBV. We speculated that vaccination could prevent reactivation of HBV in allo-HSCT recipients. Safety and efficacy of recombinant HBV vaccine in allo-HSCT recipients have already been confirmed. *Aims.* We conducted post-transplant recombinant HB vaccine intervention to immunize naïve donor immunity and determine clinical impact to prevent post-HSCT HBV-RS. *Methods.* Allo-HSCT recipients who underwent transplantation in our hospital from Feb. 1990 to Mar. 2007 and who were followed for at least 1 year after HSCT were enrolled. Thirty-eight recipients were serologically considered to have previous HBV infection in pre-HSCT evaluation. From Mar. 2003 we started hepatitis B vaccine intervention for HSCT recipient after cessation of immunosuppressant administration. Thirteen recipients who underwent transplantation after Mar. 2003 were immunized by HB vaccine by a standard 3-dose (0, 1, 6 month) regimen (vaccine group: M/F, 6/7; age, 24-68 years (median, 50 years); follow-up period, 20-60 months (median, 37 months)). Twelve recipients who underwent transplantation after Mar. 2003 were observed without intervention (non-vaccine group: M/F, 5/7; age, 22-65 years (meidan, 47 years); follow-up period, 12-55 months (median, 23 months)). Thirteen recipients who underwent transplantation before Mar. 2003 and were observed without vaccine intervention were considered as controls (historical control group: M/F, 9/4; age, 22-52 years (median, 36 years); follow-up period 17-116 months (median, 59 months)). We studied transition of anti-HBs and incidence of HBV-RS. *Results.* Progressive decreases in titers of anti-HBs were observed in all pre-HSCT anti-HBs positive recipients. Eight of the 13 historical control group recipients and 3 of the 12 non-vaccine group recipients suffered HBV-RS after loss of anti-HBs, but none of the 13 vaccine group recipients suffered HBV-RS. Cumulative risks of HBV-RS at 3-years after HSCT in the historical control, non-vaccine group and vaccine group were 41%, 39% and 0%, respectively (p=0.022). *Conclusions.* All recipients with previous HBV infection should be considered to be at high risk for RS after allo-HSCT. Intervention with recombinant HBV vaccines is effective for prevent post-transplant HBV-RS in allo-HSCT recipients.

0450

CLINICAL VALUE OF SCREENING DETECTION OF 1,3-BETA-D GLUCAN (BG) FOR EARLY DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS (IFI) IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES - RESULTS OF A PROSPECTIVE STUDY

Z. Racil,[1] I. Kocmanova,[2] B. Weinbergerova,[1] J. Winterova,[1] M. Lengerova1, J. Mayer1

[1]*University Hospital Brno and Masaryk University, BRNO;* [2]*University Hospital Brno, BRNO, Czech Republic*

Background and Aims. BG - the major cell wall component of various medically important fungi - can be used as a target for early detection of IFI. The aim of our study was to evaluate usefulness of BG detection for screening of IFI in hemato-oncological patients in routine clinical use. *Methods.* Between January 2005 and July 2007, blood samples for BG screening were obtained from patients in intermediate to high risk of IFI. The BG was detected by FungitellTM test (Associates of Cape Cod, USA). Only proven and probable cases of IFI were considered to calculate sensitivity, specificity, and positive and negative predictive values (PPV, NPV). *Results.* BG screening was performed in 1154 blood samples obtained during 104 anticancer treatment cycles: treatment of AML, n = 46 (44.2%); allogeneic HSCT, n = 40 (38.5%); autologous HSCT, n = 17 (16.4%); other, n = 1 (0.9%). The incidence of IFI in our study was 17.3% (n=18) and probable or proven IFI was detected in 9 cases (8.6%). The highest sensitivity, specificity, PPV, and NPV (88.9%, 40.7%, 13.6% and 97.2%) were obtained when cut off of 80 pg/mL and one positive result were used. For routine clinical use, positive results in two consecutive samples are usually more relevant. Thus, when consecutive positivity of the test was required, cut off of 60 pg/mL was found more useful (sensitivity 66.7%, specificity 47.7%, PPV 11.8%, and NPV 93.2%). Low PPV, caused by frequent false positive results, was identified as the main limitation of this assay. Fifty-one (59%) treatment cycles were positive if a criterion of one sample above 80 pg/mL was used. If consecutive positivity with cut off 60 pg/mL was used, 45 (52%) of them were positive. Approximately 90% of these BG positive cycles were false positive. We did not find any correlation between positive BG assay result and frequency of empirical antifungal treatment, frequency and grade of mucositis, yeast colonization, administration of selected antibiotics, or infusion solutions or bacteremia. *Summary and Conclusions.* Our comprehensive analysis reflecting routine use of BG test for panfungal screening of IFI showed limited usefulness of this assay in patients with hematological malignancies. Low sensitivity together with poor PPV are the major limitations of this test, which, based on these results, cannot be recommended for screening purposes.

0451

HIGH-DOSE ACICLOVIR FAILS TO PREVENT CYTOMEGALOVIRUS INFECTION AND DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

N. Duncan, D. Stocken, H. Osman, J. Ward, P. Moss, M. Cook, C. Craddock

University Hospital Birmingham NHS Foundation Trust, BIRMINGHAM, UK

Background. Cytomegalovirus (CMV) disease remains an important cause of morbidity and mortality following allogeneic stem cell transplantation (SCT).(1) It has been demonstrated that administration of high-dose aciclovir during the post-transplant period can reduce the incidence of CMV infection and disease(2,3) and we therefore wished to examine the impact of its use at a major regional allograft centre. *Aims*. To determine the effect of high-dose aciclovir prophylaxis on the incidence of CMV infection and disease, time to reactivation and survival in patients undergoing allogeneic stem cell transplantation. *Methods*. Data on incidence and outcome of CMV infection were collected for 144 consecutive at-risk patients undergoing allogeneic SCT between January 2002 and December 2006. Until July 2004, patients received low-dose aciclovir (200mg qds until day +35) as herpes simplex prophylaxis but no CMV prophylaxis. From July 2004 onwards, patients received aciclovir until day+100 at a dose of 800mg qds orally (sibling donors) or 500mg/m^2 tds IV until mucositis resolved, then 800mg qds orally (matched unrelated donors). *Results*. Data were collected for 144 patients, 73 of whom received high-dose aciclovir prophylaxis. The remaining 71 patients (control group) received low-dose aciclovir. The majority of patients (70%) received pre-transplant alemtuzumab for T-cell depletion. The overall incidence of CMV reactivation (up until day +100) was 40% in the high-dose aciclovir group and 44% in the control group (p=0.74). There were no significant differences between the groups in

the time to first reactivation (median 36 days in both groups), incidence of CMV disease (7% in both groups) and day +100 survival (84% *vs* 83%). Seronegative patients were found to have a low incidence of reactivation (13%) but of those patients who reactivated, 75% developed CMV pneumonitis. Multivariate (logistic regression) analysis demonstrated that three factors all significantly impacted on the risk of CMV infection: T-cell depletion [odds ratio 7.42 (2.4-21.9)], recipient CMV seronegativity [odds ratio 0.14 (0.04-0.51)] and a diagnosis of CML [odds ratio 4.58 (1.26-16.72)]. However, the use of aciclovir did not demonstrate any significant benefit. Using the model, a predictive equation for calculating probability of CMV reactivation for an individual patient was determined. The equation was subsequently validated in a cohort of 44 patients at risk of CMV reactivation. *Summary and Conclusions*. In this study, the use of high-dose aciclovir prophylaxis failed to reduce the incidence of CMV infection and disease in a high-risk population of patients undergoing allogeneic SCT and alternative prophylactic strategies should be considered. We have developed a novel method that allows the risk of CMV reactivation to be calculated for an individual patient. This predictive equation will be of value in identifying and targeting high-risk patients who require more intensive prophylactic strategies.

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Acute lymphoblastic leukemia - Biology

0452

TIME TO LEUKAEMIA (TTL) ASSESSED IN NOD/SCID MICE TRANSPLANTED WITH PRIMARY ALL LEUKAEMIA CELLS DETERMINES EARLY RELAPSE IN PATIENTS AND IS IDENTIFIED BY A SPECIFIC GENE SIGNATURE

H. Meyer,[1] S.M. Eckhoff,[1] M. Queudeville,[1] M. Zimmermann,[2] A. Schrauder,[3] G. Lahr,[1] K. Holzmann,[1] M. Schrappe,[3] K. Stahnke,[1] K.-M. Debatin[1]

[1]University of Ulm, ULM; [2]Medical School Hannover, HANNOVER; [3]University Hospital Schleswig-Holstein, KIEL, Germany

Poor response to induction therapy is the major risk factor identified in ALL and is used within the ALL BFM study to identify patients at high risk for relapse (e.g. prednisone poor response, PPR: more than 1000 blasts/µl peripheral blood after treatment with prednisone systemically for 8 days and MTX intrathecally once on day 1). Despite the efforts achieved by the stratification strategies the majority of relapses are recruited from the group of initially good responding patients (in ALL BFM the standard and medium risk, SR and MR groups) emphasising the need for additional independent stratification factors. In our study we transplanted primary leukaemia cells from 50 children with newly diagnosed B cell precursor ALL (BCP-ALL) into NOD/SCID mice. Time to leukaemia was determined for each patient sample transplanted as weeks from date of transplant to date of clinical manifestation of the disease. Leukaemia was verified in spleen and bone marrow by flow cytometry staining for human CD19. Time to leukaemia of less than 10 weeks (short TLL) was observed in 6 patient samples whereas 44 leukaemia samples took clearly more than 10 weeks until appearance of leukaemia (long TTL). A clear cut in relapse free survival (Kaplan Meier analysis, N=50, log rank: $p<0.0001$) was found for patients whose leukaemia cell samples showed short TTL (N=6, mean survival: 13.1 months, SE: 4.1, CI: 5.0-21.2) in contrast to patients with long TTL (N=44, mean survival: 50.7 months, SE: 1.9, CI: 47.1-54.4). Of note, the same distinct difference in relapse free survival was observed considering the SR and MR groups only (N=40, log rank $p<0.0001$, long TTL: 52.4 months; short TTL: 12.6 months). By multivariate analysis (N=50, relapse free survival) patients exhibiting short TTL in the xenotransplant model exhibited a strongly increased risk for relapse with a risk ratio of 44.59 (CI: 8.42-236.11, $p<0.0001$). Interestingly, patients in our cohort showing prednisone poor response known as important clinical risk factor revealed only a risk ratio of 8.71 (CI: 1.75-43.44, $p= 0.008$). None of the patients with long TTL encountered early relapse. These findings in 50 directly transplanted samples were confirmed transplanting different cryopreserved BCP-ALL samples. In order to further characterise the biological properties of the leukaemia cell in the two groups, gene expression profiles of samples with short or long TTL in the xenograft model were investigated using a human whole genome array (Affymetrix U133 Plus 2.0). We identified a signature of differentially expressed genes distinguishing both groups. The differential expression was confirmed for selected genes by quantitative RT-PCR. Taken together, estimation of time to leukaemia (TTL) of leukaemia samples transplanted onto NOD/SCID mice is a new promising factor in paediatric ALL. Using an expression array approach patient samples displaying short TTL can be discriminated from those with long TTL by a unique gene expression signature. This allows direct identification of patients with increased risk for relapse by this new independent risk factor avoiding transplant in the mouse model.

0453

UNIQUE DNA METHYLATION PATTERNS SEPARATE DISTINCT SUBTYPES OF MLL REARRANGED INFANT ALL

D.J.P.M. Stumpel,[1] P. Schneider,[1] R.X. Menezes,[1] E.H.J. van Roon,[2] T.C.M. Peters,[1] J.M. Boer,[2] R. Pieters,[1] R.W. Stam[1]

[1]Erasmus MC Rotterdam-Sophia Children's Hospital, the Netherlands, ROTTERDAM; [2]Leiden University Medical Center, Center for Human and Clinical Genetics, LEIDEN, Netherlands

Background. Acute Lymphoblastic Leukemia (ALL) in infants (<1 year of age) is characterized by chromosomal rearrangements involving the MLL gene (~80%) that are associated with a poor prognosis. The most frequent MLL translocations in infant ALL are t(4;11), t(11;19) and t(9;11). Based on gene expression profiling, MLL rearranged ALL (MLL) represents a unique type of leukemia clearly distinguishable from other ALL

subtypes. Whether MLL also displays unique epigenetic features like aberrant silencing of (tumor suppressor) genes induced by promoter CpG island hypermethylation remains unkown. *Aims.* Therefore, the present study was designed to unravel specific DNA methylation patterns associated with the different types of MLL translocations found among infant ALL patients. *Methods.* For this, we applied Differential Methylation Hybridization (DMH) using both custom spotted 9K CpG island microarrays (Huang *et al.*, 2002), and commercially available 244K CpG arrays (Agilent), on primary infant ALL samples carrying t(4;11) (n=21), t(11;19) (n=17) or t(9;11) (n=6). The obtained DNA methylation patterns were compared with the patterns found in infant ALL samples without MLL rearrangements (n=13). Healthy pediatric bone marrow samples (n=9) were included as a reference. *Results.* Both CpG microarray platforms revealed that t(4;11) and t(11;19) positive samples are characterized by abundant promoter CpG island hypermethylation as compared with normal bone marrow. This aberrant DNA methylation pattern appeared to be largely absent in t(9;11) positive samples as well as in infant patients carrying germline MLL genes. Based on unsupervised cluster analysis, these latter subgroups consistently cluster together with healthy bone marrow samples (Figure 1). Subsequently, the obtained CpG array data were compared with gene expression profiles (Affymetrix) from corresponding patients. For several hypermethylated genes we observed down-regulated gene expression. However, we also repetitively found that genes significantly hypermethylated in t(4;11) and t(11;19) positive samples show silencing in all samples tested, including healthy bone marrow. Possibly t(4;11) and t(11;19) samples display hypermethylation of genes already methylated (or otherwise silenced) among hematopoietic cells. In rare occasions we observed the opposite; despite severe hypermethylation, some genes retain high expression levels. *Summary and Conclusions.* Unique DNA methylation patterns are present among different subtypes of MLL rearranged infant ALL, separating t(4;11) and t(11;19) positive infant ALL patients from patients carrying t(9;11) and wild-type MLL genes. Clearly, translocations t(4;11) and t(11;19) are associated with severely hypermethylated leukemias, whereas translocation t(9;11) seems to be less capable of inducing aberrant DNA methylation patterns. This study provides the first epigenetic insights into the complex biology of MLL rearranged ALL in infants.

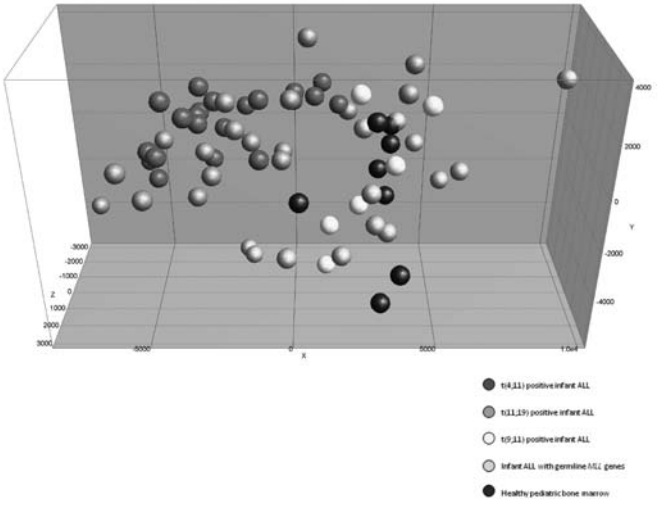

Figure 1. Unsupervised Principal Component Analysis plot

0454

HIGH-RESOLUTION GENOMIC PROFILING OF PH-POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) IDENTIFIED RECURRENT COPY NUMBER ANOMALIES IN GENES REGULATING THE CELL CYCLE AND THE B-CELL DIFFERENTIATION

I. Iacobucci,[1] E. Ottaviani,[1] A. Astolfi,[2] T. Storlazzi,[3] N. Testoni,[1] I. Luciana,[3] A. Lonetti,[1] S. Soverini,[1] S. Paolini,[1] P.P. Piccaluga,[1] C. Papayannidis,[1] P. Giannoulia,[1] F. De Rosa,[1] D. Cilloni,[4] F. Messa,[4] A. Pession,[2] F. Pane,[5] A. Vitale,[6] S. Chiaretti,[6] R. Foà,[6] M. Baccarani,[1] G. Martinelli[1]

[1]Department of Hematology/Oncology "Seràgnoli", BOLOGNA; [2]Pediatric Oncology and Hematology "L. Seràgnoli", BOLOGNA; [3]Department of Genetics and Microbiology, University of Bari, BARI; [4]Hematology, University of Turin at Orbassano, ORBASSANO, TURIN; [5]CEINGE, University of Naples Federico II, NAPLES; [6]"La Sapienza" Unversity, ROME, Italy

Background. The Ph chromosome is the most frequent cytogenetic aberration associated with adult ALL and it represents the single most significant adverse prognostic marker. The constitutively active tyrosine kinase encoded by BCR-ABL blocks the B-cell differentiation, prevents apoptosis and also causes genetic instability. Recently, the introduction of array-based analysis of single nucleotide polymorphism (SNP) has allowed the rapid determination of genome-wide allelic information at high density for a DNA sample. *Aims.* To identify, at submicroscopic level, genetic lesions which can escape standard cytogenetic observations and may provide new insights into the alternative process underlining leukemogenesis and resistance in Ph+ ALL. *Methods.* We profiled, until now, the genomes of 36 out of 55 Ph+ ALL patients. The median age was 55 years (range, 18-76) and the median blast percentage was 90% (range, 76-100%). 250 ng of genomic DNA were processed on 500K SNP array according to protocols provided by the manufacturer (Affymetrix Inc., USA). Copy number state was calculated with respect to a set of 48 Hapmap normal individuals and a set of samples obtained from acute leukaemia cases in remission using Partek® Genomics Suite. Fluorescence *in situ* hybridization (FISH), real-time quantitative PCR and western-blot analyses were performed to validate our results. *Results.* We identified region of high level amplification and homozygous deletion in all patients, with deletions outnumbering amplification almost 3:1. Copy number alterations most frequently involved chromosome 7 and 8. Lesions varied from loss or gain of complete chromosome arms (trisomy 4, monosomy 7, loss of 9p, 10q, 14q, 16q and gain of 1q and 17q) to microdeletions and microduplications targeting genomic intervals. Sub-microscopic lesions encompassing single or only few genes were observed with relative high frequency. Many of these copy number alterations were associated with cellular proliferation and/or apoptosis [e.g. CDKN2A and CDKN2B (n=8), GADD45A (n=2), FAS (n=2), BTG1(n=2)]. We also frequently observed anomalies in genes involved in early B-cell differentiation. These include the paired box gene PAX5 (9p13, n=5) which is a critical determinant of B-lineage commitment; the B-cell adapter containing a SH2 domain protein BLNK (10q23.2-q23.33, n=1); the pre-B lymphocyte gene VPREB1 (22q11.22, n=8) and the transcription factor IKZF1 (7p13-p11.1, n=9), which is required for normal lymphoid development. Reverse transcriptase-PCR and western-blot analysis for IKZF1 showed that in some cases this deletion was associated with the expression of the dominant-negative isoform Ik6 with cytoplasmatic localization. Other recurring copy number abnormalities with relevance to leukemogenesis included deletions of BTLA, TOX, MDS, PBX1, RUNX1, ETV1 and PTEN. It is noteworthy that some lesions felt in regions lacking annotated genes. *Conclusions*. High-resolution SNP-based genomic profiling is a reliable and valid method for the identification of genomic anomalies and genes relevant for the development of leukaemia. Multiple independent copy number anomalies were frequently observed in genes involved in cell cycle regulation and B-cell differentiation, suggesting that these processes could contribute to the poor prognosis of Ph+ ALL.

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0455

MONOALLELIC LOSS AND FREQUENT MUTATION OF THE SECOND ALLELE OF PAX5 ARE GENETIC HALLMARKS OF DIC(9;20) CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

S. Strehl, K. Nebral, M. König, D. Krehan

CCRI, Children's Cancer Research Institute, VIENNA, Austria

Background. About 1.5% of childhood B-cell precursor acute lymphoblastic leukemia is associated with the presence of a dic(9;20)(p13;q11) chromosome. In about 40% of the cases dic(9;20) is the sole karyotypic change suggesting that this aberration is the primary leukemogenic event. However, so far all efforts to delineate the genes involved in this specific genetic aberration have failed. This is mainly due to the observation that the breakpoints in both chromosomes are heterogeneous and thus neither a specific gene is affected on either chromosome nor has the formation of a fusion gene been observed. In general, the formation of a dicentric chromosome results in hypodiploidy, which however can be masked by nonrandom gains of chromosomes. Owing to the fact that dic(9;20) not always results in monosomy 20q the crucial gene(s) are more likely to be located at 9p. In this respect, it has been observed that dic(9;20) is frequently associated with homo- or heterozygous deletion of CDKN2A. Recently, it has been shown that hypodiploid ALL has one null PAX5 allele and a significant proportion of cases harbor point mutations in the other PAX5 allele. *Aims and Methods.*In order to determine whether deletion and/or mutation of PAX5 are associated with dic(9;20) leukemia we analyzed 7 cases for their PAX5 status by fluorescence *in situ* hybridization (FISH) using PAX5-specific probes, mutation screening of all PAX5 coding exons, and expression of PAX5 isoforms. *Results*. FISH analysis using PAX5 flanking BAC clones showed that in 7/7 cases with a dic(9;20) one allele of PAX5 was consistently deleted. Sequence analysis of all coding exons of the retained allele revealed mutations in PAX5 in 4/7 (>50%) cases. These mutations comprised two in exon 3 encoding the paired DNA-binding domain, namely P80R (2 cases) and T75A (1 case), and a T311A mutation in exon 8 encoding the N-terminal part of the transactivation domain of PAX5. To exclude inherited germline mutations remission samples of all four cases were analyzed and showed that all PAX5 mutations were indeed somatically acquired. Moreover, evaluation of the expression pattern of N- and C-terminal PAX5 isoforms revealed that in one of the unmutated cases no full-length transcript was present. All other cases expressed full-length PAX5 and different isoforms in variable patterns and levels. However, to date it remains elusive whether the expression of such PAX5 isoforms is leukemia-associated or reflects specific stages of B-cell development. Regarding the CDKN2A status, FISH analysis revealed a heterozygous in three, a homozygous in two, and a mixed pattern of homozygous and heterozygous loss in one of the cases. In the remaining case, CDKN2A was lost from the dic(9;20) chromosome but a signal was observed on a der(12) marker chromosome suggesting a complex rearrangement, which resulted in a dic(9;20) without concomitant loss of CDKN2A. *Conclusions*. Together, our data suggest that haploinsufficiency of PAX5 and mutation of the second allele may play a crucial role in the leukemogenesis of dic(9;20) leukemia.

0456

IDENTIFICATION OF CANCER STEM CELLS IN B-CELL LYMPHOID HEMATOLOGIC MALIGNANCIES

H. Nishida

The Institute of Medical Science, The University of Tokyo, TOKYO, Japan

Background. Although cancer stem cells (CSCs) have already been identified in acute myeloblastic leukemia, current data on CSCs in lymphoid hematologic malignancies are conflicting. B-cell acute lymphoblastic (B-ALL) cells capable of long-term proliferation were reported to be $CD34^+/CD10^-$ or $CD34^+/CD19^-$ cells, suggesting that these cells are progenitors of B-ALL (Castor 2005, Cox 2004). *Aims*. In order to overcome this disease, molecular targeted therapy against CSCs is essential, but specific positive markers for them other than CD34 have not been identified yet. The purpose of this study was to determine the positive CD marker for the efficient isolation of CSCs in B-ALL. *Methods*. We evaluated the stem cell properties of B-ALL cell lines and primary pediatric pre-B ALL samples *in vitro* and *in vivo*. *Results.* we first performed extensive FACS analysis of cell surface antigen markers (total 101) in 7 B-cell leukemia/lymphoma cell lines. We found that especially three B-lineage precursor leukemia cell lines; YAMN90 (t(1;19) positive), REH(t(12;21) positive), ARH77 (normal karyotype) consisted of heterogeneous populations in marker expressions. Moreover, small subpopulations of $CD9^+$/high cells in these cell lines were shown to have stem cell characteristics within the clone *in vitro* and *in vivo*. Sorting & Culture assay revealed that $CD9^-$ cells repopulated only $CD9^-$ cells, whereas $CD9^+$ cells repopulated not only $CD9^+$ cells but also $CD9^-$ cells to reconstitute the original pattern of the cell line. We then transplanted $CD9^+$ and $CD9^-$ cells into immunodeficient NOG mice to compare the tumorigenicity of each population. The mice injected with $CD9^+$ cells died of leukemia within 30-40 days, however, those injected with $CD9^-$ cells survived, which leukemic engraftements were not successful. Next, we isolated leukemia cells from spleens or bone marrow of the transplanted mice and $CD9^+$ cells were shown to be serially transplantable onto secondary, tertiary mice. For further investigation, we also examined primary samples of childhood pre B-ALL patients and discovered that $CD9^+$ cells were found in all cases of peripheral blood or bone marrow and showed significant correlation with CD34 expression in many cases. In addition, gene expression profile between $CD9^+$ and $CD9^-$ cells using Affymetrix gene chips revealed that there are several affected genes related to stem cell characters and leukemia development. *Conclusions*. our data indicate that B-ALL cells with tumorigenicity onto NOG mice express CD9, and suggest that CD9 is a new positive marker for efficient isolation of CSCs in some cases of B-cell lymphoid hematologic malignancies including B-ALL.

Acute myeloid leukemia - Biology I

0457

WILMS' TUMOR 1 (WT1) GENE MUTATIONS IN CHILDHOOD ACUTE MYELOID LEUKEMIA: CHARACTERISTICS, PROGNOSTIC VALUE AND CONSEQUENCES FOR MRD DETECTION

I.H.I.M. Hollink,[1] M.M. van den Heuvel-Eibrink,[1] M. Zimmermann,[2] T.J.C.M. Arentsen-Peters,[1] M. Alders,[3] G.J. Kaspers,[4] J. Stary,[5] A. Baruchel,[6] S.S. De Graaf,[7] U. Creutzig,[8] D. Reinhardt,[2] R. Pieters,[1] C.M. Zwaan[1]

[1]Erasmus MC - Sophia Children's Hospital, ROTTERDAM, Netherlands; [2]AML-BFM Study Group, Dept.of Pediatric Oncology, Medical High School, HANNOVER, Germany; [3]Clinicla Genetics, Academic Medical Center, AMSTERDAM, Netherlands; [4]Dept.of Pediatric Oncology/Hematology, VU University Medical Center, AMSTERDAM, Netherlands; [5]Pediatric Hematology/Oncology, 2nd Medical School, Charles University Prague, PRAGUE, Czech Republic; [6]Hematology, Saint-Louis Hospital, PARIS, France; [7]Dutch Childhood Oncology Group (DCOG), THE HAGUE, Netherlands; [8]AML-BFM Study Group, University Children's Hospital, MUNSTER, Germany

Background. Cytogenetically normal AML (CN-AML) accounts for 20-25% of childhood AML. In an earlier array-based CGH study on 43 childhood CN-AML samples, we identified an 11p13-deletion that includes the Wilms' tumor 1 (WT1) gene in one patient. Its other WT1 allele carried a mutation. Recently, 10% of adult CN-AML patients were also found to carry WT1 mutations. *Aims*. To investigate the frequency of WT1 mutations, their association with clinical characteristics and their prognostic value in a large cohort of childhood AML. *Methods.* We screened 298 diagnostic childhood AML samples using PCR-based direct sequencing of exons 7-10. We also screened exons 1-6 in a selected CN-AML subgroup. Additionally we screened 39 paired diagnostic-relapse samples. *Results.* WT1 mutations were detected in 35/298 (12%) of the samples. In 18/35 (51%) of the WT1-mutated samples, we detected more than one WT1 mutation that included another WT1 mutation (n=14), a homozygous mutation (n=3), or a deletion of the other allele (n=1). Mutations were predominantly found in exon 7, but also in exons 1, 2, 3, 8, 9 and 10. Strikingly, the mutational hotspots were located within areas used for WT1-based MRD detection. Moreover, 4/28(14%) diagnostic-relapse pairs with a wild-type WT1 at diagnosis gained a mutation at relapse, which also has consequences for MRD detection. Regarding patient characteristics, WT1 mutations clustered in the CN-AML subgroup (21/93=22.6%; p=<0.001); fewer (14/205=6.8%) being found in the other cytogenetic subgroups. WT1-mutated patients had a higher diagnostic WBC (median 57.2x10E9/l; p=0.007). No significant differences were found for median age, gender and FAB classification between WT1-mutated *vs* wild-type patients. WT1 mutations were more frequently associated with an FLT3/ITD (40%; p=0.002) and a CEBPalpha mutation (26%; p=0.007), but did not carry simultaneously NPM1 mutations (0%; p=0.09). Prognostic univariate analysis within the CN-AML patients identified WT1 mutations as a poor prognostic factor (WT1-mutated *vs* wild-type patients: CR rate 74% *vs* 91%; p=0.05, pOS 45% *vs* 72%; p=0.02 and pEFS 32% *vs* 51%; p=0.04). Multivariate analysis including other known prognostic factors in childhood AML is currently being performed. *Conclusions.* WT1 mutations cluster in childhood CN-AML, and are a new poor prognostic marker in this subgroup. The presence of these mutations may complicate the WT1-based MRD detection currently used.

0458

MIR-125B: RELEVANCE IN CHILDHOOD ACUTE MEGAKARYOBLASTIC LEUKEMIAS

K. Boehmer, J.H. Klusmann, D. Reinhardt

Hannover Medical School, HANNOVER, Germany

Background. Children with Down's syndrome (DS) are at high risk to develop transient leukemia (TL) or acute myeloid leukemia (ML-DS). Acquired mutations of the hematopoietic transcription factor GATA1 have been identified in leukemic blasts from virtually all patients with ML-DS or TL. Five miRNAs (miR-99a, miR-125b-2, miR-155 and let-7c) are known to be encoded on chromosome 21. We investigated the role of miR-125b-2 in the development of TMD and ML-DS. *Patients and Methods.* To elucidate their role in the development of TL and ML-DS, we first measured their expression level in sorted cells from patient samples [ML-DS (n = 4), TL (n = 4), non-DS AMKL (n = 4)], and in megakaryocytes from healthy donors (n = 3) by qRT-PCR. The expression of miR-125b was clearly elevated in ML-DS (10-fold), TL (5-fold) and non-DS AMKL (3-fold) compared to normal megakaryocytes (ppatients *vs* control <0.01). This was confirmed in microRNA low density array [ML-DS (n = 5), TMD (n = 5), non-DS AMKL (n = 3)]. To define the relevance of miR125b-2 for proliferation the miRNA was transduced into human $CD34^+$ stem cells. Proliferation was analysed by MegaCult and CollagenCult. Inhibition of miRNA125b was evaluated by transfection of anti-miRNA 125 into cell lines (CMK, M07, K562), blasts and stem cells. Cell cycle of the transfected cells was examined by BrdU-flow-Kit. With Gene Set Enrichment Analysis (GSEA), we analysed the regulation of putative target genes of mi-R125b-2 predicted by Targetscan and PicTar databases in the gene expression profile of ML-DS in comparison to non-DS AMKL. *Results.* By retroviral overexpression, we could show that ectopic expression of miR-125b markedly enhanced proliferation of myeloid progenitor cells (MPC, $CD34^+CD33^+$) (MPCmiR-125b) *in vitro*. In colony-forming assays, the number of MPCmiR-125b derived colonies exceeded the number of MPCvector derived colonies (empty vector) by 4-fold (<0.01). All MPCmiR-125b derived colonies contained a mixture of immature cells (CFU-GEMM) indicating an affected differentiation. This could also be confirmed by immunophenotyping of MPC cultured in liquid medium containing various cytokines (percentage $CD34^+CD33^+$ 26% *vs* 9%). The silencing of miR-125b-2 resulted in a significant inhibition of proliferation of megakaryoblastic cell lines, and primary blasts, whereas $CD34^+$ human stem cells and K562 remains unaltered (ANOVA; p<0.05). Approximately 10% of M07 cells transfected with anti-125b were in G2-cell phase comparison to 16% of control cells. GSEA of ML-DS (n=22) compared to AMKL (n=30) revealed a decreased expression of putative target genes of mi-R 125b-2. As predicted, silencing of miR-125b shows the upregulation of 347 target genes of mi-R125b-2 in sample of cells (M07, CMK). *Conclusions*. miR-125b seems to be involved in the leukemogenesis of trisomy 21 associated leukemia by mediating the proliferation of MPC while repressing differentiation.

0459

GENOME-WIDE SNP ANALYSIS IDENTIFIES GENOMIC ABNORMALITIES IN ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE

J. Kronke,[1] L. Bullinger,[1] C. Schön,[1] K. Urlbauer,[1] S. Miller,[1] C. Senger,[1] K. Holzmann,[2] R. Schlenk,[1] K. Döhner,[1] H. Döhner[1]

[1]University of Ulm, ULM; [2]Microarray Core Facility, University of Ulm, ULM, Germany

Approximately 45% of patients (pts) with acute myeloid leukemia (AML) exhibit a normal karyotype on conventional chromosome banding analysis. To further characterize this heterogeneous group of cytogenetically normal (CN)-AML, we applied the microarray-based technology of single nucleotide polymorphism (SNP) analysis which provides a powerful tool to identify copy number alterations (CNA) and uniparental disomies (UPD) that result from the exclusion of the wild-type allele without change of chromosomal copy number. We performed paired SNP-chip analysis in a total of 126 CN-AML pts: in 80 pts the Affymetrix 50k Hind and in 62 pts Affymetrix 250k Sty and Nsp (together 500k) arrays were used;.in 16 pts a comparative analysis on both the 50k and 500k platform was done. In all pts bone marrow samples taken at the time of complete morphological remission was used as an intra-individual control to distinguish acquired somatic aberrations in AML blasts from germline polymorphisms. Data analysis was performed by using the CNAG 2.0 software. In total, 42 aberrations (deletions n=16; gains n=6; UPDs n=20) were identified in 31 cases. Two small deletions, one in 2p21 (0.7 Mb) and a second in 11p13 (0.26 Mb), were detected only by the 500k platform, thereby demonstrating the superiority of the 500k over the 50k assay. Other deletions occurred in 3p13 (n=4), 12p13 (n=2), 12q21 (n=1), 12q24 (n=1), 2p21 (n=1), 4q24 (n=1), 8q21 (n=1), 8q23 (n=1) 9q21 (n=1), 11p13 (n=1), 11q23 (n=1), and 13q12 (n=1). Recurrent deletions on 3p were confirmed by fluorescence *in situ* hybridization (FISH) and the commonly deleted region was delineated to a genomic segment of approximately 2 Mb. Genomic gains were identified in 6 pts and were restricted to single cases [4q22 (n=1), 8q12 (n=1), 9p23 (n=1), 14q32 (n=1), 18q12 (n=1) and 22q11 (n=1)]. Furthermore, acquired UPDs ranging from 28 Mb to 96 Mb in size were detected in 16% of the pts [6p (n=5), 11p (n=5), 13q (n=5), 1p (n=2), 2p (n=1), 11q (n=1) and 19q (n=1)]. All pts exhibiting an UPD on chromosome 13q had homozygous FLT3-ITD (internal tandem duplication) mutations. The UPD on 19q was associated with a homozygous CEBPA mutation. Our data demonstrate the power of high resolution SNP-chip analysis in CN-AML for the detection of novel regions of interest

as well as the definition of areas affected by UPDs, thereby supporting non-random mechanism of acquired mitotic recombination events in AML.

0460

THE LEUKEMOGENIC TRANSCRIPTION FACTOR ERG COLLABORATES WITH GATA1S TO IMMORTALIZE MEGAKAROCYTIC PRECURSORS: A MOUSE MODEL FOR THE PATHOGENESIS OF SPORADIC AND DOWN SYNDROME ASSOCIATED ACUTE MEGAKARYOBLASTIC LEUKEMIAS

S. Izraeli,[1] S. Salek-Ardakani,[2] J. De Boer,[2] N.J. Sebire,[2] L. Rainis,[1] S. Lee,[2] O. Williams,[2] S. Izraeli,[3] H.J.M. Brady[2]

[1]Sheba medical center, RAMAT GAN, Israel; [2]Institute of Child Health, LONDON, UK; [3]Sheba Medical Center, RAMAT GAN, Israel

Background. Although, numerical chromosomal aberrations (aneuploidy) are prevalent in cancer, very little is known of their role in carcinogenesis. The most common constitutional aneuploidy with predisposition to leukemia is trisomy 21, also known as Down Syndrome (DS). DS children have a 20-fold increased risk of childhood acute lymphoblastic leukemia (ALL) and a 500-fold increased risk for acute megakaryoblastic leukaemia (AMKL)3. Around 10% of DS newborns have a transient myeloproliferative disorder (TMD) that resolves spontaneously but approximately 20% of TMD patients will progress to develop AMKL during early childhood. Somatic mutations acquired during fetal hematopoiesis in the GATA1 transcription factor are detected in megakaryoblasts from all the DS patients with either TMD or AMKL. *Aims.* To investigate the hypothesis that the chromosome 21 gene ERG-3 collaborates with mutated GATA1 in megakaryobastic transformation and leukemias. *Methods. In vitro* and *in vivo* transformation and transplantation assays with mouse fetal liver hematopietic progenitors (HPCs) transduced with ERG and mutated GATA1 (GATA1s). *Results.* Transduction with both ERG and GATA1s (but not with each of them alone) was necessary and sufficient for immortalization of fetal megakaryocyte progenitors with the phenotype of AMKL megakaryoblasts. We show that ERGpromotes megakaryopoiesis and acts as an oncogene and that progenitor cells harboring a GATA1s mutation plus ERG or ERG alone lead to rapid development of invasive megakaryoblastic leukemia *in vivo*. The addition of GATA1s resulted in a less differentiated AMKL compared with ERG alone. *Conclusions.* Our data support a model where trisomy 21 overexpressed genes that promote fetal megakaryopoiesis co-operate with GATA1s mutations that arrest differentiation and lead to DS AMKL. It is also the first demonstration in-vivo of the direct leukemogenic role of ERG, whose high level of expression in human myeloid leukemias are associated with particularly poor outcome.

0461

IDENTIFICATION OF THREE TYROSINE RESIDUES OF IMPORTANCE FOR SURVIVAL SIGNALING OF FLT3 ITD THROUGH GAB2

K. Masson, T. Liu, J. Sun, L. Rönnstrand

Lund University, MALMÖ, Sweden

The receptor tyrosine kinase Flt3 is normally expressed in hematopoietic progenitor cells and has been implicated as major cause of transformation in acute myeloid leukemia, where it in approximately 30% of cases is constitutively active. Intracellular signals mediated by Flt3 play pivotal roles in cell fate determination and survival and previous studies have shown that signaling via the mutant Flt3 ITD (internal tandem duplication) promotes enhanced cell growth as compared to the wild type receptor. In this study, we have identified tyrosines 768, 955 and 969 of Flt3 as phosphorylation sites and mediators of Grb2 interaction, leading to the association of the scaffolding protein Gab2 and contributing to proliferation and survival. We used murine hematopoietic Ba/F3 cells stably transfected with either the wild type Flt3 or the ITD, with or without a triple mutation of the Grb2 binding sites, and have characterized the cells in terms of proliferation, survival and apoptosis. Interestingly, we found that the Flt3 ITD promotes increased survival and when mutating the tyrosines 768, 955 and 969 to phenylalanine, this phenotype is lost. When looking into different downstream pathways, we observed that this effect is mainly caused by decreased PI3-kinase/Akt as well as STAT5 signaling, and the Flt3 ITD carrying the triple mutation of the Grb2 binding sites show less Akt and STAT5 activation as compared to the regular Flt3 ITD receptor. siRNA mediated knockdown of Gab2 in the Flt3-ITD expressing AML cell line MV4-11 strongly suppressed Erk, Akt and Stat5 phosphorylation. These findings not only reveal new phosphorylation sites in Flt3 but contribute to the understanding of the molecular mechanism by which Flt3 ITD functions in pathological conditions.

Anemia and bone marrow failure

0462

OPPOSING EFFECTS OF PROTEIN KINASE A AND C CAUSES DIFFERENTIAL PHOSPHATIDYLSERINE EXPOSURE IN A CD47 RECEPTOR MEDIATED ERYTHROCYTE APOPTOTIC PATHWAY

P. Jeremy,[1] F. Gilsanz,[2] J. Delaunay,[3] N. Avent[1]

[1]University of the West of England, BRISTOL, UK; [2]Hospital '12 de Octubre' 'Instituto National de la Salud, MADRID, Spain; [3]Service d'Hématologie, d'Immunologie et de Cytogénétique, PARIS, France

Erythrocyte apoptosis, like nucleated cell death, is characterised by phosphatidylserine exposure at the outer membrane leaflet. Our group has previously reported that ligation of a monoclonal antibody, BRIC-126 can mediate red cell apoptosis and subsequent PS exposure, through a CD47 receptor mediated pathway. We have also demonstrated that several membrane proteins are involved in this pathway and one such interaction is a direct protein: protein interaction between CD47 and protein 4.1R. Further studies have shown that red cells deficient in protein 4.1R undergo increased PS exposure in response to BRIC-126 than do normal cells, suggesting that protein 4.1R is critical to this pathway. In order to further elucidate the CD47 apoptotic pathway we have inhibited Protein Kinase A and Protein Kinase C whilst ligating CD47 with BRIC-126, as these kinases are known to interact with protein 4.1R at specific serine residues and are often implicated in cell signalling cascades, especially apoptosis. We have used flow cytometry in conjunction with an annexin V-FITC binding assay to compare mean percentage annexin V positive cells whilst inhibiting protein kinase A and protein kinase C using the cell permeable specific inhibitors Bisindolylmaleimide I, Go 6976 and KT 5720. Our findings suggest opposing effects of Protein kinase A and Protein Kinase C compared with control erythrocytes. We observed that PS exposure was decreased in cells with the specific cell permeable PKC inhibitors Bisindolylmaleimide I and Go 6976 present but increased in cells with the specific PKA cell permeable inhibitor KT5720 present. The increase in PS exposure when PKA inhibitor KT5720 is present is similar to the effect seen in individuals deficient in protein 4.1R. The difference in PS exposure between PKC inhibition and PKA inhibition suggests opposing effects of PKA and PKC during CD47 ligation and a possible interaction with protein 4.1R, this may also suggest that specific phosphorylation events on protein 4.1R are critical to the CD47 apoptotic pathway. Ongoing studies are investigating isoform specific inhibition of PKC and PKA along with 2D phosphoimmunoblots to look at changes in PI before and after ligation of BRIC 126.

0463

CHARACTERISATION OF TINF2 MUTATIONS IN A LARGE COHORT OF PATIENTS WITH DYSKERATOSIS CONGENITA AND RELATED BONE MARROW FAILURE SYNDROMES

J. Walne, T. Vulliamy, R. Beswick, M. Kirwan, I. Dokal

Institute of cell and molecular sciences, LONDON, UK

Background. Dyskeratosis congentia (DC) is a multi-system syndrome which displays marked clinical heterogeneity. Patients present with a wide range of haematological and non-haematological features including nail dystrophy, abnormal skin pigmentation, leucoplakia and aplastic anaemia (AA). Many patients can first present with AA and the diagnosis of DC may not be clinically obvious. The genetic basis of DC remains unknown in >50% of patients. Mutations have been found in genes encoding components of the telomerase complex, namely TERC (the RNA component), TERT (the enzymatic component) and dyskerin (DKC1) and NOP10 both of which encode proteins in the small nucleolar ribonucleoprotein particle. Recently TINF2, a component of the shelterin complex has also been shown to be mutated in a small number of patients with DC *Aims*. The aim of this study was to determine the contribution to the pathology that mutations in exon 6 of TINF2 may make in patients with DC and other related syndromes including aplastic anaemia (idiopathic and constitutional, IAA and CAA, respectively) and myelodysplasia/acute myeloid leukaemia. This exon was the focus of the study as previous work identified mutations only in this region of the gene. *Methods*. To date 302 families are entered into the Dyskeratosis Congenita Registry at Barts and The London, London, UK. In this large collection of patients, 185 remain genetically uncharacterised. Analysis failed on 12 patients, and the remaining 173 were screened for mutations in exon 6. Heteroduplex analysis was performed using denaturing high performance liquid chromatography following PCR amplification. Any abnormal shifts were sequenced to determine the nucleotide change involved. We also screened 111 patients with IAA, 77 with CAA, 15 with myelodysplasia/acute myeloid leukaemia and 41 with related disorders as well as 91 healthy individuals. *Results*. Coding changes were found in 31/173 previously uncharacterised DC index cases. In this group, 21/31 mutations affected amino acid arginine 282, mutated to either cysteine (n=7) or histidine (n=14). Of the remaining 10 mutations, 7 were mis-sense and 3 resulted in a frame-shift and premature stop codons. 8 coding changes were also seen in the 244 non-DC patients analysed. Of these, 5 resulted in the polymorphic Gly237Arg substitution. The remaining 3 were unique mis-sense mutations. Apart from the known polymorphism none of these changes were seen in the healthy control group. Segregation analysis in the DC families showed that of the 16 cases where both parents are available, 15 are *de novo* and in only one case is the mutation is inherited. *Summary and conclusions*. In this large series, TINF2 mutations in exon 6 account for ~11% of DC (31/290), with the majority being *de novo* cases. Patients with TINF2 mutations have severe disease with a high incidence of aplastic anaemia usually presenting below 10 years of age. In the non-DC patient group TINF2 mutations may have a minor pathological role (3/244). The prevalence of TINF2 as a cause of DC is second only to dyskerin (~30%, 86/290).

0464

EVIDENCE OF A SUB-CLINICAL LYMPHOPROLIFERATIVE DISEASE IN PATIENTS WITH 'IDIOPATHIC' AUTOIMMUNE HEMOLYTIC ANEMIA

F.R. Mauro, M.S. De Propris, I. Del Giudice, D. Armiento, S. Coluzzi, I. Della Starza, M.C. De Nicolo', M.C. Arista, L. Quattrocchi, G. Girelli, A. Guarini, R. Foa'

University "La Sapienza", ROME, Italy

Background. Idiopathic autoimmune hemolytic anemia (AHA), which accounts for about half of cases, is diagnosed in the absence of a concomitant disease. A lymphoproliferative disease is frequently associated or can follow AHA. *Aims*. The aim of this study was to better define the clinical features of cases defined as *idiopathic* AHA. *Methods*. Fifty consecutive patients with *idiopathic* AHA were diagnosed at the Institute of Hematology of "La Sapienza"University of Rome. In all patients, except for anemia associated with laboratory signs of hemolysis and a positive DAT test, no evidence of an associated disease emerged by the clinical examination and the medical history. Before starting steroid treatment, the following examinations were performed: immunohematologic study; total body CT scan or a chest XR with abdomen ultrasound; HBV and HCV serology; autoantibody screening (anti-nucleous; anti-cardiolipin; anti-gastric parietal cell; anti-beta 2-glycoprotein-I); peripheral blood (PB) immunophenotype. *Results*. The median age of the 50 patients was 66 years (range: 20-84), Thirty-two patients were females. The immunohematologic study revealed an anti-erythrocyte auto-antibody in all cases (IgG: 27; IgM:15; IgG + IgM: 7; IgA:1). An idiopathic AHA was confirmed in 26 cases (52%), while in 24 (48%) a concurrent disease was diagnosed (tumor: 4; HCV hepatitis: 2; autoimmune disease: 2). In 16 cases (32%), all with a normal lymphocyte count, the PB immunophenotype revealed the presence of a small B-lymphocyte clone, with a chronic lymphocytic leukemia phenotype, $CD5^+/CD19^+$, $CD23^+$, in 3 cases and a lymphoma phenotype, $CD5^+/CD19^+$, $CD23^-$ or $CD5^-/CD19^+$, $CD23^-$, in 13. The median number of clonal B lymphocytes in the PB was 0.5×10^9/L (range: 0.03-1.9×10^9/L). Clonality was confirmed by PCR Ig gene rearrangement analysis. The same clonal population was detected in the bone marrow aspirate of all evaluated cases. No other signs ascribable to a lymphoproliferative disease were detected, except for a patient with an enlarged abdominal lymph node. *Conclusions*. Our findings indicate that about one third of newly diagnosed patients with otherwise defined *idiopathic* AHA show an underlined B-lymphocyte clone, suggesting the presence of a subclinical lymphoproliferative disease which may play a role both in the pathogenesis of the autoimmune disorder and in the subsequent occurrence of a lymphoma. The detection of such clones should be considered in the diagnostic work-out and address the treatment strategy of patients with AHA.

0465

DOUBLE CORD BLOOD TRANSPLANTATION FOR PATIENTS WITH INHERITED AND ACQUIRED BONE MARROW FAILURE SYNDROMES

A. Ruggeri,[1] R. Peffault de Latour,[2] V. Rocha,[2] C.A. Rodrigues,[2] M. Robin,[2] J. Larghero,[3] R. Traineau,[4] P. Ribaud,[2] C. Ferry,[2] A. Devergie,[2] E. Gluckman,[2] G. Socié[2]

[1]Bone Marrow Transplantation unit, PARIS; [2]Service d'Hématologie Greffe, PARIS; [3]Unité de Thérapie cellulaire, PARIS; [4]Service d'Hémobiologie, PARIS, France

Background. The outcome of severe aplastic anemia (SAA), refractory to immunosuppressive therapy or related to Fanconi anemia (FA), is usually poor in the absence of hematopoietic stem cell transplantation. Single cord blood is an alternative stem cell source for patients without matched related or unrelated donors, but is associated with high transplant related mortality due to the low cell dose infused. *Aims.* We performed double cord blood transplantation (dCBT) in 14 patients (6 male and 8 female) with a median age of 16 years (6-31), diagnosed with bone marrow failure syndromes (BMFS) (8 FA, 5 SAA and 1 PNH), from 2004 to 2007. All patient data were collected after informed consent. *Methods.* Median disease duration before dCBT was 31 months (5-240). At transplant, median neutrophil and platelet count were 0.3×10^9/L (0.0- 1.1) and 21×10^9/L (3-93), respectively. All patients were highly transfused before transplant. Six patients (43%) received a dCBT as a rescue of previous rejected transplants (2 SAA and 4 FA). All patients received a fludarabine-based regimen, with TBI (2 Gy) for 4 patients. Cord blood units were 4/6 or 5/6, HLA A, B and DR match with the patient, except one which was 3/6. Graft versus host disease (GVHD) prophylaxis consisted in cyclosporin+mycophenolate mophetile for 5 patients. Steroids were given from day 7 to day 14 and stopped in absence of GVHD. Median cell doses infused were 4.8×10^7 NC/Kg (1.8-9.7) and 2.9×10^5 $CD34^+$cells/Kg (0.5-7.46). *Results.* Graft rejection was observed in 6 patients (3 previously allotransplanted). Among the remaining 8 patients the median time to achieve absolute neutrophils count >500 was 28 days (range 14-42) and median time to a platelet count > 20,000 was 83 days. At day 100, donor-recipient chimerism analysis was evaluable in 10 patients (72%). Seven patients (70%) showed a full donor chimerism, 2 patients (20%) were mixed chimeras and 1 patient (10%) had an autologous recovery. In six out of 8 patients, the predominant UCB unit was the first one infused. Acute GVHD grade II-III was scored in 9 patients (70%) (6 grade II, 3 grade III). One patient presented acute GVHD grade IV. All the FA group patients (8 out of 14) developed aGVHD. Six patients out of 8 developed chronic GVHD (3 limited and 3 extensive). Six patients died (2 GVHD, 2 fungal infections, 1 thrombotic microangiopathy, 1 sepsis). With a median follow-up of 13 months (range 5- 22), the overall survival (OS) was 57% (±13%) for all patients. According to disease category, the predicted 1-year OS for patients with acquired BMF (SAA and PNH) and inherited BMFS (FA and CD) was 80 (±17%) and 44 (±16%), respectively.The median survival of patients who were transplanted twice was 50% (±13%). *Conclusions.*dCBT seems to be an option to treat patients with bone marrow failure syndromes and without matched HLA donors. Those results need to be confirmed in a prospective study to warrant the inclusion of dCBT in the treatment strategy of diseases with high risk of rejection.

0466

EFFECTS OF STAT5A PHOSPHORYLATION ON PROLIFERATION AND DIFFERENTIATION OF MYELOID HEMATOPOIETIC PROGENITORS IN HEALTHY INDIVIDUALS AND IN PATIENTS WITH SEVERE CONGENITAL NEUTROPENIA

J. Skokowa, K. Gupta, A. Müller Brechlin, K. Welte

Hannover Medical School, HANNOVER, Germany

Background. Severe congenital neutropenia (SCN) is characterized by the onset of recurrent life-threatening infections in early childhood due to a *maturation arrest* of myeloid progenitors at the promyelocytic stage with a few or no mature neutrophils in the bone marrow and blood. SCN patients are at increased risk of developing AML or MDS. The cumulative incidence of AML/MDS is 21% after 10 years. The leukemic transformation occurred in both autosomal dominant (with ELA2 mutations) and autosomal recessive (with HAX1 mutations) groups of SCN patients. The pathogenesis of SCN, the mechanisms underlying malignant transformation in these patients as well as the role of G-CSF treatment in this process are still unknown. Since AML/MDS are not observed in cyclic (CyN) or idiopathic neutropenia patients treated with G-CSF, an underlying defect downstream of G-CSFR signaling rather than G-CSF therapy *per se* predisposes to malignant transformation in SCN. Recently we found that downregulation of LEF-1 transcription factor and its target gene C/EBPβ are a common pathomechanism of SCN irrespective of mutation status. *Aims.* In the present study, we aimed to evaluate the association between increased G-CSF-triggered phosphorylation of STAT5, low levels of LEF-1 and leukemic transformation of hematopoiesis in SCN. *Methods.* We investigated the effects of G-CSF on phosphorylation status of STAT5 in $CD34^+$ and in $CD33^+$ bone marrow progenitor cells from healthy individuals and SCN patients. We also compared expression patterns of LEF-1 and C/EBPβ in the presence of constitutively activated STAT5a (mutant of STAT5a, (STAT5A[1*6]) in these cells and evaluated the mechanisms of STAT5: LEF-1 interaction. *Results.* We found that G-CSF stimulation resulted in a significantly higher phosphorylation of STAT5 in $CD33^+$ bone marrow myeloid progenitors of four SCN patients (two harbouring ELA2 mutations and two with HAX1 mutations), as compared to two patients with cyclic neutropenia, two patients with idiopathic neutropenia and four healthy volunteers. Transduction of constitutive activated STAT5a in $CD34^+$ or in $CD33^+$ cells of healthy individuals resulted in 20-fold downregulation of LEF-1 expression. A screen of the 10kb upstream region of LEF-1 gene revealed two putative STAT5a binding sites (-3913 bp to -3894 bp and -3728 bp to -3709 bp). The specificity of the STAT5a binding to the LEF-1 promoter in nuclear extracts of $CD34^+$ cells and in two myeloid leukemia cell lines (HL-60, U937) was confirmed in a chromatin immunoprecipitation (ChIP) assay. More intriguingly, to analyse the mechanisms of LEF-1 inhibition, we generated reporter gene constructs containing 4000 bp upstream regulatory region with promoter of human LEF-1 gene. We found, that co-transfection of 293 HEK cells with LEF-1 cDNA and β-catenin cDNA resulted in a considerable elevation of LEF-1 promoter activity, suggesting a strong autoregulation of LEF-1 gene by LEF-1 protein. Additional co-transfection with constitutively activated STAT5a significantly abrogated LEF-1-dependent activation of LEF-1 promoter. Moreover, we found that constitutively activated STAT5a also abrogated LEF-1/β-catenin effects on TOPglow reporter construct, containing four LEF-1/TCFs binding sites. Similar inhibitory effects of STAT5A[1*6] on LEF-1 transcriptional activity has been observed by the analysis of the reporter construct of known LEF-1 target gene, cyclin D1. *Conclusion*. G-CSF induced a strong phosphorylation of STAT5 in hematopoietic progenitors of SCN patients, as compared to patients with other types of neutropenia and to healthy individuals. Constitutively activated STAT5a binds directly to the promoter of LEF-1 gene and inhibits its expression by disturbing the LEF-1-autoregulatory loop. These intracellular events may contribute to the malignant transformation of myelopoiesis in SCN.

Hodgkin's lymphoma

0467

FDG-PET FOR ASSESSMENT OF RESIDUAL TISSUE AFTER COMPLETION OF CHEMOTHERAPY IN HODGKIN LYMPHOMA REPORT ON THE 2ND INTERIM ANALYSIS OF THE PET INVESTIGATION IN THE TRIAL HD15 OF THE GHSG

M. Fuchs,[1] H. Haverkamp,[1] M. Dietlein,[1] V. Diehl,[2] A. Engert,[1] C. Kobe[1]

[1]University Hospital of Cologne, COLOGNE; [2]German Hodgkin Study Group, COLOGNE, Germany

Background and Aims. The HD15 multicenter trial of the German Hodgkin Study Group (GHSG) included advanced-stage Hodgkin lymphoma patients. Patients were prospectively randomized to either 8 or 6 cycles of BEACOPPescalated or 8 cycles time-condensed BEACOPP-baseline. Beside the reduction of chemotherapy one other main study endpoint was the prognostic value of 18F-fluorodesoxyglucose (FDG) positron emission tomography (PET) following chemotherapy. The aim was to specify the negative predictive value of PET (NPV) in patients with residual tumour mass after chemotherapy. *Methods.* Inclusion criteria for the PET question were partial remission (PR) after the end of chemotherapy with at least one involved nodal site measuring 2.5 cm or more in diameter by computed tomography (CT). Exclusion criteria included diabetes, elevated blood sugar levels and skeletal involvement with risk of instability. Calculations were restricted to those cases with either progressive disease (PD) or relapse within 12 months after PET or at least 12 months of follow-up. A total of 275 patients were eligible for this analysis. The assessment was based on those patients confirmed by an expert panel as being PET-negative. CT verification was performed to identify false positive PET findings. The NPV was defined as the proportion of patients without progression or relapse within 12 months. *Results.* 9/216 patients with PET-negative residues and 9/59 patients with PET-positive residues had PD or relapse within one year of follow-up. The NPV was 0.958% (95% CI 0.931 - 0.985%). 244/245 patients with PET-negative residual masses, received not further irradiation. 62/66 patients with PET-positive residues, received additional radiotherapy. Progression/relapse rates were significantly different between those patients with residual mass being PET-negative or PET-positive (p=0.0053). PET-negative patients, who were assessed as partial response by CT, had a prognosis similar to those in complete remission. There was no significant difference in the progression free survival in this trial and the prior GHSG trials HD12 (arms pooled) and HD9 (arm C) for advanced-stage HL (p=0.266). Importantly, the proportion of patients receiving radiotherapy decreased from 70% (HD9-C) to 39% (HD12) and 12% (HD15). *Conclusions*. The high NPV of PET suggests that radiotherapy following 6 or 8 cycles of BEACOPP might be restricted to those patients who are PET-positive after chemotherapy.

0468

ALLOGENEIC STEM CELL TRANSPLANTATION AFTER A RIC REGIMEN PROLONGS THE SURVIVAL IN PATIENTS WITH HODGKIN LYMPHOMA (HL) RELAPSED AFTER HIGH-DOSE CHEMOTHERAPY: A RETROSPECTIVE STUDY BASED ON DONOR AVAILABILITY

L. Castagna,[1] B. Sarina,[1] F. Benedetti,[2] G. Milone,[3] P. Patriarca,[4] S. Viviani,[5] M. Malagola,[6] S. Ferrari,[7] G. Sorasio,[8] L. Farina,[9] E. Todisco,[1] L. Giordano,[1] P. Corradini[9]

[1]Istituto Clinico Humanitas, ROZZANO; [2]Hematology University of Verona, VERONA; [3]BMT Unit Ospedale Ferrarotto, CATANIA; [4]Clinica Ematologica ed Unita di Terapie Cellulari Carlo Melzi, University of U, UDINE; [5]Medical Oncology, Istituto Nazionale Tumori, University of Milano, MILANO; [6]Centro Trapianti di Midollo Osseo per Adulti Cattedra di Ematologia Spedali Civi, BRESCIA; [7]Hematology, Spedali Civili Brescia, BRESCIA; [8]Hematology University of Torino, TORINO; [9]Hematology dept. Istituto Nazionale Tumori, University of Milano, MILANO, Italy

Background. Allogeneic stem cell transplantations (allo-SCT) with reduced intensity conditioning (RIC) regimens are increasingly performed in lymphomas. The number of studies on RIC allografting in Hodgkin lymphoma (HL) remains quite limited, several groups reported conflicting results, and no clear data exist on the clinical role of allo-RIC as an effective salvage option. HL patients relapsing after autologous SCT (auto-SCT) have a very dismal outcome with no therapeutic options able to obtain a durable disease control. *Aims*. To investigate the role of RIC allo-SCT in HL patients relapsing/progressing after auto-SCT. All our analysis was based on the commitment of the attending physician to perform an allo-SCT and therefore we retrospectively evaluated only those patients undergoing a HLA-typing immediately for the failure of auto-SCT. We compared patients receiving allogeneic transplantation (donor group) with those not having a suitable donor (no donor group) receiving a conventional treatment. *Patients and Methods*. 97 patients were retrospectively evaluated. For all patients, a search for a sibling or matched unrelated donor (MUD) was started at time of relapse/progression. 56 patients had a donor: 31 HLA-identical siblings (53%), 19 MUD (32%), 6 haploidentical family donor (15%). Thiotepa and fludarabine based regimens were used before allogeneic transplantation in all patients. Patients with no donor (n= 41) were treated according to the common policy of each center. All of them received chemo- and/or radiotherapy. *Results*. The median age of patient population was 30 years (17-62). After a median follow-up of 23 months (0-110), 29 patients are alive (52%) in the donor and 13 in the no donor group (32%). For all patients, the median overall (OS) and progression free survival (PFS) were 31 and 15 months, respectively. The 2-year OS and PFS were 60% and 29% respectively. The transplant-related mortality was 12% in the donor group. The 2-y OS and PFS were significantly better in the donor compared to the no donor group (OS 68% *vs* 50%, p 0.003, long rank test; PFS 31% *vs* 26%, p 0.03, Wilcoxon test). A pre allo-SCT positive PET scan correlated with a poor outcome. *Conclusions*. This retrospective study shows that RIC allogeneic transplantation is a feasible and useful option for HL patients failing an auto-SCT.

0469

EARLY INTERIM FDG-PET IN ADVANCED-STAGE HODGKIN LYMPHOMA (HL). LONG-TERM RESULTS OF THE ITALIAN-DANISH COOPERATIVE STUDY

A. Gallamini,[1] M. Hutchings,[2] L. Rigacci,[3] L. Specht,[4] F. Merli,[5] M. Hansen,[6] C. Patti,[7] A. Loft,[8] F. Di Raimondo,[9] F. D'Amore,[10] A. Biggi,[11] P. Pregno,[12] C. Stelitano,[13] R. Sancetta,[14] L. Trentin,[15] S. Luminari,[16] E. Iannitto,[17] S. Viviani,[18] I. Pierri,[19] P. Torchio,[20] A. Levis[21]

[1]Az. Ospedaliera S. Croce e Carle, CUNEO, Italy; [2]University of Copenhagen, COPENHAGEN, Denmark; [3]University Of Florence, FLORENCE, Italy; [4]Copenhagen University, COPENHAGEN, Denmark; [5]Reggio Emilia Hospital, REGGIO EMILIA, Italy; [6]Oncology Department, COPENHAGEN, Denmark; [7]Ospedale V. Cervello, PALERMO, Italy; [8]Clinical Physiology department, COPENHAGEN, Denmark; [9]Catania University, CATANIA, Italy; [10]Aarhus University, AARHUS, Denmark; [11]S. Croce Hospital, CUNEO, Italy; [12]S.Giovanni Battista Hospital, TORINO, Italy; [13]Bianchi and Melacrino Hospital, REGGIO CALABRIA, Italy; [14]S. Giovanni and Paolo Hospital, VENEZIA, Italy; [15]Padoa University, PADOA, Italy; [16]Modena University, MODENA, Italy; [17]Palermo University, PALERMO, Italy; [18]National Institute of Tumours, MILAN, Italy; [19]University of Genoa, GENOA, Italy; [20]University of Turin, TURIN, Italy

Background. FDG-PET scan has been demonstrated as the only factor able to predict treatment outcome in advanced-stage HL patients treated with ABVD with much more efficacy than International Prognostic Score (IPS) (Gallamini JCO 2007). We present hear the updated result of this trial, on behalf of Intergruppo Italiano Linfomi and Danish Lymphoma Group. *Patients and methods.* Patients prospectively enrolled in the trial were treated with standard ABVD therapy x 6 courses. Consolidation radiotherapy was given in case of bulky presentation or residual tumour mass. Conventional radiological staging and FDG-PET scan were performed at baseline (PET-0) and after two courses of ABVD (PET-2). No treatment change was allowed based on the PET-2 results except in case of overt disease progression. Positive and minimally positive PET-2 scans were centrally reviewed. PET 2 was considered negative if the scan was negative o minimally positive (MRU^+). A study was defined as MRU^+ in presence of a non-focal uptake with a SUV lower, equal or slightly higher than mediastinal blood pool structures. *Results.* Two-hundred-sixty patients were enrolled in the study and are available for analysis. After a median follow-up of 3.15 years (0.6-6.3), 203 patients were in continued CR (cCR), 2 patients were in PR; 43 patients progressed during therapy, and 12 relapsed. Fifty-two patients were PET-2 positive and 208 patients were PET-2 negative. 44/52 PET-2 positive patients (84.6%) showed treatment failure (36 showed progression and 8 relapse) while 7 were in cCR and one in PR at the latest follow-up. 198/208 PET-2 negative patients (96.6%) were in cCR and one patient in PR at the latest follow-up, while 11 patients had experienced treatment failure. The 5-year FFS for PET-2 positive patients was 24.5% and for PET-2 negative 96.0% ($p<0.0001$). The sensitivity, specificity and overall accuracy of PET-2 for predicting 2-year PFS were 77%, 96%, and 92%, respectively. The positive predicting value (PPV) was 85% and the negative predicting value (NPV) was 95%. In univaried analysis the factors confirmed to be significant for FFS were PET scan, IPS (0-2 *vs* > or =3), stage IV, bulky disease and extranodal sites. In multivariate analysis the factors remaining significant were stage IV (HR 2.2) and PET (HR 21.8). In a matched 2-year PFS analysis, this study showed no prognostic value of IPS when the information from PET-2 is added. *Conclusions.* Since most if not all HL treatment failures are recorded within 3 years from diagnosis, PET-2 is able to predict with great efficiency the majority of treatment failures. For these reasons we propose to consider ABVD-treated HL patients with a PET-2 positive a distinctive HL subset characterized by a very poor outcome, requiring very aggressive therapy as early as possible during the course of the disease.

0470

CNS HODGKIN LYMPHOMA: AN INTERNATIONAL PRIMARY CNS LYMPHOMA COLLABORATIVE GROUP (IPCG) REPORT OF 18 CASES

R. Gerstner,[1] L.E. Abrey,[2] D. Schiff,[3] A.J.M. Ferreri,[4] A. Lister,[5] S. Montoto,[5] R. Tsang,[6] E. Thiel,[7] F. Graus,[8] D. Behringer,[9] G. Illerhaus,[10] S. Weaver,[11] P. Wen,[12] N.L. Harris,[1] T.T. Batchelor[1]

[1]Massachusetts General Hospital, BOSTON, MA, USA; [2]Memorial Sloan-Kettering Cancer Center, NEW YORK CITY, USA; [3]University of Virginia, CHARLOTTESVILLE, USA; [4]San Raffaele H Scientific Institute, MILAN, Italy; [5]St. Bartholomew's Hospital, LONDON, UK; [6]Princess Margaret Hospital, TORONTO, Canada; [7]Charité-Campus Benjamin Franklin, BERLIN, Germany; [8]Hospital Clinic, BARCELONA, Spain; [9]Augusta-Kranken-Anstalt, BOCHUM, Germany; [10]University Hospital Freiburg, FREIBURG, Germany; [11]Albany Medical Center, ALBANY, USA; [12]Dana Farber Cancer Institute, BOSTON, USA

Background. Hodgkin lymphoma (HL) involves the central nervous system (CNS) in only 0.2-0.5% of all cases. Most published reports consist of single cases so there is little guidance for clinicians on how to manage this rare disease. *Aims.* To better understand the natural history of CNS involvement of HL. *Methods.* We collected detailed clinical data on 18 patients with meningeal or parenchymal CNS involvement of HL (CNS-HL) treated at 12 international centers from 1972-2007. *Results.* All 18 patients had classical HL: 8 classic HL not otherwise specified, 7 nodular sclerosis HL, and 3 mixed cellularity HL. Fourteen of the 18 patients had histological confirmation of CNS-HL by biopsy, resection, or autopsy and 4 patients had neuroimaging findings consistent with secondary CNS-HL. Only 1 patient had primary CNS-HL. In 7 patients, CNS-HL was discovered simultaneously with or prior to the diagnosis of systemic HL. For the remaining 10 patients, CNS-HL appeared an average of 10.3 months (range 4-189 months) after the diagnosis of systemic HL. Four of these 10 were in *systemic* CR when CNS-HL was discovered. Patients presented with a variety of symptoms at the time of CNS-HL diagnosis: pain/sensory symptoms (5/18), weakness (4/18), altered mental status (3/18), headache (4/18), and seizure (3/18). Lumbar puncture was performed in 11 patients and 6 had elevated protein in the CSF. Two patients had *atypical* cells, in 1 of these 2 the cells stained positive for CD30. Thirteen patients had intraparenchymal disease and 5 had dural-based lesions without parenchymal involvement. Multifocal disease was found in 5 patients, 2 of whom had brain, spinal cord, and meningeal involvement. Seven patients were treated with chemotherapy and radiation resulting in 2 PRs and 5 CRs; 5 received chemotherapy alone resulting in 3 CRs and 2 PDs; 4 received radiation alone (3 WBRT, 1 stereotactic radiosurgery) resulting in 3 CRs and 1 PR; 1 patient underwent gross total resection alone, and 1 patient had an unknown treatment history. In addition, 4 patients were given intrathecal chemotherapy with methotrexate or cytarabine and 2 patients received PBSCT. Radiographic response to any type of treatment was observed in 15/17 patients. Ten patients have died and the median overall survival is 55.8 months (range 4.5-235 months) after first diagnosis of HL (either systemic or CNS) and 39 months (range 2.2-46.8 months) after diagnosis of CNS-HL. Seven patients died from progressive HL, 1 patient died of a pulmonary embolism with evidence of systemic and CNS-HL at the time of autopsy, and 2 died of unrelated causes in CR. Only 2 of the 10 patients with CNS-HL at relapse are alive at 35 and 273 months of follow-up. *Summary and Conclusions.* CNS-HL is rare and our series of 18 patients is the largest one reported to date. Our data indicate that CNS-HL can affect any part of the CNS and can occur in the setting of widespread disease or when disease is well controlled. Although there is no established standard of care for CNS-HL, 15/17 treated patients achieved a radiographic response and long-term survival is possible.

Presidential Symposium

0471

MUTATION IN TMPRSS6, A SUPPRESSOR OF HEPCIDIN GENE EXPRESSION, IN FAMILIAL IRON DEFICIENCY ANEMIA

M.A. Melis,[1] M. Cau,[1] R. Congiu,[1] S. Barella,[1] A. Cao,[1] M. Westerman,[2] M. Cazzola,[3] R. Galanello[1]

[1]University of Cagliari, CAGLIARI; [2]Intrinsic LifeSciences, LA JOLLA, USA; [3]University of Pavia, PAVIA, Italy

Background. While genetic hemochromatosis is a common inborn error of iron metabolism, inherited disorders with defective iron absorption are extremely rare in humans. *Aims*. We studied a large Sardinian family in which microcytic anemia due to defective iron absorption and utilization is inherited as a recessive character, with the aim of identifying the mutant gene. *Case report and methods*. Three of the five patients were referred to hospital between 8 and 12 months of age because of anemia, while the other two were found to be moderately anemic during the study of the family. Anemia was severely microcytic (MCV from 50 and 55 fL) and hypochromic (MCH from 15 to 17 pg). Evaluation of body iron status showed low serum iron, normal TIBC and normal serum ferritin. Serum transferrin receptor levels were elevated, indicating a defective iron supply to the erythroid marrow. This condition is refractory to oral iron, and only partially responsive to parenteral iron administration. *Results*. Genome-wide scanning by microsatellites and single nucleotide polymorphisms showed a multipoint LOD score of 5.6 on chromosome 22q12.3, where the TMPRSS6 gene is located. Its murine counterpart (Tmprss6) has been recently found to be a suppressor of hepcidin gene expression, required for normal uptake of dietary iron. In fact, a splicing error in Tmprss6 has been detected in Mask mice, which have a recessive chemically induced phenotype characterized by progressive loss of body hair and severe iron deficiency due to reduced absorption. Sequencing analysis of TMPRSS6 revealed a homozygous mutation, predicting a splicing error, in affected members of the Sardinian family. Despite iron deficient erythropoiesis, a condition in which hepcidin concentration is expected to be almost undetectable, homozygous subjects had normal to high serum hepcidin levels. *Conclusions*. These observations suggest that the TMPRSS6 mutation leads to overproduction of hepcidin, and in turn to defective iron absorption and utilization. More generally, findings of this study confirm in humans the inhibitory effect of TMPRSS6 on hepcidin synthesis already demonstrated in Mask mice.

0472

BIALLELIC MUTATIONS IN THE TELOMERASE COMPONENT NHP2 CAUSE THE PREMATURE AGEING SYNDROME DYSKERATOSIS CONGEINTA

J. Vulliamy,[1] R. Beswick,[1] M. Kirwan,1 A. Marrone,[2] M. Digweed,[3] A. Walne,[1] I. Dokal[1]

[1]Barts and The London School of Medicine and Dentistry, LONDON, UK; [2]Imperial College School of Medicine, LONDON, UK; [3]Institut fur Humangenetik, BERLIN, Germany

Background. Dyskeratosis congenita (DC) is a bone marrow failure syndrome characterized by a triad of muco-cutaneous features. A range of other somatic abnormalities may also be observed, including signs of premature ageing (early greying, dental loss), osteoporosis and malignancy. DC is genetically heterogeneous, but is unified by the fact that the mutations identified to date all affect molecules involved in telomere maintenance. These include components of the telomerase complex (dyskerin, the RNA component TERC, the reverse transcriptase component TERT and NOP10) which is responsible for maintaining telomere length. More recently, mutations have also been identified in a component of shelterin (TIN2), the telomere-associated protein complex. *Aims*. Many DC patients remain genetically uncharacterized. Here we describe the analysis of two other proteins, NHP2 and GAR1 that together with dyskerin and NOP10 are key components of telomerase and small nucleolar ribonucleoprotein (snoRNP) complexes. *Methods*. Mutation analysis has been performed by denaturing HPLC screening followed by direct DNA sequencing. Telomere lengths have been measured by Southern blot analysis. TERC levels and the effects of siRNA knockdown of gene transcripts have been assessed by quantitative real-time PCR measurements. *Results*. We have identified the first biallelic mutations in NHP2 and shown that can cause autosomal recessive DC. In contrast, we have not found any GAR1 mutations. Patients with NHP2 mutations, in common with patients bearing dyskerin and NOP10 mutations have short telomeres and low TERC levels. SiRNA mediated knockdown of NHP2 in human cells also led to a reduction in TERC levels, but this was not observed following GAR1 knockdown. *Summary and conclusions.* These findings suggest that in human cells GAR1 has a different impact on the accumulation of TERC compared to dyskerin, NOP10 and NHP2. Most of the mutations so far identified in patients with classical DC impact either directly or indirectly on the stability of RNAs. Specifically, patients with dyskerin, NOP10 and now NHP2 mutations have all been shown to have low levels of telomerase RNA in their peripheral blood providing direct evidence of their role in telomere maintenance in humans.

0473

SUPERIOR EFFICACY WITH BORTEZOMIB PLUS MELPHALAN-PREDNISONE (VMP) VERSUS MELPHALAN-PREDNISONE (MP) ALONE IN PREVIOUSLY UNTREATED MULTIPLE MYELOMA (MM): RESULTS OF THE PHASE III MMY-3002 VISTA STUDY

J. San-Miguel,[1] R. Schlag,[2] N. Khuageva,[3] M. Dimopoulos,[4] O. Shpilberg,[5] M. Kropff,[6] I. Spicka,[7] M. Petrucci,[8] A. Palumbo,[9] O. Samoilova,[10] A. Dmoszynska,[11] K. Abdulkadyrov,[12] R. Schots,[13] B. Jiang,[14] M. Mateos,[1] K. Anderson,[15] D. Esseltine,[16] K. Liu,[17] A. Cakana,[18] H. Van de Velde,[18] P. Richardson[15]

[1]Hospital Universitario de Salamanca, SALAMANCA, Spain; [2]Praxisklinik Dr. Schlag, WÜRZBURG, Germany; [3]SP Botkin Moscow City Clinical Hospital, MOSCOW, Russian Federation; [4]University of Athens School of Medicine, ATHENS, Greece; [5]Rabin Medical Center, PETAH-TIQVA, Israel; [6]University of Münster, MÜNSTER, Germany; [7]University Hospital, PRAGUE, Czech Republic; [8]University La Sapienza, ROMA, Italy; [9]Universita di Torino, TORINO, Italy; [10]Nizhnii Novgorod Region Clinical Hospital, NOVGOROD, Russian Federation; [11]Medical University of Lublin, LUBLIN, Poland; [12]St Petersburg Clinical Research Institute of Hematology & Transfusiology, ST PETERSBURG, Russian Federation; [13]Myeloma Study Group Belgian Hematological Society, BRUSSELS, Belgium; [14]People's Hospital, Peking University, PEKING, China; [15]Dana-Farber Cancer Institute, BOSTON, United States of America; [16]Millennium Pharmaceuticals, Inc,, CAMBRIDGE, United States of America; [17]Johnson & Johnson Pharmaceutical Research & Development, L.L.C., RARITAN, United States of America; [18]Johnson & Johnson Pharmaceutical Research & Development, BEERSE, Belgium,

Background. MP has been the standard of care for newly diagnosed MM patients ineligible for high-dose therapy with stem cell transplantation (HDT-SCT). However, rates of complete response (CR) are low and median overall survival (OS) is typically 2-3 years. In a phase I/II trial of VMP in 60 elderly newly diagnosed patients, CR rate was 32%, and 3-year OS rate was 85%. *Aims*. The phase III VISTA study compared VMP with MP in previously untreated MM patients ineligible for HDT-SCT. Off-label use: bortezomib in front-line MM in a novel combination. Methods: Between December 2004 and September 2006, 682 patients from 151 centers in 22 countries in Europe, North and South America, and Asia were randomized (1:1) to 54 weeks treatment with VMP (N=344) or MP (N=338); stratification was by baseline β2-microglobulin and albumin, and region. All patients provided written, informed consent. Patients received nine 6-week cycles of melphalan 9 mg/m^2 and prednisone 60 mg/m^2, days 1-4, alone or with bortezomib (1.3 mg/m^2: days 1, 4, 8, 11, 22, 25, 29, 32, cycles 1-4; days 1, 8, 22, 29, cycles 5-9). The primary end point was time to progression (TTP). M-protein analyses were performed by a central laboratory. Progression was determined using European Group for Blood and Marrow Transplantation (EBMT) criteria. *Results*. Median age was 71 years; 30% of patients were aged ≥75 years, 34% had KPS ≤70%, 33% had β2-microglobulin >5.5 mg/L, and 34% had ISS Stage III disease. Based on a protocol-specified interim analysis, the Independent Data Monitoring Committee recommended the study be stopped, as the pre-specified statistical boundary for the primary end point (TTP) had been crossed. M-protein objective responses (≥50% reduction) were seen in 82% and 50% of patients receiving VMP and MP, respectively, including 35% and 5% immunofixation-negative M-protein CR. VMP was superior to MP for all time-to-event end points, including TTP (median 24.0 *vs* 16.6 months; HR=0.483, $p<0.001$), OS (median not reached in either group, HR=0.607, $p=0.0078$), and time to subsequent therapy (median not reached *vs* 20.8 months, HR=0.522, $p<0.001$). TTP superiority was seen consistently across patient subgroups defined by age, sex, race, region, β2-microglobulin level, albumin level, and ISS stage. VMP efficacy (M-protein CR rate - see table, TTP, and OS) was not affected by age (<75 *vs* ≥75 years), cytogenetics (high-risk vs standard risk), or creatinine clear-

ance (≥60 *vs* <60 mL/min). VMP was well tolerated; patients remained on therapy for a median of 46 weeks (8 cycles) versus 39 weeks (7 cycles) with MP. The rate of serious AEs was higher with VMP (46% *vs* 36%), but rates of grade 4 AEs (28% *vs* 27%) and treatment-related deaths (1% *vs* 2%) were similar. Grade 3/4 sensory neuropathy occurred in 13% of VMP patients; 74% of peripheral neuropathy events resolved/improved in a median of 2 months. Conclusions: VMP is significantly superior to MP in newly diagnosed MM patients ineligible for HDT-SCT. These results establish VMP as a new standard of care in this patient population.

Table 1.

VMP patients	M-protein CR rate
All patients (N=336)	35%
Aged ≥75 years (n=106)	33%
Aged <75 years (n=230)	36%
High-risk cytogenetics (n=26)	35%
Standard-risk cytogenetics (n=142)	32%
Creatinine clearance <60 mL/min (n=182)	35%
Creatinine clearance ≥60 mL/min (n=154)	34%

0474

UNIQUE ACTIVATING MUTATIONS OF JAK2 IN THE ACUTE LYMPHOBLASTIC LEUKEMIAS OF DOWN SYNDROME - BIOLOGICAL AND THERAPEUTIC APPLICATIONS

S. Izraeli,[1] I. Ganmore,[1] D. Bercovich,[2] L.M. Scott,[3] G. Wainreb,[4] A. Elimelech,[2] G. Cazzaniga,[5] A. Biondi,[5] G. Basso,[6] G. Cario,[7] M. Schrappe,[7] M. Stanulla,[8] S. Strehl,[9] O.A. Haas,[9] G. Mann,[9] V. Binder,[10] A. Borkhardt,[10] H. Kempski,[11] J. Trka,[12] S. Avigad,[13] B. Stark,[13] B. Bielorei,[1] O. Smith,[14] N. Dastugue,[15] J.P. Bourquin,[16] A.R. Green,[3] S. Izraeli[17]

[1]Sheba medical center, RAMAT GAN, Israel; [2]MIGAL, KYRIAT SHMONA, Israel; [3]Cambridge Institute for Medical Research, CAMBRIDGE, UK; [4]Tel Aviv University, TEL AVIV, Israel; [5]Centro Ricerca Tettamanti, MONDZA, Italy; [6]University of Padova, PADOVA, Italy; [7]University Hospital Schleswig-Holstein, KIEL, Germany; [8]Hannover Medical School, HANNOVER, Germany; [9]St. Anna Kinderspital, VIENNA, Austria; [10]Heinrich Heine University, DÜSSELDORF, Germany; [11]Great Ormond's Street Hospital, LONDON, UK; [12]Charles University Prague, PRAGUE, Czech Republic; [13]Schneider's Children's Hospital, PETACH TIQVA, Israel; [14]Trinity College, DUBLIN, Ireland; [15]Centre Hospitalier Universitaire, TOULOUSE, France; [16]University Children's Hospital, ZURICH, Switzerland; [17]Sheba Medical Center, RAMAT GAN, Israel

Background. Children with Down Syndrome (DS) have a markedly increased risk for acute megakaryoblastic (AMKL) and lymphoblastic (ALL) leukaemias. DS-AMKL is uniquely characterized by an acquired mutation in GATA1. The existence of similar collaborating mutations in DS-ALL has remained elusive. Constitutive activation of JAK/STAT pathway occurs in several hematopoietic malignancies. We hypothesized that mutations in JAK2 might be a common molecular event in DS-ALL. *Aims*. To characterize the prevalence, the specificities and functional consequences of JAK2 mutations in DS-ALL. *Methods*. We analyzed JAK2 DNA analysis by denaturing high-performance liquid chromatography and sequencing was performed on 89 diagnostic DS-ALL and on 216 sporadic ALL, DS-AMKL and essential thrombocythemia samples obtained from major European Leukemia Groups. The functional consequences of identified mutations were studied in mouse pro-B cells. *Results*. Acquired JAK2 mutations were identified in 16 (18%) DS-ALL patients; The only non-DS ALL patient with JAK2 mutation had an iso-chromosome 21q, later shown to be a DS mosaic. Children with a JAK2 mutation were significantly younger (mean 4.5 *vs* 8.5 yrs, $p<0.001$) at diagnosis. Five mutant alleles were identified, each affecting a highly conserved arginine residue (R683). Modeling of JAK2 pseudokinase domain revealed that R683 is situated in an exposed conserved patch separated from the one involved in MPDs. The mutations caused constitutive JAK/STAT activation and cytokine independent growth of BaF3 cells that was sensitive to treatment with JAK2 inhibitors. *Conclusions*. Somatically acquired R683 JAK2 mutations define a distinct ALL subgroup which is uniquely associated with trisomy 21. JAK2 inhibitors may be useful for treatment of these leukemias. There is a striking genotype-phenotype association between the type of somatic mutation within the JAK2 pseudokinase domain and either B-lymphoid or myeloid neoplasia.

0475

IFN-α PROMOTES PROLIFERATION OF DORMANT HSCS *IN VIVO*, MAKING THEM SUSCEPTIBLE TO ELIMINATION BY CHEMOTHERAPY

M.A.G. Essers,[1] S. Offner,[2] W.E. Blanco-Bose,[2] Z. Waibler,[3] U. Kalinke,[3] M. Duchosal,[4] A. Trumpp[2]

[1]ISREC, School of life sciences, EPFL, EPALINGES, Switzerland; [2]ISREC, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), EPALINGES, Switzerland; [3]Paul Ehrlich Institute, LANGEN, Germany; [4]Centre Hospitalier Universitaire Vaudois, LAUSANNE, Switzerland

The life-long maintenance of blood is dependent on the activity of bone marrow haematopoietic stem cells (HSCs), which are multipotent and display long term self-renewal capacity. In the mature organism, stem cell activity is retained in a reservoir of dormant HSCs, which do not significantly contribute to the daily haematopoiesis. However, upon haematopoietic injury these cells can be efficiently recruited into the cell cycle actively supporting the recovery of the haematopoietic system. Up to now, the signals, which promote the exit of HSCs out of the dormant stage, are largely unknown. Here we show that HSCs efficiently exit G0 and enter the cell cycle in response to treatment with interferon-alpha (IFNα) *in vivo*. IFNα treatment results in the phosphorylation of STAT1 and PKB/Akt, and expression of IFNα target genes in HSCs, as well as stem cell antigen (Sca-1) up-regulation at their surface. HSCs lacking IFNAReceptor, STAT1 or Sca-1 do not show increased proliferation in response to IFNα stimulation. Priming of HSCs with IFNα followed by treatments with the anti-proliferative chemotherapeutic agent 5-FU causes lethality due to HSC exhaustion, indicating that 5-FU resistant dormant HSCs can be sensitized to this drug by pre-treatment with IFNα *in vivo*. These results demonstrate a novel role for IFNα on activation of dormant stem cells in vivo and, since dormancy is thought to be a typical feature of certain haematological tumour cells as well, may help to clarify the so far unexplained clinical effects of IFNα on dormant leukaemic stem cells.

POSTER SESSION II

Acute lymphoblastic leukemia - Biology

0476

LCK IS A CRITICAL SIGNALING EFFECTOR IN NUP214-ABL1 POSITIVE T-ALL

K. De Keersmaecker, M. Porcu, O. Gielen, J. Cools

K.U.Leuven, LEUVEN, Belgium

Background and Aims. T-ALL is characterized by the presence of different types of mutations leading to cell cycle defects, impaired differentiation, abnormal proliferation and unlimited self-renewal. Fusion genes involving the tyrosine kinase ABL1 were identified as mechanisms that provide a proliferation and survival advantage to leukemic cells. The NUP214-ABL1 fusion gene is the most common ABL1 fusion in T-ALL, found in 6% of T-ALL patients. NUP214-ABL1 is sensitive to the ABL1 kinase inhibitor imatinib, and proliferation of NUP214-ABL1 expressing Ba/F3 and ALL-SIL cells is inhibited by imatinib. However, treatment of NUP214-ABL1 positive patients with imatinib might result in development of imatinib resistance, similarly to what has been described in imatinib treated BCR-ABL1 positive patients. In this study, we aimed at identifying critical signaling proteins downstream of NUP214-ABL1 that could be used as alternative therapeutic targets for treatment of NUP214-ABL1 positive T-ALL. *Results.* The SRC family kinase LYN is known to be required for BCR-ABL1 induced B-ALL development. We observed that the NUP214-ABL1 positive T-ALL cell line ALL-SIL was sensitive to the SRC family kinase inhibitor PP2, suggesting also a critical role for one or more SRC family kinases in NUP214-ABL1 signaling. Analysis of expression and phosphorylation of all SRC family members in ALL-SIL cells suggested that the PP2 induced effects in this cell line were due to inhibition of LCK and/or FYN. We confirmed that LCK phosphorylation was inhibited by PP2, and that siRNA mediated knock-down of LCK resulted in a marked reduction of cell viability, similar to the effect observed by inhibition or knock-down of NUP214-ABL1 itself. In contrast, siRNA mediated knock-down of FYN only resulted in a weak inhibitory effect on the proliferation of ALL-SIL, indicating that this SRC family kinase was not critically required. PP2 treatment or knock-down of LCK expression had no significant effect on T-ALL cell lines not transformed by NUP214-ABL1, suggesting that LCK activation is specific to NUP214-ABL1 transformed T-cells. *Summary and conclusions.* Although imatinib efficiently inhibits NUP214-ABL1 activity, we expect that NUP214-ABL1 positive T-ALL patients treated with this drug might develop imatinib resistance. Our data indicate that LCK activity is critical for NUP214-ABL1 signaling and that LCK could represent an alternative therapeutic target for treatment of NUP214-ABL1 positive T-ALL. Further *in vivo* analysis of the potential of LCK as a target in NUP214-ABL1 positive T-ALL is warranted.

0477

UNINTENTIONAL TRANSPLANTATION OF T CELL LYMPHOBLASTIC LEUKEMIA OF THE DONOR TO THREE DIFFERENT ORGAN RECIPIENTS WITH SIGNIFICANT HLA INCOMPATIBILITIES

W. Jedrzejczak,[1] J. Dwilewicz-Trojaczek,[1] A. Dmoszynska,[2] L. Konopka,[3] B. Ziarkiewicz-Wroblewska,[1] E. Chmarzynska-Mroz,[1] G. Charlinski,[1] A. Gronkowska,[1] B. Kaczmarek,[1] H. Stanczak,[1] M. Nowaczyk,[1] A. Sadowska[1]

[1]Medical University of Warsaw, WARSAW; [2]Medical University of Lublin, LUBLIN; [3]Institute of Hematology and Transfusion Medicine, WARSAW, Poland

Background. Despite the performance of hundred thousands organ transplantations in the world we failed to find a report of the transplantation of leukemia of organ donor together with transplanted solid organ. It is commonly assumed that unlike cells of solid tumors, leukemic cells are readily available to the immune system of the recipient, that under conditions of multiple HLA incompatibilities is still able to destroy them, despite posttransplant immunosuppression. *Description of cases*. In June 2005 four patients received organs from 19-year old previously healthy male donor that died accidentally. No donor autopsy was performed but examination of some adipose tissue adjacent to the transplanted liver revealed the presence of T cell lymphoma $CD3^+$,Tdt^+, MIB^+, $CKMNF^-$, $CD10^-$,$CD20^-$, while liver itself seemed not to be affected. The heart recipient died soon thereafter, but recipients of the liver (30 yr old male with Wilson disease) and both kidneys (32 yr old male and 59 yr old female with renal insufficiency) survived. In all these patients in January 2006 (i.e. 6-7 months after transplantation) T cell lymphoblastic leukemia bearing the same phenotype as T cell lymphoma earlier found in donor tissue was diagnosed. In two patients it was also found that this leukemia (HLA type of the donor) had the same cytogenetic abnormalities [46, XY, t(1;14)(p32;q11),del (9)(q32)], while one of these recipients was a female. HLA typing has revealed that between the donor and all recipients existed some shared specificities. Between the donor and recipient of the liver there was compatibility of one class I antigen, between the donor and female kidney recipient compatibility of one class I and one class II antigen. Finally, between the donor and male kidney recipient there was compatibility of one class I and two class II antigens. This means that the recipients have been incompatible with the donor at 5/6, 4/6, and 3/6 antigens respectively, and yet leukemia developed in all of them at almost the same time. All patients after transplantation received tacrolimus in standard doses. All have been treated for leukemia with chemotherapy, and all promptly achieved complete remission. This remission is still maintained in the recipient of the liver. Male recipient of the kidney had one relapse and is in the 2nd complete remission, while female kidney recipient died of fungal infection during an attempt to induce the fourth complete remission. Transplanted organs are functioning in two surviving patients and also in succumbed patient the kidney was functioning almost until death in January 2007. *Conclusions.* These data indicate, that T lymphoblastic leukemia is transplantable despite large HLA incompatibilities and low tumor load. Immune system seem to be unable to reject such leukemia at least when depressed by tacrolimus.

0478

GENOME PROFILE OF PH POSITIVE ALL IN ADULT PATIENTS

A. Chanalaris,[1] D. Brazma,[1] L. Rai,[1] A. Chanalaris,[1] J. Howard,[1] M. Valgañón,[1] C. Grace,[1] L. Foroni,[2] E.P. Nacheva[1]

[1]Royal Free & UC Medical School, LONDON; [2]Imperial College London, Hammersmith Campus, LONDON, UK

Background. Acute lymphoblastic leukemia (ALL) accounts for 15% of all leukaemias in adults. Philadelphia positive (Ph^+) ALL is the most common form of adult ALL accounting for 15-30% of adult ALL. Ph^+ ALL patients have the most unfavourable prognosis with overall disease free survival of less than 10%. *Aims.* As there is limited information in the genomic profile of adult ALL we employed aCGH to complete a genome profile of Ph^+ ALL. *Methods.* 21 patients with Ph^+ ALL were investigated by comparing DNA from presentation and remission bone marrow samples using oligonucleotide array CGH at resolution of 65kbp. FISH and RT-PCR assessed the presence of BCR/ABL1 fusion in all presentation samples. Additionally, G-banding analysis was carried out in 5 patients. *Results.* Copy number aberrations (CNA) were detected in all samples. Six genome segments, ranging in size from 120kb to over 1Mb were recurrently affected and over 200 single locus imbalances were found in 3 or more (~30%) samples. Some 65% of all CNA were found to involve regions within the resolution of the cytogenetic analysis. In order of frequency the whole chromosome CNA found are: gain/loss of X (8/14); gain of 8 (6/14); gain of 4 (5/14); gain of 14(5/14); gain of 21 (3/14). Lack of correlation between the aCGH and karyotype data is apparent in many samples, but further work is required to build up a precise map of these discrepancies. Among the most frequent segmental changes is the loss of a common region within 14q32 (105.350-105.480) that contains part of the Immunoglobulin heavy chain locus, as seen in 9/21 samples. In further 7 samples the loss included the TCR-alpha chain sequences (14q32 141.94-141.99). Deletions at 9p21, covering a common region (21.36-22.69) were found in 5/21 samples. The 135kb region, that houses the genes CDCN2A&2B, interferon α 1,2,8 & e1 and the BC015960 (p16) genes, is several fold smaller than the 9p loss identified recently by 1Mbp BAC aCGH in 58 patients with B and T/ ALL (Strefford *et al.*, 2007). Loss of 9q34 and 22q11 sequences consistent with deletions of der(9)t(9;22) were found in 2/21 cases (~10%), while genome gains associated with double Ph chromosome were less than expected (14%). In addition to gains of either the whole chromosome 8 or the 8q arm, an ~ 700kb segment from 8q24.3 (145.00-145.70) was found amplified in two samples. Containing several genes this amplicon differs from the ones described in AML (Storlazzi *et al.*, 2005) and CML (Brazma *et al.*, 2007). Finally, over 15 single locus imbalances detected in 6 or more patients, have been reported as polymorphic markers (CNVs) in disease free individuals. Their identification as secondary aberrations in sequential Ph^+ ALL samples adds new aspects to the cur-

rent concept about the biological role of this polymorphism. *Conclusions*. These findings generate an unique genome profile for Ph+ ALL, substantially different from both ALL and CML to merit further investigations.

0479

IDENTIFICATION BY OLIGONUCLEOTIDE ARRAYS OF A SUBSET OF T-ALL PATIENTS WITH MYELOID-LIKE GENE PATTERN: POTENTIAL MECHANISMS OF TRANSFORMATION

S. Chiaretti, M. Messina, S. Tavolaro, V. Fulci, I . Della Starza, R. Maggio, A.D. Negulici, V. Di Maio, A. Vitale, G. Macino, A. Guarini, R. Foà

University Sapienza, ROMA, Italy

Background. Until recently, few molecular aberrations were recognized in acute lymphoblastic leukemia of T-cell origin (T-ALL). The scenario is rapidly changing; novel lesions are emerging and they include rearrangements (i.e. SIL/TAL1, CALM/AF10 and NUP214/ABL), gene overexpression (i.e. LYL, TLX1, TLX3, TAL1) and mutations (NOTCH1, PTEN). Furthermore, it is now clear that there is a certain degree of overlap between acute myeloid leukemia (AML) and T-ALL: it was recently described that CEBPA hypermethylation induce, in a subset of AML patients, an increased expression of T-cell-related genes. In this context, gene expression profiling is useful in identifying previously unrecognized subentities. *Aims*. To identify, using gene profiling, novel entities of T-ALL and to investigate their clinico-biologic features. *Methods*. Within the MILE study (Microarray Innovations in LEukemia, sponsored by Roche Molecular Systems) we evaluated, using HGU133 Plus 2.0 arrays, 52 adult patients with T-ALL at diagnosis: 3 patients harbored SIL/TAL, 2 BCR/ABL, 1 NUP/ABL1, 1 CALM/AF10 and 2 a rearrangement involving ALL1. Statistical analysis ,performed using the dChip software, was based on unsupervised analysis; real-time quantitative PCR (Q-PCR) was used to validate the results obtained on a set of genes. *Results*. By unsupervised clustering we identified at least 3 T-ALL subgroups. The first distinct cluster included SIL/TAL+ patients; the second cluster included all cases exhibiting high levels of HOXA gene expression: in agreement with previous reports, the 2 ALL1+ and the CALM/AF10+ patient were included in this branch. Finally, the third group (defined for simplicity *myeloid T-ALL*) included 5 patients whose gene profile resembled that of AML cases. In fact, they were characterized by the overexpression of a large set of myeloid-related genes: 1) surface antigens; 2) transcription factors, namely CEBPA, CEBPB, CEBPD, MNDA and MAFB; 3) granule proteins, as myeloperoxidase (MPO) and lysozime. Flow cytometry confirmed that they were undoubtedly of T derivation, being all CyCD3 and CyCD7+. Cytogenetic analysis was not available; however, molecular screening did not reveal the presence of typical myeloid fusion products. In order to validate gene expression results, we performed Q-PCR on MPO, CEBPA and CEBPB: for all the 3 genes teste, and their overexpression was confirmed (T-ALL *vs* myeloid T-ALL p=0.0001, 0.005 and 0.0002, respectively). Given the overexpression of several members of the CEBP family, and in particular of CEBPA, we also sought to evaluate the expression levels of miR-223, known to be regulated by CEBPA and to have a role in myeloid differentiation: these cases had significantly higher levels of miR-223 when compared to remainder T-ALL (p=0.0002), comparable to those observed in AML. *Conclusions*. By gene profiling it is possible to identify a subset of T-ALL patients, representing 10% of those analyzed, who display myeloid features. Notably, these cases were not recognized by standard approaches, underlying the importance of gene profiling. Since these cases are characterized by the overexpression of CEBPA and miR-223, further studies are warranted to understand the relation between these 2 RNAs and to evaluate potential epigenetic modifications involved.

**Equal contribution*

0480

ETV6/RUNX1 INTERFERES WITH SPINDLE CHECKPOINT FUNCTION AND TP53 SURVEILLANCE THEREBY PROMOTING TETRAPLOIDIZATION

E. Panzer-Grümayer,[1] A. Inthal,[1] R. Joas,[1] L. Orel,[1] G. Fuka,[1] A. Haas,[2] G. Krapf[1]

[1]Children's Cancer Research Institute, VIENNA; [2]St. Anna Kinderspital, VIENNA, Austria

Background. The chromosomal translocation t(12;21)(p13;q22) occurs in about 25% of childhood B cell precursor (BCP) ALL and generates the ETV6/RUNX1 fusion gene (also known as TEL/AML1). This gene rearrangement is an initiating event in leukemia development but requires further genetic alterations for the clinical manifestation of the leukemia. Unlike all other BCP ALL subtypes ETV6/RUNX1 positive ALL may display a near tetraploid karyotype suggesting that the fusion protein and/or its ensuing secondary mutations interfere with cell cycle regulation and TP53 surveillance. The most important control for mitotic failures during cell division is the spindle-assembly checkpoint (SAC). Its activation in pro- and promethaphase delays onset of anaphase via inhibition of the anaphase promoting complex/cyclosome until all kinetochors are attached to microtubules. The aim of this study was to elucidate the mechanism by which ETV6/RUNX1 impacts on SAC thereby causing polyploidization. Experimental set up: Stably ETV6/RUNX1 expressing clones of the murine pro-B cell line Ba/F3 and human leukemic cell lines (REH, AT-1, AT-2, which carry the ETV6/RUNX1 fusion gene) were used as model systems. To assess the functionality of the major control systems that prevent aneuploidy SAC activation and TP53 protein phosphorylation were evaluated. Furthermore, key regulatory proteins of SAC were analyzed upon chemical activation by microtubule-altering drugs (i.e. nocodazole and paclitaxel). TP53 activation was studied via double strand break induction using the TopoII inhibitor VP16. *Results.* Expression of ETV6/RUNX1 induced tetraploidization in Ba/F3 clones while controls containing several other constructs remained diploid. We then went on to study the effects of ETV6/RUNX1 on SAC activity but also on protein levels of its key components. SAC was attenuated in fusion gene carrying leukemic cell lines upon chemical activation, as indicated by a reduced mitotic index. Furthermore, these cells also showed *slippage* into G1 phase without increase in apoptosis. At the protein level, ETV6/RUNX1 expressing cells had reduced amounts of MAD2L1 and BUBR1, the major key players of the mitotic checkpoint complex, compared to controls. In keeping with recent reports indicating that reduced MAD2L1 and BUBR1 protein levels inhibit also DNA damage-induced mitotic arrest and consecutive cell death, we showed that this could also be the case for ETV6/RUNX1 positive cells based on reduced phosphorylation of TP53 at S15 after DNA damage. Based on our findings we assume that the chimeric protein ETV6/RUNX1 attenuates the spindle checkpoint and TP53 activity and thereby sensitizes cells to polyploidization. As only a small proportion of t(12;21) positive ALL have a near tetraploid karyotype, it is tempting to speculate that ETV6/RUNX1 leads to the manifestation of this phenotype only in collaboration with particular secondary events or predisposing factors. Studies are under way to test this hypothesis.

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0481

CO-ADMINISTRATION OF 2-DEOXY-D-GLUCOSE ALLOWS DEXAMETHASONE DOSAGE REDUCTION IN THE INDUCTION THERAPY OF LYMPHOBLASTIC LEUKEMIA

K. Eberhart,[1] K. Renner,[2] I. Ritter,[2] C. Hellerbrand,[3] B. Timischl,[2] R. Kofler,[4] P. Oefner[2]

[1]University of Regensburg and Tyrolean Cancer Research Institute, REGENSBURG, Germany; [2]University of Regensburg, Institute of Functional Genomics, REGENSBURG, Germany; [3]University of Regensburg, Department of Internal Medicine, REGENSBURG, Germany; [4]Tyrolean Cancer Research Institute, INNSBRUCK, Austria

Background. Glucocorticoids (GC) are used in the therapy of acute lymphoblastic leukemia (ALL) in combination with other chemotherapeutics because of their ability to induce apoptosis and cell cycle arrest. However, GC exert severe side effects and resistance develops frequently. Hence, there is a demand for improved treatment protocols. Our gene expression profiles of patients undergoing GC monotherapy suggested that the enzyme phosphofructokinase/fructosebiphosphatase-2 (PFKFB2) may play a crucial role in GC-induced apoptosis via alterations in glucose utilization. *Aims.* The development of a new treatment to reduce the effective dose of GC by amplifying the glycolytic disturbances and harm preferentially cancer cells. To this end, we investigated the combinatory treatment of GC and 2-deoxy-D-glucose (2-DG), in a concentration tolerated by cancer patients, when systemically administered. 2-DG is a nonmetabolizable glucose analog, which accumulates intracellularly as 2-deoxy-glucose-6-phosphate (2-DG-P). *Methods.* Acute lymphoblastic T-cells (CCRF-CEM-C7H2) and pre-B ALL cells (preB-697) were treated with 100 nM dexamethasone and either 3 mM or 1 mM 2-DG, respectively. Apoptosis was determined by Annexin V/PI stain and sub G1-peak detection. Intracellular accumulation of 2-DG-P was determined by CE-ESI-MS. Lactate and glucose concentrations in the supernatant were analyzed by enzymatic assays, ATP by a luciferase assay. Mitochondrial function was examined by high-resolution respirometry. Hexokinase II expression was analyzed by RT-PCR and

immunoblotting after cellular fractionation. Primary lymphoblasts of patients were co-cultured with fibroblasts. *Results.* T- and preB-ALL cell lines were dramatically sensitized to GC-triggered apoptosis by the co-administration of 2-DG, allowing a 20-fold reduction of the effective dose of GC. 2-DG treatment resulted in a 2-fold doubling time. Human primary resting and activated lymphocytes showed no effect on sole 2-DG treatment and no synergistic effect on the combinatory treatment. 2-DG accumulated intracellularly 2-DG-P (200 nM/mg protein). The combination of 2-DG and GC resulted in a reduction of glycolysis, mitochondrial oxidative phosphorylation and ATP levels. This ATP drop might be partially responsible for the synergism of 2-DG and GC, since restoring ATP level by the administration of pyruvate could prevent this effect in preB cells. In T-ALL cells we could not elevate ATP concentration by the administration of pyruvate. Mitochondrial bound hexokinase II expression was reduced at mRNA and protein levels, leading to membrane alterations and release of pro-apoptotic factors. Preliminary data suggest additional alterations in the lipid composition of the mitochondrial membrane. Hexokinase II reduction in combination with the observed decrease in glucose up-take might explain the decline in energy metabolism. First patient samples treated *ex vivo* are under investigation. *Conclusions.* GC as well as 2-DG disturb the energy metabolism, thereby amplifying each others effects. Since cancer cells are strongly dependent on glycolysis, the observed glycolytic changes suggest that cancer cells are harmed preferentially. The co-administration of 2-DG and GC holds the promise of an improved therapy protocol for the treatment of ALL.

0482

RARE INCIDENCE BUT EXTREME DIVERSITY OF PAX5 FUSION GENES IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

K. Nebral,[1] M. König,[1] G. Mann,[2] O.A. Haas,[2] S. Strehl[1]

[1]CCRI, Children's Cancer Research Institute. St. Anna Kinderkrebsforschung, WIEN; [2]St. Anna Children's Hospital, WIEN, Austria

Background. PAX5 encodes the B-cell lineage specific activator protein (BSAP) and is required for B-cell development and lineage maintenance. Pax5 fulfills a dual role by activating B-cell specific genes and simultaneously repressing lineage-inappropriate genes. In B-cell non-Hodgkin's-Lymphoma with a t(9;14)(p13;q32), PAX5 is juxtaposed to the IGH locus, which results in overexpression of PAX5. In acute lymphoblastic leukemia (ALL) it has been shown that PAX5 can fuse to FOXP1 (3p13), AUTS2 (7q11), ELN (7q11), ETV6 (12p13), PML (15q24), ZNF521 (18q11), and C20orf112 (20q11). *Aims and Methods.* To determine the overall incidence of PAX5 rearrangements in childhood ALL dual-color split-apart FISH assays using BAC clones flanking the PAX5 gene as well as PAX5 exon-specific cosmid probes were used. This FISH approach allows for the detection of all PAX5 rearrangements independent of the partner gene, including also those resulting in juxtaposition of PAX5 under regulatory elements of a partner gene. Novel fusion partners were identified by 5' and 3' RACE. The presence of a specific fusion gene was verified by RT-PCR and sequence analysis or fusion gene-specific FISH. *Results*. In a retrospective study, more than 400 patients enrolled in the Austrian ALL-BFM 2000 study were screened for PAX5 rearrangements. Six patients showed a separation of the PAX5 FISH probes and three showed PAX5-3' deletions as well indicating PAX5 rearrangements. Out of these 9 patients, one each has been previously shown to harbor a dic(9;12)(p13;q13)/PAX5-ETV6 and a t(9;15)(p13;q24)/PAX5-PML aberration. Of the remaining 7 cases, in one patient showing a PAX5-3' deletion, fusion gene-specific FISH and RT-PCR detected a PAX5-C20orf112 fusion. In four patients 5' or 3' RACE identified novel PAX5 chimeric transcripts and subsequent RT-PCR confirmed that PAX5 was fused to HIPK1 (1p13), POM121 (7q11), DACH1 (13q21), and BRD1 (22q13.33). The remaining two cases were extensively analyzed by FISH, RT-PCR and RACE, but no fusion gene could be delineated so far. All hypothetical fusion proteins fuse the C-terminal region or even the entire protein of the fusion partner to the PAX5 paired DNA-binding domain, or parts of the transactivation domain. Thus, all PAX5 fusion proteins are predicted to retain the ability to bind to PAX5 target genes, but would no longer provide normal transcriptional regulatory functions. *Conclusions*. We could determine that in childhood ALL PAX5 rearrangements occur at an incidence of about 2% and have identified the fusion partners in seven cases, which included four with novel PAX5 in-frame fusions to HIPK1, POM121, DACH1, and BRD1. Our data show that PAX5 fusions, though a rare event, fuse PAX5 to a broad range of different partner genes with diverse functions including not only transcription factors but also structural proteins.

0483

NK-LIKE HOMEODOMAIN PROTEINS ACTIVATE TRANSCRIPTION OF POLYCISTRONIC MIR-17-92 AND INHIBIT ETOPOSIDE-INDUCED APOPTOSIS IN T-ALL CELLS

S. Nagel,[1] L. Venturini,[2] G.K. Przybylski,[3] P. Grabarczyk,[4] C. Corinna,[1] H.G. Drexler,[1] C.A. Schmidt,[4] R.A.F. MacLeod,[1] M. Scherr[2]

[1]DSMZ, BRAUNSCHWEIG, Germany; [2]Medical School Hannover, HANNOVER, Germany; [3]Polish Academy of Sciences, POZNAN, Poland; [4]University of Greifswald, GREIFSWALD, Germany

Background. Homeobox genes code for transcription factors, impacting developmental processes and oncogenesis if mutated or dysregulated. The NK-like familiy of homeobox genes includes TLX1, TLX3 and NKX2-5 which are ectopically activated in T-cell acute lymphoblastic leukemia (T-ALL) cells mostly via chromosomal aberrations. The pathologic function of these closely related genes is still unclear. Here we analyzed their effect on the C13orf25 gene, containing the miR-17-92 cluster. Micro RNAs (miRNAs) are a class of deeply conserved small non-coding RNAs which regulate gene expression by hybridization to complementary sequences usually located in the 3´untranslated region of coding transcripts. The primary transcripts (pri-mRNA) are processed to short mature miRNAs, mediating either inhibition of mRNA translation or mRNA cleavage. Aberrant expression of specific miRNAs is involved in oncogenesis as recently described for several human malignancies. The miR-17-92 polycistron encodes miRNAs which reduce E2F1 and PTEN protein expression. Transcription of both E2F1 and miR-17-92 is induced by MYC, itself a target of E2F1, generating a highly regulated interactive network. Depending on the cellular context, E2F1 performs conflicting tasks by triggering proliferation or inducing apoptosis. *Aims.* Here we investigated expression and function of the miR-17-92 cluster in T-ALL cells. *Results.* Real-time RT-PCR analysis of both miR-17-92 pri-mRNA and mature miRNAs revealed different expression levels in these cells, suggesting a possible implication of the NK-like homeodomain proteins in the regulation of the miR-17-92 cluster in T-ALL. HELA cells transfected with TLX1 or NKX2-5 expression constructs showed elevated miR-17-92 pri-mRNA expression, demonstrating an activating effect. Lentiviral-mediated overexpression of NKX2-5 in the T-ALL cell line MOLT-4 consistently showed increased miR-17-92 pri-mRNA levels and decreased E2F1 protein amounts. For functional analysis of these downstream targets, another T-ALL cell line (PEER) was lentivirally transduced with expression constructs for either miR-17-92 or E2F1, resulting in reduced or elevated E2F1 protein levels, respectively. Overexpression of miR-17-92 or E2F1 did not significantly influence cell proliferation. However, induction of apoptosis by treating these cells with etoposide, an inhibitor of topoisomerase II, indicated that overexpression of miR-17-92 and E2F1 resulted in enhanced and reduced cell viability, respectively, as analyzed by MTT assay. Furthermore, expression analysis of miR-17-92 pri-mRNA in primary T-ALL samples positive for TLX1/3 expression, respectively, displayed elevated levels in comparison to control samples. *Conclusions*. In summary, these data indicate an activatory effect of oncogenic NK-like homeodomain proteins on miR-17-92 expression, reducing E2F1 protein levels and thereby enhancing survival of leukemic T-cells.

0484

CHARACTERIZATION OF NUP214-ABL1 POSITIVE T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA REVEALS GENOMIC HETEROGENEITY OF THE FUSION GENE PRESENTATION

C. Graux,[1] N. Dastugue,[2] M. Lafage,[3] F. Mugneret,[4] S. Struski,[5] M.J. Grégoire,[6] N. Nadal,[7] E. Lippert,[8] M. Stevens-Kroef,[9] P. Vandenberghe,[10] L. Michaux,[10] A. Bosly,[1] I. Wlodarska,[10] I. Lahortiga,[10] K. De Keersmaecker,[10] J. Cools,[10] A. Hagemeijer,[10] H.A. Poirel[1]

[1]UCL, YVOIR, Belgium; [2]Hôpital Purpan, TOULOUSE, France; [3]Institut Paoli-Calmettes, MARSEILLES, France; [4]CHU Dijon, DIJON, France; [5]CHU Strasbourg, STRASBOURG, France; [6]CHU Nancy, NANCY, France; [7]CHU St Etienne, ST ETIENNE, France; [8]CHU Bordeaux, BORDEAUX, France; [9]Academisch Ziekenhuis, NIJMEGEN, Netherlands; [10]KUL, LEUVEN, Belgium

Background. The t(9;22) generating the BCR-ABL1 fusion is the hallmark of CML, and also characterizes 25% of B-ALL cases. BCR-ABL1 is extremely rare in T-ALL, but we recently identified a NUP214-ABL1 fusion in about 6 % of T-ALL cases. A unique feature of NUP214-ABL1 is its presence on extrachromosomal amplified material (episomes). To

date, only 1 NUP214-ABL1 positive T-ALL case has been described without episomes, but with an intrachromosomal amplification of the fusion gene on chromosome 2. In the current study we analysed 16 additional NUP214-ABL1 positive T-ALL cases and 3 T-ALL cell lines to characterize their clinical and genetic presentations. *Methods*. In collaboration with the GFCH, we retrospectively collected 346 T-ALL bone marrow, blood or pleural fluid samples from 15 different centres. FISH and/or RT-PCR were performed to determine the presence and genetic presentation of the fusion gene. *Results*. We identified 16 NUP214-ABL1 positive T-ALL cases. Median age was 15,5 years (3 to 45) with a male predominance (12:5). The 12 samples with available information showed an HOX11 or HOX11L2 abnormality as assessed by FISH or RT-PCR confirming the specific occurrence of NUP214-ABL1 with HOX11 or HOX11L2. Among the 14 cases with available data on outcome, we observed 1 primary refractory and 5 relapse cases of which 4 occurred early. FISH analysis of these cases revealed a variable pattern of NUP214 and ABL1 signals. Thirteen of the 15 samples that could be evaluated by FISH showed episomal amplification. In 1 case, episomal and intrachromosomal amplification of the NUP214-ABL1 fusion was observed. One other case was characterized by episomes, intrachromosomal amplification and an additional insertion of the non amplified NUP214-ABL1 fusion on 14q. One case only demonstrated intrachromosomal amplification. Finally, 1 case showed no detectable rearrangement of NUP214 or ABL1 by FISH, but was positive by RT-PCR. Three cell lines with the NUP214-ABL1 fusion only showed the presence of an intrachrosomal amplified NUP214-ABL1 fusion on 9q34. *Conclusions*. Our work stresses the interest of combining both FISH and RT-PCR for the identification of the NUP214-ABL1 fusion, since the fusion gene may only be present in a fraction of the cells. Our data confirm that the NUP214-ABL1 fusion is in most cases associated with episomal amplification, but that also intrachromosomal amplification or insertion into random chromosomes can occur. This heterogeneous presentation of the NUP214-ABL1 fusion provides insight in the mechanisms of episome formation, amplification and optionnal reintegration. It underlines also that NUP214-ABL1 is supported by episomes that have the property to amplify and we have to take this into account when discussing the interest of adding imatinib to the treatment of these patients. The NUP214-ABL1 fusion is mainly detected together with HOX11 or HOX11L2 positive T-ALL, and could influence the outcome in these subgroups. Our data raise the question of the impact of variable NUP214-ABL1 genomic presentations on the outcome of this subgroup of T-ALL. Finally, during the screening we did not detect any other ABL1 fusion variants, indicating that the NUP214-ABL1 fusion is the most frequent ABL1 abnormality in T-ALL.

0485

ALTERNATIVE SPLICING OF IKAROS MRNA IS RESPONSIBLE FOR THE EXPRESSION OF DIFFERENT ABERRANT ISOFORMS IN PHILADELPHIA CHROMOSOME-POSITIVE (PH+) ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) PATIENTS

A. Lonetti,[1] I. Iacobucci,[1] R. Zuntini,[2] S. Ferrari,[2] D. Cilloni,[3] F. Messa,[3] E. Ottaviani,[1] F. Arruga,[3] F. Salmi,[1] S. Paolini,[1] C. Papayannidis,[1] P.P. Piccaluga,[1] P. Giannoulia,[1] S. Soverini,[1] G. Saglio,[3] F. Pane,[4] A. Vitale,[5] S. Chiaretti,[5] R. Foà,[5] M. Baccarani,[1] G. Martinelli[1]

[1]Department of Hematology/Oncology L. and A. Seràgnoli, BOLOGNA; [2]Medical Genetics Unit, S.Orsola-Malpighi University Hospital, BOLOGNA; [3]Department of Clinical and Biological Science, University of Turin, ORBASSANO, TURIN; [4]CEINGE and Department of Biochemistry and Medical Biotechnology, NAPLES; [5]Department of Cellular Biotechnologies and Hematology, "La Sapienza" Universit, ROME, Italy

Background. Ikaros is a zinc finger transcription factor required for normal hemopoietic differentiation and proliferation, particularly in the lymphoid lineages at multiple stages, that acts both potentiating and repressing gene expression. By means of alternative splicing, Ikaros encodes several proteins that differ that differ in their abilities to bind to a consensus DNA-binding site. Shorter, DNA non-binding isoforms exert a dominant negative effect, inhibiting the ability of longer heterodimer partners to bind DNA. An excess of short DNA nonbinding isoforms has been described in different human haematological malignancies. *Aims.* To detect and quantify different Ikaros transcript variants in Philadelphia-positive (Ph+) adult acute lymphoblastic leukaemia (ALL) patients by performing a high-throughput method based on capillary electrophoresis. *Methods*. We analyzed 46 adult *de novo* Ph+ ALL patients. Ikaros cDNA, obtained from bone marrow or peripheral blood, was amplified with two pairs of oligonucleotides, the forward primer of each couple conjugated with a fluorescent dye (fluorescein) at its 5' end. PCR products were then loaded on the ABI Prism 3730 DNA Analyzer for automated capillary gel electrophoresis and the results were plotted with the AbiPrism GeneMapper v3.5 software (Applied Biosystems). The GeneMapper electrophoretograms display information about transcript length, peak height and peak area. The emitted fluorescence at each peak position reflects the number of amplified target sequence: peaks heights are correlated to the quantity of amplified PCR product and used as an indication of the Ikaros level expression in a sample. *Results.* In 19/46 (41%) of patients we identified a single peak of 255 bp corresponding to DNA non-binding isoform Ik6. In the remaining 59% patients we observed the co-expression of many fragments, corresponding to the different functional (Ik2) and non functional (Ik4, Ik4A, Ik5A, Ik6, Ik6Δ, Ik8) Ikaros transcript variants. Furthermore, a recurring 60-bp insertion immediately downstream of exon 3, was frequently detected in Ik2 and Ik4 isoforms either alone or together with an in-frame 10-amino acid deletion, due to a 30- bp deletion at the end of exon 7. Both the alterations are due to the selection of alternative splice sites which determine an impairment ability to form dimers and to bind the DNA target sequence. *Summary and conclusions.* Taken together, our findings demonstrated that alterations of the transcription factor Ikaros, involving both short spliced oncogenic isoforms and aberrant full-length isoforms are a common feature of Ph+ ALL. Because of the leukemic role of short and/or aberrant Ikaros splice variant, it is more important to use a sensitive method to detect them: in this study we develop a fast, sensitive, high-throughput method, to both detect and quantify splice variants.

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0486

GLUCOCORTICOID-MEDIATED EFFECTS IN LEUKEMIA CELLS: INVOLVEMENT OF MICRORNAS

R. Mitlohner, P. Rhein, R. Ratei, W.D. Ludwig, L. Karawajew

RRK, Helios Klinikum Berlin, Charité, BERLIN, Germany

Background. Glucocorticoids (GCs) are the principal therapeutic agents in acute lymphoblastic leukemia (ALL), but molecular targets of GCs are still under discussion. MicroRNAs (miRNAs) are a recently discovered class of small noncoding RNAs with the potential to target the messages of protein-coding genes. *Aims.* Identification and function of miRNAs involved in GC-specific signaling in human precursor B-cell ALL (PBC-ALL). *Methods.* This study has been performed using human ALL cell lines (TOM-1, REH), primary ALL blasts at initial diagnosis, and ALL engrafted in NOD/SCID mice. miRNA profiling of GC treated *vs* untreated cells was performed using a set of 157 human miRNA assays. Expression of candidate miRNAs was validated by real-time RT-PCR. For the functional analysis, cells were nucleofected with miRNA precursor oligonucleotides. MS-5 stromal cells have been used for culturing of nucleofected primary cells. *Results.* miRNA expression profiling of ALL cells has demonstrated GC-induced modulation of miRNA expression in different cell systems. GC-induced upregulation (1.6-3.5 fold) of miR-29b has been a common event in GC-sensitive (TOM-1, primary and NOD/SCID engrafted leukemia cells) but not in GC-resistant (REH) cells. In TOM-1 cells, an overexpression of miR-29b alone was sufficient to induce apoptosis 24h after transfection. Moreover, considerable apoptosis rates (17%) have been achieved in NOD/SCID engrafted cells after transfection with miR-29b precursor molecules. Of note, overexpression of miR-29b was able to induce apoptosis in REH cells and, therefore, to overcome GC-resistance in PBC-ALL. *Conclusions*. Our data demonstrate a pro-apoptotic activity of miR-29b which is potentially involved in GC-specific signaling.

0487

IDENTIFICATION OF MULTIPLE NOVEL IGH@ TRANSLOCATIONS IN ADOLESCENT ACUTE LYMPHOBLASTIC LEUKAEMIA

L. Russell, C.J. Harrison

Newcastle Universtiy, NEWCASTLE UPON TYNE, UK

Chromosomal translocations lead to oncogene activation or gene fusion in hematological malignancies, where they play an important role both in diagnosis and as an indicator of prognosis. Chromosomal translocations involving the immunoglobulin heavy chain locus (IGH@) at chromosome band 14q32 are frequently observed in mature B-cell neoplasia, although a number are now emerging in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). The partner genes differ from those observed in mature B-cell ALL, although their expression is always dereg-

ulated due to the juxtaposition of transcriptional enhancers within the locus. We have identified an increasing number of partners, including recurrent translocations involving 6p (ID4) (n=13) and 19p (EPOR) (n=2), as well as individual cases involving 4p (ZNF595), 5q (novel gene), 7q (TRG@), 15q (novel gene or MEIS2) and 17q (IGF2BP1). Fluorescence *in situ* hybridization and/or long-distance inverse polymerase chain reaction identified partner genes with upregulation of target gene mRNA shown by quantitative real-time PCR. A novel subgroup of 13 IGH@ positive BCP-ALL patients with t(6;14)(p22;q32) showed deregulated expression of ID4 in cooperation with loss of CDKN2A and PAX5, frequently as a result of i(9)(q10). Breakpoints were scattered over a 19kb region centromeric of ID4. Two patients showed a recurrent, reciprocal translocation involving IGH@ with the breakpoint at 19p13, in one patient, 3kb centromeric of EPOR. Analysis of EPOR expression showed increased mRNA levels of this receptor associated with the specific IGH@ translocation, t(14;19)(q32;p13), at diagnosis and relapse in one patient. Overexpression of BCL2L1, CIS and PIM-1 in this patient suggested activation of the JAK-STAT pathway signifying a block in apoptosis and increased proliferative advantage, the two main hallmarks of cancer. Individual patients were also identified by FISH mapping to have IGH@ translocations involving multiple chromosomes and target genes highlighting the promiscuity of this locus, in which all partners may be linked to common pathways in the pathogenesis of leukaemia. The age of these patients ranged from 1-48 yrs, 4 were <10 yrs, 12 were 10-19yrs and 4 were >20 yrs (median - 15yrs) at diagnosis. This is consistent with previous findings of IGH@ translocations being frequently observed in adolescents and young adults. Although patient numbers are small, preliminary survival data suggest that this subgroup may be associated with a good response to therapy.

0488

WHICH TECHNIQUE(S) TO USE WHEN STUDYING MINIMAL RESIDUAL DISEASE AT DAY 35 IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: A MULTICENTER APPROACH

B.J. de Moerloose,[1] K. Swerts,[1] M. Bakkus,[2] A. Uyttebroeck,[3] N. Boeckx,[3] A. Van Damme,[2] P. Vandenberghe,[3] B. Verhasselt,[1] J. Philippé,[1] Y. Benoit[1]

[1]Ghent University Hospital, GHENT; [2]University Hospital Brussel, BRUSSELS; [3]University Hospital Leuven, LEUVEN, Belgium

Background. Detection and quantification of MRD in pediatric ALL patients is important for monitoring response and detection of early relapse. Currently, different methodologies are available (multiparameter flow cytometry (FC), detection of fusion genes using real-time quantitative (RT-)PCR, and competitive PCR-based quantification of clonal immunoglobulin or T-cell receptor gene rearrangements (genescan)). *Aims.* In this multicenter study, MRD detection rates of these techniques were compared and relationships between MRD results and clinical outcome were studied. *Methods.* At the end of induction (day 35), bone marrow samples from 260 patients, treated according to EORTC protocol 58951, were analyzed using at least one method (see above). *Results.* More MRD positive patients were identified by (RT-)PCR (8/78; 10%) or FC (21/258; 8%) compared to genescan (13/214; 6%) or cytomorphology (6/254; 2%). 13/21 patients positive by FC were positive by at least one other technique; likewise 5/8 and 7/13 children positive by (RT-)PCR or genescan scored positive for at least one other method. 14 patients suffered from early relapse, whereas CCR above 30 months was reached in 155 children (median follow-up: 69 months). MRD results of these 169 patients were used to calculate positive predictive value (PPV) and negative predictive value (NPV) at day 35. PPV of FC, (RT-)PCR and genescan was 36%, 33% and 50%. Highest PPV (60%) was reached combining FC and genescan. NPV's of single or combined tests were at least 94%. *Conclusions*. Genescan analysis is superior to FC or (RT-)PCR in identifying patients at risk of early relapse. MRD rates by (RT-)PCR and FC are higher than those obtained by genescan. However, FC and (RT-)PCR have a relatively high false positive rate (PPV=33% and 36%) and MRD by (RT-)PCR detection of fusion genes was only possible in 30% of our patients. Therefore, it is advisable to combine techniques in order to optimize the predictive value of MRD testing.

0489

MOLECULAR CHARACTERIZATION OF CHROMOSOMAL ALTERATIONS IN T-CELL NEOPLASMS BY COMBINING OF FT-CGH AND LM-PCR

G.K. Przybylski,[1] K. Dittmann,[2] P. Grabarczyk,[2] S. Gesk,[3] W. Siewert,[3] R. Siebert,[3] C.A. Schmidt[2]

[1]Institute of Human Genetics, POZNAN, Poland; [2]Universität Greifswald, GREIFSWALD, Germany; [3]Institut für Humangenetik, KIEL, Germany

Background. Dysregulation of gene expression due to chromosomal aberrations plays a key role in malignant transformation. Translocations are routinely found in cancer cells using cytotogenetic methods, but only few of them have been further characterized on the molecular level due to time and labour consuming genomic cloning methods. To better understand the pathogenesis of malignant diseases novel rapid high throughput approaches for determination of the sequence of chromosomal breakpoints are needed. *Aims.* In this study combination of fine tiling comparative genomic hybridization (FT-CGH) and ligation mediated PCR (LM-PCR) was used for cloning and molecular characterization of novel chromosomal breakpoint regions and gene rearrangements in primary T-cell acute lymphoblastic leukaemia (T-ALL) and in a T-cell Non-Hodgkin Lymphoma cell line (K384). *Methods.* A custom fine-tiling oligonucleotide array of 385,000 oligonucleotides (NimbleGen) covering 24 Mb of different genomic areas, including the T-cell receptor alpha/delta locus (TCRAD) on 14q11.2, was designed for comparative genomic hybridization (CGH). All DNA losses within TCRAD were further analyzed by LM-PCR. Since chromosomal translocation involving the TRAD locus are usually accompanied by DNA losses due to TRAD rearrangements, we assumed that using LM-PCR T-cell acute lymphoblastic leukemia we will be able to amplify unknown translocation partners. *Results.* FT-CGH analysis revealed several mono- and biallelic DNA losses in the TCRAD locus in the primary T-ALL sample and in the K384 cell line. Using LM-PCR with sets of nested primers located at the borders to the deleted regions amplification products were obtained differing from the germline control. Sequence analysis of the non-germline LM-PCR products revealed two physiological TCRD rearrangements, TRDV2-J3 and TRDD2-J3, and a 4.6 kb deletion next to the TRAV41 region. In the T-ALL sample on one allele a normal TRAV30-J42 rearrangement was found, while on the other allele an error occurred during the TCRD deleting rearrangement, which is a pivotal step in alpha/beta T-cell development and generates the T-cell receptor excission circles (TREC). The TCRD recombining element (dREC) and the TRAJ61 segment, which normally are joined together, were abberantly cross fused with regions located on chromosome 12q23. Sequence analysis reveald that the rearrangement disrupted the hypothetical gene C12orf42, and was accompanied by a 1.3 kb deletion between the breaks. The identification of a reciprocal translocation t(12;14)(q23;q11.2) was in line with a split observed with fluorescence in-situ hybridization (FISH) probes for the TRAD locus in 39% of cells. *Summary and Conclusions.* We have shown that the combination of FT-CGH and LM-PCR allows the amplification and molecular characterization of gene rearrangements and chromosomal translocation in cell lines and even in patient samples with limited percentage of malignant cells harboring a distinct chromosomal aberration.

0490

TAF1-NUP214 IS A NEW RECURRENT FUSION IN A SUBSET (~3%) OF ADULT T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

P. Gorello,[1] L. Elia,[2] R. La Starza,[1] L. Brandimarte,[1] E. Varasano,[1] A. Vitale,[2] V. Pierini,[1] M. Longinotti,[3] G. Del Poeta,[4] M.F. Martelli,[1] R. Foà,[2] C. Mecucci[1]

[1]Hematology, IBiT Foundation, University of Perugia, PERUGIA; [2]Hematology, University La Sapienza, ROME; [3]Hematology, University of Sassari, SASSARI; [4]Hematology Division, Sant'Eugenio Hospital, Tor Vergata University, ROME, Italy

Background. In T-cell acute lymphoblastic leukaemia (T-ALL) oncogene deregulation occurs through TCRB or TCRA/D juxtaposition or gene fusions. Cytogenetic mechanisms underlying oncogene activation are often cryptic and are detected only by molecular techniques such as FISH, CGH, array-CGH, and gene expression profiling. *Aims.* To characterize the molecular events underlying a cryptic del(9)(q34) in two cases of T-ALL and to establish its incidence in adult T-ALL. *Methods.* A panel of 15 FISH probes was used in a pilot study on 29 adults with T-ALL. A cryptic del(9)(q34)emerged in 2 cases. To delineate the cryptic del(9)(q34)/ABL1, we used four DNA clones for 9q34 ordered as fol-

lows from centromere to telomere: RP11-216B9, RP11-550J21, RP11-143H20, RP11-544A12. For PCR analyses TAF-I-NUP214 fusion transcripts were amplified using primers SET_540F (exon 6) (GAAGAGGCAGCATGAGGAAC) + CAN_2916R (exon 21) (TACTTTGGGCAAGGATTTGG) for the first amplification round and SET_747F (exon 7) (TGACGAAGAAGGGGATGAGGAT) + CAN_2601R (exon 19) (ATCATTCACATCTTGGACAGCA) for nested PCR. Isoform-specific PCR was also performed using CAN_2916R (exon 21) as reverse primer and either TAFIα42F (GAAACCAAGACCACCTCCTG) for the TAF-I alpha isoform, or TAFIβ38F (AGCTCAACTCCAACCACGAC) for TAF-I beta. Reverse primer CAN_2601R (exon 19) and either TAFIα42F or TAFIβ38F were used for the nested PCR. The PCR products were sub-cloned into the pGEM-T easy vector (Promega), sequenced and analyzed using the Blast sequences program (NCBI, http:\\www.ncbi.nim.nih.gov/) and BLAT Search Genome (http:\\genome.ucsc.edu/cgi-bin/hgBlat). To include TAF-I-NUP214 fusion in molecular screening we set-up a multiplex RT-PCR using primers SET-540F and CAN-2916R for the first reaction step and SET_747F and CAN_2601R for the second. Our assay was applied to study 73 patients with T-ALL who were enrolled in the Italian Multicentric GIMEMA studies LAL-0496, LAL- 2000, and LAL-0904. *Results*. The 5'TAF-I and the 3'NUP214 appeared to be juxtaposed as the four 9q34 DNA clones defined the del(9)(q34) endpoints centromerically at the 3' of TAF-I and telomerically at the 5' of NUP214. RT-PCR gave a 802 bp product in one case and a 643 bp product in the other. In the second round a 280 and 121 bp amplification product respectively was detected. Cloning experiments and sequence analysis revealed fusion of nucleotide 813 (exon 7) of TAFI with nucleotide 2389 (exon 17) in 1 case and with nucleotide 2548 (exon18) of NUP214 in the second. To our knowledge, TAFI exon 7/ NUP214 exon 17 fusion has never been described. Moreover the presence of TAFI-alpha/NUP214 and TAFI-beta/NUP214 fusion transcripts was observed in both patients. Multiplex-PCR detected 1/73 TAF-I-NUP214 positive T-ALL. *Conclusions*. Cryptic interstitial del(9)(q34)TAF-I-NUP214 produces the TAF-I-NUP214 fusion transcript which is a new recurrent molecular lesion in adult T-ALL. Since we found 3 cases of TAF-I-NUP214 positive T-ALL when a total of 102 adult patients were screened, the incidence was estimated as 2.9%.

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0491

THE GENERATION OF SENSITIVE, PATIENT SPECIFIC MOLECULAR PROBES IS FEASIBLE IN MOST ADULT ALL PATIENTS AND ALLOWS THE DEFINITION OF A RISK ADAPTED POSTCONSOLIDATION TREATMENT STRATEGY

O. Spinelli, M. Tosi, B. Peruta, T. Intermesoli, E. Oldani, R. Bassan, A. Rambaldi

Ospedali Riuniti, BERGAMO, Italy

Background. Several studies have shown the importance of a molecular evaluation of minimal residual disease (MRD) to predict outcome in childhood acute lymphoblastic leukemia (ALL) but in the setting of adult ALL, only a few studies define a risk adapted treatment strategy on the basis of MRD results. *Aims*. To generate at least 1 sensitive allele specific oligonucleotide (ASO) probe derived from TCR and IG gene rearrangements for RQ-PCR evaluation of MRD in adult ALL patients lacking a translocation derived chimeric transcript. The availability of sensitive molecular probes were instrumental for the definition of risk adapted post consolidation therapy. *Methods*. One hundred and eighty patients with B-lineage ALL, 107 T-lineage ALL, 5 B-and 18 T-Lymphoblastic Lymphomas enrolled in a Phase II clinical study (ALL 09/2000) of the Northern Italy Leukemia Group (NILG) were evaluated following the ESG-MRD-ALL (European Study Group on MRD detection in ALL) methodology. TCR and IG gene rearrangements were identified by PCR amplification, heteroduplex analysis and direct sequencing of clonal bands. Two or more ASO primers were designed on the specific junctional regions and their sensitivity were evaluated at different annealing temperatures (from 60 to 69°C) on serial dilution of diagnostic material by quantitative PCR. *Results*. TCR gamma (TCRG) gene rearrangements were detected by PCR in 49% (n=88) of B-lineage ALL and in 73% (n=77) of T-lineage ALL, while TCRD gene rearrangements in 40% (n=72) and in 47% (n=50) of B- and T-ALL respectively. IgH gene rearrangements were detected in 59% (n=106) of B-lineage and in 4% (n=4) of T-lineage ALL cases. IGK-Kde gene rearrangements were investigated only in B-lineage ALL and the percentage of positivity for clonal bands was 32% (n=57). TCR beta clonality was studied only in T-ALL patients and it was found in 65% (n=64) of cases. The distribution of the clonal rearrangements were similar in the lymphoblastic lymphomas. A total of 484 ASO primers were designed and tested by quantitative PCR at different temperatures to reach a sensitivity suitable for MRD evaluation. We obtained a sensitivity of 10^{-5} in 48% (n= 232) of tested ASO primers, 10^{-4} in the 35%(n=167) and 10-3 in 16% (n=79). Overall, we were able to monitor MRD in 81% of the studied cases, with 2 or 1 ASO probes in 49% (n=135) and 32% (n=90) of cases, respectively. *Conclusions*. In combination with probes generated on translocation derived chimeric genes, more than 90% of adult ALL enrolled into the NILG ALL 09/2000 protocol were suitable for a molecular evaluation of MRD. Therefore, our data show the feasibility of a MRD based definition of risk adapted post consolidation treatment strategy in adult ALL.

0492

ADDITIONAL GENETIC FEATURES OF NUP214-ABL1 POSITIVE PEDIATRIC T-ALL

M. Pisecker,[1] M. König,[1] R. Ullmann,[2] S. Strehl[1]

[1]CCRI, VIENNA, Austria; [2]MPIMG, BERLIN, Germany

Background. T-cell acute lymphoblastic leukemia (T-ALL) is a high-risk malignancy of thymocytes, and accounts for 10-15% of pediatric ALL cases. In T-ALL genetic analyses have elucidated an enormous heterogeneity in genetic abnormalities including chromosomal translocations, deletions, amplifications, and mutations. In about 5-10% of the cases an episomal amplification of a 500 kb DNA fragment normally located at 9q34 that fuses NUP214 and ABL1 has been observed. This genetic aberration is always accompanied by either TLX3 or TLX1 rearrangement. *Aims and Methods*. This study was aimed at the identification and characterization of genetic events accompanying the occurrence of the NUP214-ABL1 fusion in pediatric T-ALL. Genomic DNA of 102 childhood T-ALL patients and 12 matched relapse samples was subjected to BAC tiling path array CGH. Samples showing an amplification of 9q34 were further analyzed by FISH and RT-PCR to confirm the amplification/fusion event. In addition, the TLX3 rearrangement-status in these patients was investigated by FISH and other associated genetic events determined by array CGH were subjected to bioinformatic analysis. *Results*. Array CGH analysis revealed a gain of 9q34 in 5 samples, two of which were relapse samples and showed the amplification only at this stage of the disease. Subsequent FISH analysis confirmed these results and detected a reintegration of the NUP214-ABL1 amplicon into the genome in one of the relapse samples. Amplification of the NUP214-ABL1 by RT-PCR revealed that the fusion gene was also present in those two diagnostic specimens in which array CGH failed to detect the amplification and FISH analysis showed ambiguous results with a minor fraction (<3%) of positive cells. At diagnosis all five patients showed a TLX3 rearrangement by FISH in more than 60% of the cells. Detailed analysis of the array CGH data revealed five regions of minimal overlap (RMO) shared by at least two cases located at 1p36, 6q16, 9p21 and 11p13 (losses) and 18q21 (gain), which together contained 34 known genes. In four of the cases CDKN2A was homozygously deleted, whereas the remaining case showed a heterozygous deletion. The del(11p13) encompassed only the WT1 gene, which has tumor suppressive function, and was present in three patients two of which had a relapse. These two cases showed the deletion also at diagnosis, a stage of the disease in which the NUP214-ABL1 fusion was not detectable by array CGH. *Conclusions*. Neither FISH nor array CGH are the appropriate methods to unambiguously uncover the presence of a NUP214-ABL1 fusion, and thus reliable detection of this genetic aberration requires high-sensitive RT-PCR approaches. Moreover, together our data suggest that fusion and amplification of NUP214-ABL1 is not a primary genetic event in the development of pediatric T-ALL.

0493

T-LYMPHOCYTE LEUKEMIA CELLS MOLT-4: RESISTANT TO TRAIL, BUT VERY SENSITIVE TO IONIZING RADIATION. COULD LOW DOSES OF RADIATION INCREASE SENSITIVITY TO TRAIL?

M. Rezacova

Charles University, Faculty of Medicine in Hradec Kralove, HRADEC KRALOVE, Czech Republic

Aims and Background. T-lymphocyte leukemia cells MOLT-4 are resistant to TRAIL, but very sensitive to ionizing radiation. The aim of the study was to evaluate effect of ionizing radiation on senzitization of MOLT-4 cells to TRAIL. *Methods and Results.* T-lymphocyte leukemia cells MOLT-4 are very sensitive to ionizing radiation. We determined D0

value (the dose after which survives 37% of cells) 0,8 Gy. As soon 2 min after irradiation by the dose of 1.5 Gy we detected ionizing radiation inducing foci containing gammaH2A.X, 53BP1 and Nbs1, which were formed around double-strand breaks of DNA. However, we did not detect phosphorylated form of Nbs1. Inability to phosphorylate Nbs1 could be the reason of high radiosensitivity and low repair capacity. High doses of ionizing radiation (5 and10 Gy) caused decrease of repair proteins Mre11, Rad50 and Nbs1. Our study further demonstrates that cells of acute T-lymphoblastic leukemia MOLT-4 do not express receptors for TRAIL (tumor necrosis apoptosis inducing ligand) DR5 and are TRAIL resistant. Ionizing radiation in the therapeutically achievable dose of 1 Gy significantly sensitizes TRAIL-resistant cells MOLT-4 to the TRAIL-induced apoptosis by increase in death receptor for TRAIL DR5. *Conclusions*. When TRAIL is applied after the irradiation in the time of increased DR5 positivity induced by ionizing radiation (1 Gy) more efficient cell killing by apoptosis is achieved and resistance to TRAIL is nullified.

0494

INCREASED RISK OF ACUTE LYMPHOBLASTIC LEUKAEMIA IN BRAZILIAN INDIVIDUALS WITH NAD(P)H:QUINONE OXIDOREDUCTASE 1 (NQO1) AND CYTOCHROME P450 A1 (CYP1A1) GENE DEFECTS

G. Yamaguti,[1] G. Lourenco,[1] V. Silveira,[2] L.G. Tone,[2] L.F. Lopes,[3] C.S.P. Lima[1]

[1]State University of Campinas, CAMPINAS; [2]School of Medicine of Ribeirao Preto, University of Sao Paulo, RIBEIRAO PRETO; [3]Hospital do Cancer A. C. Camargo, SAO PAULO, Brazil

Background. Acute lymphoblastic leukaemia (ALL) has been linked to chronic exposure to benzene and tobacco's polycyclic aromatic hydrocarbons (PAH). The NAD(P)H:quinone oxidoreductase 1 (NQO1) is an enzyme that detoxifies benzene-derived quinones and reduces oxidative stress on hematopoietic cells. A C'T substitution polymorphism at nucleotide 609 of the NQO1 gene has been linked to a decreased activity of the coded enzyme. On the other hand, PAH are bioactivated by the cytochrome P450 A1 (CYP1A1) enzyme. An A'G substitution polymorphism at nucleotide 4889 and a T'C substitution at nucleotide 6235 of the CYP1A1 gene lead to the production of an enzyme with increased activity in PAH metabolism. Moreover, environmental-related diseases resulting from exposure to benzene and tobacco-related diseases have been described as serious health problems in south-eastern Brazil. *Aims.* The aim of this study was to evaluate the influence of the NQO1 C609T and CYP1A1 A4889G and T6235C polymorphisms for ALL risk in individuals of the south eastern region of Brazil. *Methods.* Genomic DNA from 93 ALL patients (median age: 4.33, range: 0.4-17; male: 47, female: 46; Caucasian: 82, African-American: 11) and 93 gender and race-matched controls (median age: 53, range: 40-60; male: 47, female: 46; Caucasian: 82, African-American: 11) were analysed using the polymerase chain reaction (PCR) and enzymatic digestion. The differences between groups were calculated by the chi-square or Fischer exact test. Logistic regression analysis was used to obtain gender and ethnic origin adjusted odds ratios (ORs). *Results.* Controls' samples but not patients' samples were in Hardy-Weinberg (HW) equilibrium for the NQO1 locus (X2=2.04, p=0.15, X2=5.16, p=0.02, respectively). The frequency of the NQO1 609CT+TT genotype was significantly higher in ALL patients than in controls (49.5% *vs* 50.5%, p= 0.001). Carriers of the NQO1 variant allele (T) were under a 2.8-fold increased risk of ALL (95% CI: 1.53-5.29). Both controls' and patients' samples were in HW equilibrium for the CYP1A1 A4889G and T6235C loci (X2=0, p=1.00, X2=0.46, p= 0.50; X2= 3.38, p=0.07, X2=0.64, p=0.42; respectively). Similar frequencies of the CYP1A1 4889AG+GG and 6235TC+CC genotypes were seen in patients and controls (38.7% *vs* 26.9%, p=0.09; 39.7% *vs* 31.2%, p=0.22; respectively). Controls and patients with the distinct genotypes of the CYP1A1 A4889G (OR=1.72, 95% CI:0.92-3.19) and T6235C (OR=1.46, 95% CI: 0.80-2.67) were under similar risks for ALL. However, the frequency of the combined variant genotypes NQO1 609CT+TT and 4889AG+GG was higher in patients than in controls (33.3% *vs* 7.5%, p< 0.001). Carriers of the two variant alleles were under a 8.6-fold increased risk for ALL (95% CI: 3.35-21.86). Conclusion: These results suggest a role for the NQO1 609TT+CT and CYP1A1 4889GG+AG genotypes in increased risk for ALL in individuals of south eastern Brazil. Since the variant alleles of NQO1 C609T and CYP1A1 A4889G polymorphisms are prevalent in the general population, the identification of individuals with the variant genotypes may provide a useful public health approach for prevention of ALL. Financial support: CNPq

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DETECTION OF PROTEIN PATTERNS IN PLASMA OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA USING FUNCTIONALIZED MAGNETIC BEADS AND LINEAR MALDI-TOF MASS SPECTROMETRY

N. von Neuhoff,[1] T. Oumeraci,[1] B. Hirsch,[1] M. Kostrzewa,[2] M. Schrappe,[3] B. Schlegelberger,[1] G. Cario[3]

[1]Hannover Medical School, HANNOVER; [2]Bruker Dalton GmbH, LEIPZIG; [3]University Hospital Schleswig-Holstein, Campus Kiel, KIEL, Germany

Background. Proteomic profiling of body fluids is a promising novel tool for the identification of protein signatures in order to stratify the risk and to monitor treatment outcome. Different methods have been developed to isolate and fractionate plasma proteins and to determine discriminative protein signatures. However, it is still challenging to obtain highly reproducible and distinctive protein patterns for the reliable detection of disease specific biomarkers. *Aims.* The present study describes a novel technology for plasma proteome analysis using functionalized beads to fractionate proteins and peptides, matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) and specialized software tools. This has resulted in distinctive and highly reproducible plasma proteome signatures from children with acute lymphoblastic leukemia (ALL) investigated at four timepoints before and during chemotherapy. *Methods.* EDTA plasma samples from 12 patients with childhood ALL treated according to the ALL-BFM2000 protocol were taken before and at different timepoints during treatment (day 0, week 1, week 5, week 12). Plasma proteins were fractionated using functionalized magnetic beads. Beads with different surface functionalities (e.g. hydrophobic interaction, cationic exchange and anionic exchange) were used to enrich and to purify different protein/peptide subclasses. Proteomic profiles were acquired in a linear microflex MALDI-TOF mass spectrometer. To ensure reproducibility, each purified sample was processed independently four times and each fraction was spotted four-fold onto MALDI targets. For spectra acquisition, 500 single shots were accumulated from each of the corresponding target positions. The acquired patterns were analyzed using the ClinProTools 2.1 software (Bruker Daltonik, Leipzig) to discriminate data sets and discover reliable candidate proteins. Data sets obtained by different bead functionalities were analyzed separately. Depending on the sample and magnetic bead functionality, up to 343 masses were detected in the range from 1 kDa to 10 kDa. *Results.* Distinct signatures were identified for all applied magnetic bead functionalities. Comparing the spectra from four independently processed fractions of each sample showed a very high reproducibility of the obtained data. The best classification was achieved after cationic exchange fractionation of the plasma samples. Using this technology, an almost complete separation of the four timepoints before and during chemotherapy was achieved. The discrimination was based on up to 12 different masses and resulted in a recognition capability of 100% overall and of 94.4% in cross-validation. *Conclusions*. Based on a novel technology for the fractionation of plasma proteins using functionalized magnetic beads, MALDI-TOF-MS and specialized software tools, proteome signatures were reliably able to distinguish plasma probes from children with ALL obtained at four different timepoints before and during chemotherapy. Work is in progress to identify the discriminative peptides and proteins and to clarify their cellular function and possible role in chemotherapy response. Further studies will focus on the potential value of measuring these plasma protein signatures for monitoring individual treatment response in childhood ALL.

Acute myeloid leukemia - Biology II

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TIME-DOMAIN OPTICAL IMAGING OF AML XENOGRAFTS WITH NEAR-INFRARED CONJUGATED MONOCLONAL ANTIBODIES

E. Mc Cormack, M. Mujic, B.T. Gjertsen

Institute of Medicine, BERGEN, Norway

Background. The 5-year survival for acute myeloid leukaemia (AML) has changed little in the last two decades and while molecular targeted and neoadjuvant therapies have provided some promise more recently, the clinical impact upon survival has been limited. The methods employed to authenticate these prospective targets *in vivo* tend to be quite protracted with validation and preclinical testing remaining the rate-limiting step in translating these treatments to the clinic. Molecular imaging has now become a critical facet of not only drug development but also clinical and preclinical disease monitoring. *Aims.* Using an innovative mode of *in vivo* optical imaging we have described a novel methodology of time domain (TD) optical imaging and its implementation in preclinical detection and longitudinal monitoring of fluorescent proteins in AML. However, imaging of fluorescent proteins or bioluminescent enzymatic reporters is problematic due to high tissue absorption at visible wavelengths. Thus we propose the development of novel imaging strategies in relevant animal models of AML, which exploit the near-infrared region (NIR) of light. Through development monoclonal antibodies (mAb)s targeted NIR optical probes we propose to interrogate AML biology and monitor treatment efficacy with TD optical imaging. *Methods.* Using mouse anti-human mAbs against human myeloid markers (CD45, CD13 and HLA-ABC) we conjugated NIR dyes and employing imaging phantoms in a commercial TD optical imaging system we optimised the degree of labelling for *in vivo* imaging. We established several preclinical xenograft models of AML (NB4, HL60, MOLM-13) and compared the ability to monitor disease progression with fluorescent/bioluminescent proteins and our NIR-mAb probes with TD optical imaging. *Results.* The combined use of TD optical imaging and NIR labelled mAbs proved to be superior to both fluorescence and bioluminescence for earlier and more quantitative imaging of xenograft models of AML. TD imaging of distinct fluorescence lifetimes of NIR labelled mAbs also permitted the discrimination of specific and random binding of the fluorescent probe. *Summary*. The combined use of TD optical imaging and NIR labelled mAbs provided an excellent mode of disease detection and longditudinal monitoring of human xenograft models of AML. In the future it should be possible to use this technology to detect distinct subsets of AML populations *in vivo* and monitor functional therapeutic response e.g. Immunotherapy.

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COAGULATION ACTIVATION IN ACUTE PROMYELOCYTIC LEUKEMIA TRANSGENIC MICE MODEL HCG-PML-RAR?

M.C.T. Pintao, B.A.A. Santana-Lemos, F.G. Mangolini, L.L. Figueiredo-Pontes, F.P. Saggioro, C.L.A. Silva, P.S. Scheucher, G.A.S. Santos, M.S. Baggio, A.B. Garcia, E.M. Rego

Medical School of Ribeirão Preto - University of São Paulo, RIBEIRÃO PRETO, Brazil

Background. Acute promyelocytic leukemia (APL) is associated with a coagulopathy characterized by disseminated intravascular coagulation (DIC), fibrinolysis and proteolysis. Primary hyperfibrinolysis is considered to be responsible for the severe bleeding in APL. However, the bulk of evidence, particularly elevated levels of D-dimer, suggests that excess fibrinolysis is secondary to excess thrombin generation. In addition, properties of the malignant leukemic cells themselves and their interactions with host defense mechanisms, including increased expression of procoagulant activity, expression of fibrinolytic and proteolytic properties and secretion of proinflammatory cytokines, play a role in APL coagulopathy. *Aims*. We took advantage of the transgenic mice (TM) hCG-PML-RARα generated by Pandolfi´s group (He *et al.*, 1997) to characterize coagulation and cytokine pattern in leukemic (leu) in comparison to non-leukemic-TM (non-leu) and wild type (WT) controls. *Methods*. Blood was obtained by cardiac punction of 12 leu-TM, 14 non-leu-TM and 12 WT mice using syringe with citrate 3.2% with proportion adjusted to 9:1 (blood:citrate). Plasma was separated by centrifugation and stored at -80° until analysis. Brain, kidney, lungs, heart, spleen, liver and bone marrow (BM) where obtained for histological analysis. Coagulation activation was analyzed by measurement of thrombin-antithrombin complex (TAT-ELISA), fibrinolysis was analyzes by measurement of plaminogen activator inhibitor-1 (PAI-1) activity (ELISA) and interleukins (IL-6, IL-10, MCP-1, INF-Á, TNF and IL-12p-70) where measured by BD Cytometric Bead Array mouse inflammation kit (CBA). Fibrin deposition where evaluated by histochemistry using Mallory's phosphotungstic acid-hematoxilin (PTAH) stain. Statistical analysis was performed by ANOVA. *Results*. Leu-TM presented anemia, thrombocytopenia and leucocytosis associated with BM infiltration by leukemic cells resembling promyelocytes (56±19%; 9,5±3.6%; and 8.4±2.4% in leu-TM, non-leu-TM and WT, respectively. Spleen size (median± SD) was higher in leu-TM (0.956± 0.583g), compared to non-leu TM (0.136±0.071g) and WT mice (0.094± 0.011g). PAI-1 activity was higher in leu-TM in comparison to non-leu-TM and WT (mean±SD): 16.5±11.2 ng/mL; 2.1± 6.0 ng/mL and 2.4±5.2 ng/mL (p<0.001), respectively. TAT levels were non significantly higher in leu-TM, but since the detected levels in all 3 groups were higher than the reported in literature, we can not rule out that there was activation during heart puction. In addition, intravascular fibrin deposition was not detected by histochemic evaluation. Analysis of interleukin pattern showed elevated TNF in leu-TM in comparison to non-leu-TM and WT (mean±SD): 20.50±9.2 pg/mL; 4.4±0.8 pg/mL (p<0.001) and 4.6±1.4 pg/mL (p<0.001), respectively. IL-6 was also elevated in leu-TM in comparison non-leu-TM and WT (mean±SD): 6.9±5.8 pg/mL; 2.5±0.7 pg/mL (p<0.001) and 3.4±2.0 pg/mL (p<0.05). *Summary and conclusions*. In conclusion, the leukemia developed by hCG-PML-RARαTM is associated to elevated PAI and elevated IL-6 and TNF cytokines, thus reinforcing the importance of fibrinolysis and cytokines in APL associated coagulopathy. Our results demonstrate that the TM model that mimics the APL coagulopathy and is a very useful tool for testing new drugs and understanding the physiopathology of the disease.

0498

A ONE-MUTATION MATHEMATICAL MODEL CAN EXPLAIN THE AGE INCIDENCE OF AML WITH MUTATED NUCLEOPHOSMIN (NPM1)

A. Liso,[1] F. Castiglione,[2] A. Cappuccio,[2] F. Stracci,[3] R.F. Schlenk,[4] S. Amadori,[5] C. Thiede,[6] S. Schnittger,[7] P.J.M. Valk,[8] K. Döhner,[4] M.F. Martelli,[9] M. Schaich,[6] J. Krauter,[10] A. Ganser,[10] M.P. Martelli,[9] B. Löwenberg,[8] T. Haferlach,[7] G. Ehninger,[6] F. Mandelli,[11] H. Döhner,[4] F. Michor,[12] B. Falini[9]

[1]University of Foggia, FOGGIA, Italy; [2]CNR, ROME, Italy; [3]University of Perugia, Dept. Surg. Med. Spec. and Public Health, PERUGIA, Italy; [4]Department of Internal Medicine III, University of Ulm, ULM,, Germany; [5]Institute of Hematology, University of Tor Vergata, ROME, Italy; [6]Laboratory for Molecular Diagnostics, University Hospital Carl Gustav Carus,, DRESDEN, Germany; [7]MLL-Munich Leukemia Laboratory, MUNICH, Germany; [8]Department of Hematology, Erasmus University Medical Center, ROTTERDAM, Netherlands; [9]University of Perugia, Institute of Hematology, PERUGIA, Italy; [10]Department of Hematology, Hemostasis and Oncology, Hannonver Medical School, HANNOVER, Germany; [11]Institute of Hematology, University La Sapienza, ROME, Italy; [12]Computational Biology Center, Memorial Sloan Kettering Cancer Center, NEW YORK, USA

Background and Aims. The nucleophosmin (NPM1) gene mutation is one of the most common genetic alterations in adult acute myeloid leukemia (AML), accounting for 50-60% of AML cases with normal karyotype. Despite molecular heterogeneity, all variants lead to common changes in the NPM1 protein causing an increased nuclear export of the nucleophosmin leukemic mutant and its aberrant accumulation in the cytoplasm. AML with mutated NPM1 shows distinctive biological and clinical features, suggesting that AML with mutated NPM1 represents a new disease entity. Experimental evidence of the oncogenic potential of the nucleophosmin mutant is, however, still lacking, and it is unclear whether other genetic lesion(s), such as FLT3-ITD, cooperate with NPM1 mutations in generating the leukemic phenotype. The multi-step theory of carcinogenesis was conceived after mathematical modelling demonstrated that the increasing cancer incidence with age can be explained by several stochastic events needed for tumorigenesis. An analysis of the age-specific incidence, together with mathematical modelling of AML epidemiology, can help to uncover the number of genetic events needed to cause leukemia. *Methods*. National registry-based AML incidence data with details of NPM1 mutation status are not available. Therefore, we collected data sets at five major European Institutions: the Laboratory of Cytogenetic and Molecular Diagnostic, University Hospital Ulm, representing the German-Austrian AML study Group; the Laboratory of Hemopathology, Institute of Hematology, University

of Perugia, representing GIMEMA group; the Laboratory for Molecular Diagnostics, University Hospital Carl Gustav Carus, Dresden, Germany, representing the Deutsche Studieninitiative Leukämie; the Munich Leukemia Laboratory, and the Department of Hematology, Erasmus University Medical Center. We determined the age-specific incidence of AML with mutated NPM1 (a total of 1444 cases) for each country. We then adapted a previously designed mathematical model of hematopoietic tumorigenesis to analyze the age incidence of AML with mutated NPM1. Finally, we confermed the predictions of equations with direct computer simulation of the stochastic process. *Results*. Linear regression of the curves representing age-specific rates of diagnosis per year showed similar slopes of about 4 on a doubly logarithmic scale. The one-mutation model generated slopes similar to real age-specific incidence curves from patients (Figure 1) from Germany, The Netherlands, and Italy. Since FLT3-ITD frequently associates with NPM1 mutations and appears to abrogate the favourable prognostic effect of NPM1 mutations in AML, we determined whether the age incidence of NPM1-mutated AMLs with FLT3-ITD differs from cases with wild-type FLT3. No significant difference emerged in the slopes of FLT3-ITD-positive and -negative AML with mutated NPM1. *Summary and Conclusions*. The model fits the NPMc+ AML age-specific incidence curve for plausible parameter choices supporting the hypothesis that a single genetic event, or two sinchronous events, are sufficient to cause this type of leukemia. No difference can be detected between the slopes of the age specific incidence of FLT3-ITD-positive and -negative NPMc+ AML, supporting the view that NPMc+ AML is a homogeneous group irrespective of the FLT3 mutational status. Since NPM1 mutations are associated with haploinsufficiency of wild-type NPM in leukemic cells, an attractive hypothesis would be that the two alterations act together to cause NPMc+ AML.

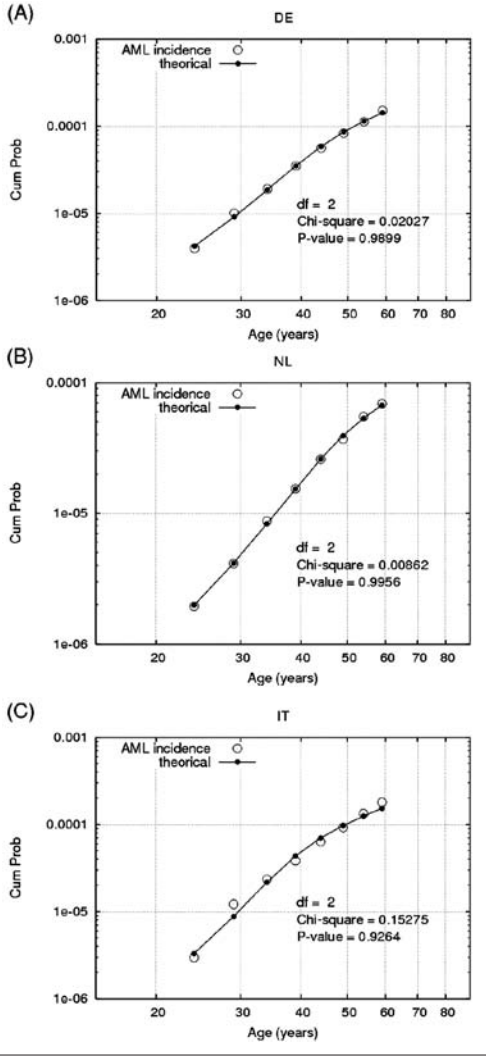

Figure 1. Age-specific incidence curves in NPMc+AML

0499

COMPLEX AMPLIFICATION OF CHROMOSOME 21 MASKING A NOVEL CRYPTIC RUNX1 GENE FUSION IN AN AML CELL LINE (OCI-M2)

S. Chen,[1] B. Schneider,[2] S. Nagel,[2] R. Geffers,[3] H. Quentmeier,[2] M. Kaufmann,[2] H. Drexler,[2] R. MacLeod[2]

[1]DSMZ'German Collection of Microorganisms and Cell Cultures, BRAUNSCHWEIG; [2]DSMZ'German Collection of Microorganisms and Cell Cultures, BRAUNSCHWEIG; [3]Helmholtz Centre for Infection Research, BRAUNSCHWEIG, Germany

Background. Recently, a novel complex recurrent genomic amplification targeting part of the long arm region of chromosome - amp21q2 - was described in a subset of acute myeloid leukemia (AML) patients (Baldus *et al.*, Proc Natl Acad Sci USA 101: 3915, 2004). Array comparative genomic hybridization (CGH) combined with expression profiling highlighted several gene amplification targets on 21q, notably APP (amyloid beta precursor protein), while RUNX1 (alias AML1) at 21q21 was excluded as a direct target. *Aims.* We have identified an erythromegakaryocytic cell line - OCI-M2, established from a 56-year old patient with AML-M6 secondary to myelodysplastic syndromes (MDS), to serve as a model for investigating this novel leukemic entity. *Methods.* OCI-M2 Cells were subjected to conventional and molecular cytogenetic analysis by fluorescence *in situ* hybridization (FISH) using BAC and fosmid clones. Array-CGH was performed using 100k arrays, and expression profiling with U133 Plus 2.0 arrays (Affymetrix). Gene expression was confirmed by reverse transcription (RT)-PCR, and quantified by qRT-PCR. Random amplification of cDNA ends (RACE) was used to detect RUNX1 fusion transcript. *Results.* Conventional cytogenetic analysis showed the following karyotype: 51,XX,der(X)t(X;8)(q23;q23),+6,+9,der(9)add(9)(p23)del(9)(p12p21)t(9;21)(p23;q22)t(21;19)(q21.2;q13)add(19)(hsr),t(10;12)(p12;p12),del(17)(q11q21.1),+20,der(21)add(21)(q22)(hsr),+3mar. FISH analysis revealed extensive amplification of material from chromosomes 19 and 21 on both der(9) and der(21) marker chromosomes forming homogeneously staining regions. Array-CGH showed that amplification of chromosome 21 peaked (~4X) the ~37-47Mbp, followed by 15-18 Mbp and 27-35 Mbp (both ~3X), but spared the interval closest to RUNX1. Thus, chromosome 21 amplification in OCI-M2 closely resembles that described by Baldus *et al.* FISH using BAC and fosmid clones confirmed that the 21q21 breakpoint inside der(9) lay within RUNX1, close to intron 6. Both der(9) and der(21) markers carried extensive homogeneously staining regions. FISH using chromosome 19 BAC/fosmid clones showed both chromosomes 19 and 21 to be amplified within these markers. The chromosome 19 amplicon was focused on the 33-45Mbp interval flanked by breakpoints on der(9) at 19q12 (33.0 Mbp) inside a gene-poor region, and at 19q13 (45 Mbp) near RPS16 - a ubiquitously expressed 40S ribosomal protein gene. Discrepant bands from 3´-RACE using RUNX1 templates were excised and sequenced. Sequencing confirmed fusion of 5´-RUNX1 with 3´-RPS16. Western blotting with N-terminus antibody to RUNX1 confirmed the translation of both wild type and enlarged 70 kDa mutant bands, consistent with formation of a novel fusion protein. RPS16 belongs to the emergent family of ribosomal proteins recently implicated as a target of 5q deletions in AML/MDS. *Conclusions.* Taken together, our findings: 1) document a novel RUNX1 translocation; 2) increase the number of RUNX1 translocations documented in AML cell lines from four (partnering ETO at 8q22, ETV6 at 12p13, EVI1 at 3q26, LRP16 at 11q13) to five; 3) implicate a new ribosomal protein gene in leukemogenesis; 4) provide a novel tool for investigating the role of RUNX1 rearrangement in leukemogenesis; and 5) identify a cell line model for characterizing the gene target(s) of chromosome 21 amplification in AML.

0500

VEGF MRNA LEVELS, PROTEIN CONCENTRATION AND MICROVESSEL DENSITY IN AML PATIENTS, AT DIAGNOSIS AND IN REMISSION

A. Avgitidou,[1] E. Ioannidou,[1] A. Tsiga,[1] E. Vlachaki,[1] F. Klonizakis,[1] St. Dimoudis,[1] M. Diamantidis,[1] S. Haralambidou,[1] I. Venizelos,[2] I. Klonizakis[1]

[1]Hippokration Hospital, AUTH, THESSALONIKI; [2]Department of Pathology, Hippokration Hospital, THESSALNIKI, Greece

Background. Angiogenesis is the formation of new blood vessels from pre-existing vessels and is involved in the growth of solid tumours. Haematologic malignancies do not develop in the same way as solid tumours so the requirement of angiogenesis has not been as clearly

recognised as for other malignancies. However, in the last years, many studies evaluated the importance of angiogenesis in heamatologic malignancies, like AML, indicating a possible role in its pathogenesis. Although, many angiogenic factors have been identified in AML, the most important is Vascular Endothelial Growth Factor (VEGF). VEGF plays an essential role in normal and pathologic angiogenesis, but its clinical role in AML remains unclear. *Aims.* The aim of our study was to investigate the expression of VEGF in the bone marrow of AML patients at diagnosis and in remission, and thus to assess the role that angiogenesis play in the progression of the disease. *Methods*. Total RNA was isolated from bone marrow cells of 50 AML patients (median age 54,4 years) and 10 individuals, of the same age, with normal haematopoiesis that used as controls. Thirty one patients were at the onset of the disease, whereas 19 patients were in complete remission (<5% bone marrow blasts). Real-Time Semi-Quantitative RT-PCR assay was performed in order to detect the mRNA levels of VEGF. The regulation of the target gene was estimated as an expression ratio. Serum concentrations of VEGF in 50 AML patients and in 10 control samples were measured by ELISA assay. Bone marrow biopsies from 23 newly diagnosed AML patients were assayed for microvessel density (MVD), using anti-CD34 monoclonal antibody. MVD was estimated by counting the number of vessels per 400x high power field (HPF) using light microscopy. *Results.* Increased expression levels of VEGF were found in the serum from patients with AML at diagnosis (365,65pg/μl, $p<0,05$) and in remission (579,08pg/μl, $p<0,05$) compared with the levels found in control group (69,48pg/μl). Interestingly, there is a significant increase ($p<0,05$) in VEGF concentration at patients in remission compared to patients at diagnosis. Immunohistochemical staining showed a significant increase of the bone marrow MVD in newly diagnosed AML patients (6,276 vessels/HPF, $p<0,05$) compared with controls (1,6 vessels/HPF). Finally, the mRNA levels of VEGF were significantly lower in newly diagnosed untreated patients (1,37 fold, $p<0,001$) compared to controls. There is no significant difference between remission patients (1,097 fold, $p>0,05$) and controls, but VEGF mRNA levels were significantly higher in remission patients compared with newly diagnosed patients ($p=0,001$). *Summary and Conclusions.* There is evidence of increased MVD in the bone marrow of AML patients, which supports the hypothesis of an important role of angiogenesis in AML. Our data show a discrepancy between bone marrow angiogenesis and VEGF expression, both in mRNA and protein level. This suggests that angiogenesis in AML likely represents a response to bone marrow microenvironmemt and that VEGF expression in not an intrinsic property of leukemic cells but can be produced also by normal cells. Further investigation in this field is recommended in order to better determine VEGF function in AML.

0501

SECONDARY GENETIC ABBERATIONS AND THEIR EFFECT ON PROGNOSIS IN CORE BINDING FACTOR - ACUTE MYELOID LEUKEMIA (CBF-AML)

J. Markova,[1] Z. Trnkova,[2] J. Maaloufova,[2] J. Stary,[3] P. Cetkovsky,[2] J. Schwarz[2]

[1]Institute of Hematology & Blood Transf., PRAGUE 2; [2]Institute of Hematology & Blood Transfusion, PRAGUE 2; [3]Dept. of Pediatric Hematology & Oncology, Faculty Hospital Motol, PRAGUE 5, Czech Republic

Background. Patients with CBF-AML carrying AML1/ETO or CBFb/MYH11 are considered to have good prognosis, albeit about 50% of them relapse. Secondary genetic aberrations associated with poor outcome occur in this patient subset. Mutations in FLT3 and particularly in C-KIT receptor tyrosine kinases have been frequently described in CBF-AML. Mutations in exon 17 of C-KIT more often occur in patients with the AML1/ETO fusion and according to some authors, negatively affect both the incidence of relapse and overall survival (OS), while a strong association between C-KIT exon 8 mutations and CBFb/MYH11+ AML has been reported. *Aims.* To perform mutational analysis of C-KIT in order to check its incidence and prognostic relevance in patients with CBF-AML. *Patients and Methods.* A cohort of 60 CBF-AML cases was studied. The median patients' age was 29.3 years (range: 1.6-72.2), the male/female ratio was 38/22. The presence of AML1/ETO and CBFb/MYH11 at diagnosis (and futher during treatment) was detected by real-time RT-PCR. C-KIT mutations (in exons 8, 9, 10, 11, 17 and 18) and FLT3/ITD were determined by gel electrophoresis and direct sequencing. FLT3 D835 point mutations were analysed by restriction analysis using EcoR V and verified by direct sequencing. To detect the JAK2 V617F mutation, an allelic discrimination real-time RT-PCR assay was employed. *Results.* The median WBC count of CBF-AML patients was 20.5 G/L. Cases with the AML1/ETO fusion had lower WBC than those with CBFb/MYH11 (medians: 13.3 *vs* 66.0, $p=0.001$). Of 34 patients with AML1/ETO, 4 had C-KIT exon 17 mutation, involving C-KIT D816 in 75% cases. Two patients had ITD in exon 11 and only one presented with a mutation in exon 8. FLT3 ITD and D835 mutations were found in 3 and 2 cases, respectively. Two patients had JAK2 V617F mutation. C-KIT mutations were detected in 14/26 patients with CBFb/MYH11: 9 of them in exon 8, 5 in exon 17 (in one patient, mutation was present in both exons). No mutation was detected in exon 11. Four patients with CBFb/MYH11 had a point mutation in FLT3 D835 and one FLT3/ITD was found in this group. JAK2 V617F mutation was not detected in CBFb/MYH11-positive patients. Although CBFb/MYH11-positive patients with a C-KIT mutation had higher median WBC counts, this was not significant ($p=0.24$). The C-KIT mutation did not affect WBC counts in AML1/ETO-positive cases. Of 33 CBF-AML patients with unmutated C-KIT, 12 (36%) relapsed ; of C-KIT mutated cases, 7/17 (41%) relapsed ($p=0.37$). No difference in the mutational status of C-KIT gene was found when AML1/ETO and CBFb/MYH11-positive patients were evaluated separately ($p=0.36$ and $p=0.43$, respectively). Of 3 AML1/ETO cases with the exon 17 D816 mutation, 2 relapsed ($p=0.10$). *Conclusions.* We were unable to confirm any major prognostic impact of assaying C-KIT mutations in CBF-AML patients. The only result indicating a trend to prognostic significance applies to AML1/ETO patients with the D816 mutation in exon 17. However, in our experience, these patients are quite rare (3 of 34 patients). In the future, we plan to test solely the D816 mutation in AML1/ETO positive patients.

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0502

ESCAPE-MECHANISM IN CHILDHOOD ACUTE MEGAKARYOBLASTIC LEUKEMIAS

O. Hummel, I. Fränzel, K. Reinhardt, D. Reinhardt

Hannover Medical School, HANNOVER, Germany

Background. About 5 to 10% of the children with Down Syndrome showed a transient leukemia and had 400-fold risk to suffer myeloid leukemia (ML-DS) within the first 4 years of life. Both leukemias are characterized by megakaryoblastic differentiation and mutations of the hematological transcription factor GATA1. The incidence of acute megakaryoblastic leukemias (AMKL) in children without Down syndrome is 6% only. In ML-DS and AMKL severe myelofibrosis is a typical problem, whereas TL could be complicated by a liver fibrosis. As previously shown transforming growth factor beta (TGFb), secreted preferentially by megakaryoblasts, was supposed to induce myelofibrosis. As a TGFb usually inhibited cell proliferation, we hypothesized that the dominating leukemic blasts, used an *escape* -mechanism as proliferation advantage. *Methods.* Expression of the TGFb-receptors I,II,III (TBR I,II,III), the pseudoreceptor BMP and activin membrane-bound inhibitor (BAMBI), thrombopoietin-receptor (c-mpl), cytokine.receptor CXCR4 and BMP2 were analysed by qPCR, gene expression profiling and immunophenotyping (if applicable) in primary AML-blasts and megakaryoblastic cell lines (CMK derived from a TL, M07 derived from AMKL, HL60 as control). Further we analysed the TGFb-receptor regulation by increasing concentrations of TGFβ(10 to 50 ng/mL) in patient derived bone marrow stroma-cell - CMK/M07/HL60/blasts/CD34$^+$ stem cell co-cultures. *Results.* Myeloid blasts from patients with TL, ML-DS and AMKL showed significantly decreased expression of TBR III, SDF-1 and CXCR4 and an increased expression of BAMBI, c-mpl and BMP2. Further, the WNT-, TGFb- and Cadherin-signalling pathways were activated in all three entities. In co-cultures, proliferation of CMK and M07 cell line was slightly inhibited. A TGFb dependent down regulation of TBR III and an up regulation of Bambi could be demonstrated. Interestingly, CXCR4 expression was strongly induced in both CMK cells and bone marrow stroma cells. *Conclusions.* The preliminary results support the hypothesis that TGFb is in involved in an escape mechanism of childhood megakaryoblastic leukemias

0503

MOLECULAR CHARACTERIZATION OF THE MLL-SEPT6 FUSION GENE IN ACUTE MYELOID LEUKAEMIA: IDENTIFICATION OF NOVEL FUSION TRANSCRIPTS AND CLONING OF GENOMIC BREAKPOINT JUNCTIONS

N. Cerveira,[1] F. Micci,[2] J. Santos,[1] M. Pinheiro,[1] C. Correia,[1] S. Lisboa,[1] S. Bizarro,[1] L. Norton,[1] A. Glomstein,[2] A.E. Åsberg,[3] S. Heim,[2] M.R. Teixeira[1]

[1]Portuguese Oncology Institute, PORTO, Portugal; [2]Radiumhospitalet-Rikshospitalet Medical Center, OSLO, Norway; [3]St. Olav University Hospital, TRONDHEIM, Norway

Background and Aims. Abnormalities of 11q23 involving the MLL gene are found in several haematological malignancies, including acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML). One of the more than 52 different MLL fusion partners so far identified is the SEPT6 gene, which belongs to an evolutionarily conserved family of genes, the septins. In this work we aimed to characterize at both the RNA and DNA levels three AML cases with chromosome banding and/or molecular cytogenetics evidence of a rearrangement between 11q23 and Xq24 (the SEPT6 locus). *Design and Methods.* Three AML cases were studied by a combination of cytogenetic (chromosome banding and/or fluorescence *in situ* hybridisation), molecular (RT-PCR, LD-PCR, HN-PCR and sequencing), and bioinformatic methodologies. *Results.* Karyotyping and molecular cytogenetics showed, in all cases, a chromosomal recombination between 11q23 and Xq24. Molecular analysis led to the identification of several MLL-SEPT6 fusion transcripts in all cases, including a novel MLL-SEPT6 rearrangement (MLL exon 6 fused with SEPT6 exon 2) and several alternative splicing variants. Genomic DNA breakpoints were found inside or near Alu or LINE repeats in the MLL breakpoint cluster region, whereas the breakpoint junctions in the SEPT6 intron 1 mapped to the vicinity of GC-rich low-complexity repeats or near Alu repeats. In addition, a topoisomerase II consensus cleavage site was detected in SEPT6 intron 1. *Interpretation and Conclusions.* We characterized a novel MLL-SEPT6 rearrangement in childhood AML. Our findings suggest that a non-homologous end-joining repair mechanism may be involved in the generation of MLL-SEPT6 rearrangements in AML.

0504

INHIBITION OF THE PROLIFERATIVE AND ANGIOGENIC ACTIVITY OF FLT3 INTERNAL TANDEM DUPLICATION MUTANTS FROM ACUTE MYELOID LEUKEMIA CELL LINES BY A TYROSINE KINASE INHIBITOR

L.A. Luis Arístides,[1] N. Barbarroja,[2] M.J. Luque,[2] R.M. Carretero,[2] A. Torres,[3] V. Velasco,[3] Ch. Lopez-pedrera[2]

[1]Hospital Reina Sofía, CÓRDOBA; [2]Unidad de Investigación, CÓRDOBA; [3]Servicio de Hematología, Hospital Reina Sofía, CÓRDOBA, Spain

The Flt3 gene encodes a haematopoietic class III receptor tyrosine kinase (RTK) involved in the proliferation and differentiation of the haematopoietic stem cells. Activation of the Flt3 by different types of mutations plays an important role for proliferation, resistance to apoptosis, and prevention of differentiation of leukemic blasts in acute myeloid leukemia (AML). At least one type of such mutations -an internal tandem duplication in the Flt3 juxtamembrane domain (Flt3/ITD)- has been associated with an unfavourable prognosis. RTK signaling pathways are normally highly regulated. However, their over activation has been shown to promote the growth, survival, and metastasis of tumour cells. Over expression of vascular endothelial growth factor (VEGF, a major angiogenesis regulator), and its specific tyrosine kinase receptors, VEGFR1 (Flt1) and VEGFR2 (KDR), are detected in a variety of haematological malignancies, including AML, were they contribute to poor survival and negative progression of the malignancy. In the last years, many laboratories embarked on projects aimed at generating compounds that specifically inhibited the activity of these aberrant signalling cascades triggered by tyrosine kinases involved in tumorigenesis. The aim of the present study was to analyze the molecular and cellular effects of Flt3 inhibition in AML cell-lines representative of the different Flt3 genotypes, by using both chemical inhibitors (AG1296 and AG1295) or gene silencing (by using a lentiviral system). Four cell lines were analyzed: NB4 and THP-1 (Flt3/wt), Molm13 (Flt3ITD/wt), and MV411 (Flt3ITD/-). *Results.* AG1296 selectively and potently inhibited autophosphorylation of constitutively activated Flt3 in Flt3-ITD cell lines (MV411 and Molm13) in a dose-dependent manner. Concomitantly, cell proliferation was prevented and apoptosis was induced. Moreover, the constitutive activation in AML cells of Flt3-mediated intracellular kinases (ERK, AkT and STAT5) was inhibited in the cell lines holding the mutated receptor. Furthermore, AG1296 was shown to be involved in the blockage of VEGF/VEGFRs expression, resulting in inhibition of VEGFRs activity. Combined RNAi-induced downregulation of Flt3 expression and treatment with the chemical inhibitors of RTK activity, leaded to higher efficiency in the inhibition of both cell proliferation and angiogenesis, as well as to the prevention of the apoptosis induction in AML cells. *Conclusions*. Their activity against Flt3 and their potent antiangiogenic activity, suggest a potential therapeutic application of AG1296 or similar drugs, in combination with RNAi-induced down-regulation of Flt3 expression, in the treatment of AML involving deregulated Flt3-tyrosine kinase activity.

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0505

AKT AND PKC ARE POSSIBLE REGULATORS OF TELOMERASE ACTIVITY DURING DIFFERENTIATION OF MYELOID LEUKEMIC CELLS

O. Yamada,[1] K. Ozaki,[1] M. Nakadake,[2] M. Akiyama,[3] K. Kawauchi,[4] R. Matsuoka[1]

[1]Tokyo Women's Medical University, TOKYO, Japan; [2]Institut Gustave Roussy, VILLEJUIF, France; [3]Jikei University School of Medicine, TOKYO, Japan; [4]Tokyo Women's Medical University Medical Center East, TOKYO, Japan

Background. Telomerase is active in immature somatic cells and tumor cells, but is suppressed in differentiated cells. Several mechanisms of telomerase regulation, including transcriptional, translational, and post-translational mechanisms, have been reported, suggesting that the regulation of telomerase activity is a complex process. *Aims.* To determine the mechanisms modulating telomerase activity during granulocytic and monocytic differentiation of hematopoietic cells. *Methods.* A human acute myeloblastic leukemia cell line (HL60) was induced to undergo monocytic differentiation by exposure to VD3, while granulocytic differentiation was induced by exposure to ATRA or Am80 (an RAR-α/β-selective retinoid that does not bind and activate RAR-γ and RXRs). Changes of several signaling proteins during differentiation were examined. Telomerase activity, expression of human telomerase reverse transcriptase (hTERT) protein and mRNA, and epigenetic factors within the telomerase promoter region were also examined. *Results.* Rapid down-regulation of telomerase transcription occurred during early differentiation of HL60 cells into both lineages prior to G1 arrest. Akt kinase activity was transiently suppressed after 6 hours of differentiation along with inhibition of telomerase activity. Because significant suppression of Akt activity occurred while telomerase protein expression was maintained, the post-translational regulation of telomerase activity was suggested. Each of the three differentiation agents caused a significant increase of signaling proteins (including Akt, PKC-α, mTOR, Rictor, and p21) and a decrease of bcl-2 at 3 days after the initiation of differentiation. To further assess the transcriptional regulation of telomerase, a chromatin immunoprecipitation (ChIP) assay was performed. This showed that acetyl-Histone H4, which binds to the hTERT promoter, underwent deacetylation during differentiation. *Conclusions.* There was a decrease of telomerase activity and hTERT (protein and mRNA) expression during granulocytic and monocytic differentiation stimulated by ATRA and Am80 or VD3, respectively. Active forms of Akt, PKC-α, mTOR, and mTOR-associated Rictor protein showed an increase at 3 days after the induction of differentiation. It has been reported that Akt promotes the transcription of hTERT and post-translational activation of telomerase. Modulation of acetyl-Histone H4, which regulates transcription of the telomerase gene, was observed before the activation of Akt occurred at 3 days after the start of differentiation, which suggests that epigenetic control of telomerase transcription takes place before Akt activation occurs during differentiation. PKC was also reported to activate telomerase post-translationally, and recombinant PKC-α caused a dose-dependent increase of telomerase activity in HL60 cells. In differentiated cells, telomerase protein disappeared before the activation of PKC-α occurred. These results indicate that telomerase activity is regulated by at least two mechanisms during granulocytic and monocytic differentiation, with one being transcriptional and the other being post-translational and involving Akt and PKC.

0506

TAP- AND PROTEASOME-DEPENDENT ENDOGENOUS ANTIGEN LOADING OF HLA CLASS II IN AML BLASTS INTRODUCES 'REVERSE' CROSS-PRESENTATION AND A PROMISING NEW TARGET FOR GENERATING LEUKEMIA-SPECIFIC CD4+ T CELLS

M.M. van Luijn,[1] M.E. Ressing,[2] E.J. Wiertz,[2] S. Ostrand-Rosenberg,[3] Y. Souwer,[4] G.J. Ossenkoppele,[1] A.A. Van de Loosdrecht,[1] S.M. Van Ham[4]

[1]VU Medical Center, AMSTERDAM, Netherlands; [2]Leiden University Medical Center, LEIDEN, Netherlands; [3]University of Maryland, BALTIMORE, USA; [4]Sanquin Research and Landsteiner Laboratory, AMSTERDAM, Netherlands

Background. According to classical HLA class II antigen loading, exogenous antigens are processed in the endosomal/lysosomal pathway and associate with HLA class II after exchange with the class II-associated invariant chain peptide (CLIP). For this, the Invariant Chain (Ii) is required for targeting of HLA class II to the lysosomes. We recently showed that Ii knock-down in AML blasts resulted in a decline in CLIP amount per HLA-DR molecule (Van Luijn *et al.*; Abstract EHA 2008). Absolute HLA-DR amount was also lowered on these blasts, which is in line with the need of Ii for exogenous antigen loading on HLA class II. In other types of AML blasts however, Ii knock-down did not affect HLA-DR expression. *Aims.* To elucidate the Ii-independent pathway of HLA class II-restricted antigen presentation in AML. *Methods.* Ii expression was silenced in HLA-DR+ KG-1 and Kasumi-1 AML cell lines by RNA interference. Both cell lines were additionally transduced with UL49.5, a BHV-1-encoded protein that blocks peptide transport into the endoplasmic reticulum (ER) by interfering with the function of the transporter associated with antigenic processing (TAP). After FACS-sorting of TAP-deficient HLA-DR- and HLA-DR+ cell populations, Ii expression was determined by flow cytometry. Furthermore, MG-132 was used to abrogate proteasome activity and confirm endogenous antigen processing. *Results.* Ii silencing in the Kasumi-1 cell line lead to a drastic decline in HLA-DR expression on the plasma membrane (13.9-fold decrease in MFI). Surprisingly, HLA-DR levels on KG-1 cells were hardly affected by Ii silencing. As HLA-DR expression does require peptide binding, Ii-independency may be achieved by endogenous antigen loading. To test this hypothesis, supply of endogenously derived peptides into the ER was blocked by viral proteins that interfere with the function of TAP. In line with previous findings, TAP inhibition in KG-1 cells by UL49.5 (which degrades TAP and changes it conformation) resulted in a significant down-regulation of HLA class I (7.7-fold MFI decrease). Most interestingly, this modulation also lead to a clear HLA-DR- (52.3%; MFI=1.4) KG-1 population next to HLA-DR+ (36.5%; MFI=288.9) KG-1 cells. Upon sorting of both populations, TAP-deficient HLA-DR- and HLA-DR+ cells contained a respectively lower (2.4-fold MFI decrease) and higher (3.0-fold MFI increase) amount of intracellular Ii as compared to wild type cells. Moreover, silencing of Ii expression in TAP-deficient HLA-DR+ cells caused a strong decline in HLA-DR expression, both intracellularly (1.5-fold MFI decrease) and extracellularly (2.3-fold MFI decrease). Finally, specific inhibition of the proteasome also resulted in a marked down-regulation of HLA-DR expression on KG-1 cells in contrast to Kasumi-1. At the less toxic concentration, MG-132 treatment caused a 2.1- and 2.7-fold MFI decrease, respectively. This confirms that a least part of the antigens presented by HLA class II in KG-1 are derived from endogenous sources. *Summary and Conclusions.* These data reveal a TAP- and proteasome-dependent 'reverse' cross-presentation pathway of endogenous antigens on HLA class II molecules. Modulation of this pathway in AML blasts might enhance HLA class II-restricted presentation of leukemic antigens, leading to leukemia-specific activation of CD4+ T effector cells.

0507

DISRUPTION OF TGF-β SIGNALING IN ACUTE PROMYELOCYTIC LEUKEMIA DEPENDS ON NUCLEAR PML FUNCTION

L.L. Figueiredo-Pontes, B.A.A. Santana-Lemos, R.H. Jácomo, A.S.G. Lima, A.I. Dore, F.U. Ferreira, A.A. Goes, R.A. Panepucci, D.T. Covas, A.M. Fontes, R.P. Falcao, E.M. Rego

Medical School of Ribeirao Preto, RIBEIRAO PRETO, SAO PAULO, Brazil

PML is a tumor suppressor that, among its physiological roles, acts as a regulator of the TGFβ pathway. Recently, Lin, Bergman and Pandolfi have demonstrated that Pml-null mouse embryonic fibroblasts are resistant to TGFβ-dependent growth arrest, apoptosis and cellular senescence. In the absence of PML, induction of TGFβ target genes and phosphorylation of TGFβ-signaling proteins Smad2 and Smad3 are impaired. Restoration of a cytoplasmic isoform of PML (cPML) in these cells reverts TGFβ defects, suggesting that it is an essential modulator of TGFβ signaling. In acute promyelocytic leukemia (APL), the expression of the PML-RARα oncoprotein leads to PML delocalization and functional impairment. Therefore, deregulation of TGFβ pathway may play a role in APL pathogenesis. However, PML-RARα is expressed mainly in the nucleus, and its effect on TGFβ and TGFβ-target genes transcription is unknown. In this context, our aim was to study the role of nuclear PML-RARα in TGFβ signaling. A conditional retroviral model in which PML-RARα expression is regulated by doxycycline was generated in the NIH/3T3 fibroblastic murine lineage. In this model, PML-RARα full-length cDNA was cloned in a retroviral vector containing a tetracycline-responsive element. Both the responsive recombinant vector and a regulatory vector containing the reverse tetracycline transactivator were transfected in the EcoPack293 packaging cell line. The resulting virus-containing supernatants were used to serially infect NIH/3T3 target cells, which were then treated and selected with vector resistant antibiotics. After recovery, transduced NIH/3T3 cells were submitted to treatment with doxycycline (2000ng/mL) during 72 hours. Total RNA was obtained and reverse transcribed. Real Time PCR for the expression of PML-RARα, Tgfβ and Smad3 was performed. Results showed that doxycycline induced an eight fold increase of PML-RARα gene expression. In the presence of PML-RARα, the expression of Tgfβ and its mediator and target Smad3 was repressed, thus suggesting that the transcription of TGFβ is PML-RARα dependent. Western blot analysis of Tgfβ expression in the nuclear fraction of doxycycline-treated and untreated controls revealed no difference. Therefore, PML-RARα oncoprotein can antagonize not only cPML but also nuclear PML (nPML) function leading to disruption of TGFβ signaling in both nucleus and cytoplasm. We can hypothesize that, in the nucleus, PML-RARα may interrupt the interaction of nPML with Smad2/3/4 complex leading to blockade of TGFβ transcriptional activity. Although the disruption of TGFβ pathways itself is not sufficient to initiate malignant transformation, it may be a critical second step that contributes to leukemia progression. In this context, the modulation of TGFβ signaling may have therapeutic interest in APL.

0508

POLYMORPHISMS OF THE DNA REPAIR GENE XRCC1 (399) AND XPD (751) CORRELATES WITH RISK OF ACUTE MYELOID LEUKEMIA IN TURKISH POPULATION

A. Ozcan,[1] M. Pehlivan,[2] E. Karaca,[1] C. Ozkinay,[1] F. Ozdemir,[1] S. Pehlivan[3]

[1]Ege University, IZMIR; [2]Gaziantep University department of hematology, GAZIANTEP; [3]Gaziantep University Department of Medical Biology, GAZIANTEP, Turkey

Background. The DNAs of all organisms are continuously under the threat of internal and external factors causing DNA damages. The aim of DNA repair mechanism is to protect genome integrity. Polymorphisms that occur in DNA repair genes affect DNA repair capacity and constitute a risk factor in hematological malignities. X-ray repair cross-complementing group 1 (XRCC1) and Xeroderma pigmentosum complementation group D (XPD), the DNA repair genes, work in the base excision repair (BER) mechanism and the nucleotide excision repair (NER) mechanism. Polymorphisms of XRCC1 gene codon 399 and XPD gene codon 751 in polymorphic regions affect DNA repair capacity. *Methods.* In this study, X-ray repair cross-complementing group 1 (XRCC1) gene codon 399 and Xeroderma pigmentosum complementation group D (XPD) gene codon 751 polymorphisms were researched by the polymerase chain reaction-enzyme cutting (PCR-RFLP) method in 100 patients with hematological malignity (HM) (36 acute myeloid leukemia [AML], 64 lymphoid malignities [LM]) and 100 healthy controls and the distributions of genotypes and allels were compared in patient and control groups. *Results.* 46 of the patients with HM are females while 54 of them are males and the median age is 46 (16-82). When the genotype and allel frequencies among the HMs and healthy controls, XPD-751Gln variant, were arranged and compared according to age and sex, it was detected that Gln/Gln genotype, reported as a protector, decreased significantly in AML (p=0.042) and that there was no relation between allel frequencies (p=0.054). In XRCC1-399, it was detected that Gln/Gln genotype decreased significantly in AML (p=0.014) and in all HMs (p=0.033) and that Gln allel was present at a lower ratio in AML (p=0.046). No statistical significance was found according to age and sex in the distribution of polymorphisms of both genes. In leukemias with early relapse, XPD 751 Lys/Lys genotype was observed to be at a statistically higher ratio (p=0.042). When both genes were evaluated togeth-

er, the presence of a relationship among MHs was detected upon a decrease in Gln/Gln+Lys/Gln haplotype frequency (p=0.048). *Conclusions.* With this study, it was put forth that a decrease in Gln/Gln genotype and Gln allel reported to be a protector among XRCC1 codon 399 and XPD codon 751 polymorphisms in AML and an increase in Lys/Lys genotype in acute leukemias were related to early relapse.

0509

PROGNOSTIC SIGNIFICANCE OF MOLECULAR MARKERS IN NORMAL KARYOTYPE -AML: A SINGEL CENTER EXPERIENCE

O. Spinelli, M. Tosi, V. Guerini, P. Zanghì, M. Franchi, I. Taboni, M. Magri, C. Zanotti, T. Intermesoli, E. Oldani, R. Bassan, A. Rambaldi

Ospedali Riuniti, BERGAMO, Italy

Background. Cytogenetic alterations well define the outcome of Acute Myeloblastic Leukemia (AML). In Normal Karyotype (NK) AML other molecular markers are needed to predict the prognosis and to drive clinical decisions. In the last decade some molecular markers have been proposed, but the clinical significance of some of them is still not well defined. *Aims*. To verify the clinical significance of the presence or absence of molecular markers in predicting the outcome of NK-AML patients enrolled in sequential clinical studies in our center. *Methods*. Diagnostic samples derived from 105 NK-AML patients (age<60y) enrolled in 4 sequential clinical study (STT, BXIII. BXIV, LAM2000) were analyzed for FLT3-ITD, FLT3-TKD, NPM1, WT1 (exon 7 and exon 9), MLL-PTD and CEBPA mutations. *Results*. The prevalence of each mutation was the following: FLT3-ITD 32% (30/94), FLT3-TKD 7% (7/97), NPM1 49% (44/90), WT1 9% (7/77), MLL-PTD 6% (3/53), CEBPA 16% (8/51). The CR rate of this group of patients was 88%. With a median observation time of 21,1 months the Overall Survival (OS) and Disease Free Survival (DFS) of this group of NK-AML patients were 40% and 43%, respectively. By univariate analysis, OS was decreased in the presence of FLT3-ITD (26% *vs* 48%, p=0.015) while it was apparently increased by the presence of CEBPA mutation (100% *vs* 44%). Patients carrying WT1 gene mutations showed an inferior OS (28% compared to 43% of WT1 negative patients) but this difference did not reach a statistical significance (p=0. 4). The DFS was significantly inferior in FLT3-ITD positive patients (30% *vs* 51%, p= 0.006) and a 75% DFS was seen in CEBPA positive patients compared to 48% in CEBPA negative patients (p= 0.2). The probability to achieve a CR was superior in NPM1 positive patients (93% *vs* 78%, p=0.04) while no significant difference was associated with the presence of other mutations. By multivariate analysis (CEBPA and MLL-PTD were excluded for the limited number of patients), the presence of FLT3-ITD independently predicts a worse DFS (HR 2.19, CI 1.03-4.66, p=0.041). On the contrary, the presence of NPM1 mutation predicts a better CR rate (OR 6.17, CI 1.01-37.52, p= 0.048) and showed also a positive trend for a better DFS (HR 0.48, CI 0.22-1.06, p= 0.07) and a better OS (HR 0.54, CI 0.26-1.1, p=0.09). The presence of WT1 gene mutations was associated with a worse OS (HR 2.8, CI 0.86-9.5, p= 0.08). *Conclusions*. In a cohort of NK-AML patients, sequentially treated with intensive chemotherapy protocols in a single center, we confirmed the adverse prognostic impact of FLT3-ITD and WT1 gene mutations, and the favorable outcome associated with CEBPA and NPM1 gene mutations. All these markers should be identify in each case in order to better define the prognostic profile of each patient and the best post remission consolidation treatment strategy.

0510

PTK 787/Z222485, A POTENT INHIBITOR OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR TYROSINE KINASE PHOSPHORYLATION, MIGHT CONSTITUTE A NEW EFFECTIVE THERAPY TO ACUTE MYELOID LEUKEMIA

N. Barbarroja,[1] L.A. Torres,[2] M.J. Luque,[2] R.M. Carretero,[2] A. Torres,[3] F. Francisco,[4] Ch. López-Pedrera[2]

[1]Hospital Reina Sofia, CÓRDOBA; [2]Unidad de Investigacion, CORDOBA; [3]Servicio de Hematología, CORDOBA; [4]Servicio de Hematologia, CORDOBA, Spain

Angiogenesis plays an important role in the pathogenesis of acute myeloid leukemia (AML) and the vascular endothelial growth factor (VEGF) is a crucial, positive regulator of this process. The biological activity of VEGF is mediated by two different receptor tyrosine kinases: VEGFR-1/Flt-1 and VEGFR-2/KDR. Many studies have shown that high levels of VEGF protein are correlated with short survival and poor prognostic of patients with AML. This fact has encouraged the study of specific inhibitors of VEGF and its receptors as alternative therapy. Currently, new antitumoral drugs inhibitors of VEGFRs activity are being analyzed. PTK787/ZK 222584 is an oral angiogenesis inhibitor targeting vascular VEGF receptor tyrosine kinases, including VEGFR-1, VEGFR-2, VEGFR-3/Flt-4, the platelet-derived growth factor receptor tyrosine kinase and the c-kit protein tyrosine kinase. Clinical trials have demonstrated the efficiency of this compound in the cell proliferation inhibition and in the induction of the cell apoptosis in various solid tumours. However, there are still unknown its effects in AML blast. *Objective*. To investigate the role of the VEGFRs inhibitor PTK787/ZK222584 on cell proliferation, survival and angiogenesis in AML cells, and the effect of the combined treatment with a chemotherapeutic drug, idarrubicin. *Material and Methods.* Four AML cell lines (MV4-11, MOLM-13, NB4 and THP-1) were treated with PTK787 (2.5, 5, 10, 20 and 40 μM), given alone or combined with idarrubicin (2 ng/mL) for 24 and 48 hours. Then, cell apoptosis was analyzed by flow cytometry, and cell proliferation was detected using an XTT colorimetric assay. The effects on the activation of VEGFRs and several intracellular pathways (ERK, Akt and STAT5) were studied by western blot. The VEGF levels in the cellular supernatants were evaluated by ELISA assay. *Results.* PTK787 reduced the cell proliferation and induced apoptosis in a dose-dependent manner in the four cell lines. At the molecular level PTK787 inhibited the constitutive activation of ERK, Akt and STAT5. These effects were stronger in MV4-11 and MOLM-13 cells. Moreover, PTK787 reduced the VEGF expression levels and inhibited the phosphorylation of VEGFRs. On the other hand, the combined treatment with PTK787 and idarrubicin promoted an additive effect in the induction of cellular apoptosis, inhibition of cell proliferation and reduction of VEGF expression. *Conclusions.* Our overall results encourage the use of PTK787/ZK 222584 in association with low doses of chemotherapic agents in clinical trials for the treatment of AML.

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0511

CHOLESTEROL-RICH DOMAINS REGULATE FLT-1 ACTIVATION SIGNALS IN ACUTE MIELOID LEULKEMIA CELLS

C. Casalou, J.F. Moura Nunes, S. Dias

Portuguese Institut of Oncology, LISBON, Portugal

Cholesterol-rich domains (i.e. lipid rafts and caveolae) have been implicated in several cellular processes. By concentrating several molecular components they function as platforms for cell signaling. Caveolin-1, the major protein component of caveolae, has been implicated in intracellular cholesterol transport. We have found that vascular endothelial growth factor receptor-1 (FLT-1) and caveolin-1 mRNA expression levels are up-regulated by PLGF/VEGF binding in AML cells. Also, FLT-1 mRNA expression level is also regulated by cellular-cholesterol content. Increased cellular cholesterol levels on AML cells up-regulate FLT-1 and caveolin-1 expression. Moreover, FLT-1 protein phosphorylation is abolished by treatment of AML cells with β-cyclodextrin, a cholesterol depletion agent or by nystatin, an inhibitor of lipid raft endocytosis. Co-localization FLT-1 with caveolin-1 in AML cells is also affect by cholesterol cellular content. In addition, by sucrose-gradient fractionation we have found that FLT-1 co-sediments with lipid-raft protein components together with Rac-1 and Hsp90 proteins. These results show that on leukaemia cells FLT-1 Is associated with lipid rafts/caveolae and this association is crucial for FLT-1 expression and signaling on acute leukaemia cells. Furthermore, a soluble portion of caveolin-1 was found in the subcellular-fractionation of AML cells and by immuno-electron microscopy we localized caveolin-1 primarily inside mitochondria of PLGF-stimulated leukemia cells. Analysis of mitochodrial extracts showed that cholesterol depletion removes caveolin-1 from mitochondria and by opposition, a large acumulation of caveolin-1 inside leukemia cell mitochondria was found after increased cellular cholesterol levels. These studies suggest a regulation of cholesterol intracellular-trafficking exerted by FLT-1-activation after VEGF/PLGF stimulus and implicate an unexpected role for this signaling pathway in mitochondria function (i.e. aerobic metabolism).

0512

VASCULAR ENDOTHELIAL GROWTH FACTOR PLAYS AN IMPORTANT ROLE FOR THE RECOVERY OF NORMAL HEMATOPOIESIS AFTER CHEMOTHERAPY IN ACUTE MYELOGENOUS LEUKEMIA CASES

H. Haruko, M. Noguchi, R. Shirasaki, T. Sugao, Y. Oka, K. Kawasugi, Y. Akiyama, N. Shirafuji

Teikyo University School of Medicine, TOKYO, Japan

Objective. The serum vascular endothelial growth factor (VEGF) level is decreased in aplastic anemia cases. We investigated VEGF system at the onset and after chemotherapy of acute myelogenous leukemia (AML) cases, and determined whether VEGF system influenced the prolonged bone marrow suppression observed in some of these cases. *Materials and Methods.* Sera and bone marrow cells were prepared from 40 informed AML patients including 12 cases of AML (M3) at the onset of the disease, and the recovery periods after chemotherapy including the prolonged hematopoietic suppression periods observed in some of the cases, and the concentration of VEGF in sera of the patients and in the conditioned media obtained from non-adherent bone marrow-cell cultures was measured with ELISA kit (Quantikine; R&D Systems). The expression of VEGF, VEGF receptor type-1 (VEGFR-1) and VEGF receptor type-2 (VEGFR-2) was analyzed with RT-PCR. The biological effect of VEGF on the bone marrow cells which showed the prolonged suppression after chemotherapy was analyzed with colony-formation assay. *Result and Discussion*. As was reported previously, VEGF levels were significantly increased in all of M3 cases. In other types of AML cases the levels of VEGF varied. When patients were given chemotherapy and the sustained bone marrow suppression was observed, the production of VEGF was significantly decreased less than that observed in AML cases with normal bone marrow recovery. In M3 cases that were treated with all-trans retinoic acid and the prolonged bone marrow-suppression was observed, VEGF production was also significantly suppressed. In these sustained suppression cases, when normal hematopoiesis was observed after the long duration, VEGF levels were recovered to be increased. The expression of VEGFR-2 was demonstrated in bone marrow cells from prolonged bone marrow suppression cases; however, the expression of VEGFR-1 was not observed. In these cases, when bone marrow cells were cultured with VEGF, synergistic effects with G-CSF, EPO and Stem Cell Factor and FLT-3 Ligand were observed with colony-formation assay. These observations indicate that VEGF works on the important role for the normal hematopoietic recovery after chemotherapy in AML cases.

0513

PROTEOMIC MODIFICATIONS INDUCED BY VALPROIC ACID IN AML1/ETO POSITIVE LEUKEMIA CELLS

V. Santini,[1] F. Buchi,[2] G. Ferrari,[2] E. Spinelli,[2] A. Gozzini,[2] T. Lunghi,[2] A. Bosi[2]

[1]University of Florence, FIRENZE; [2]AOU Careggi, University of Florence, FLORENCE, Italy

Background. Alterations in chromatin organization are a common mechanism in leukemogenesis. Many drugs affecting epigenetic modulation of gene expression have been proposed as therapy of haematopoietic neoplasms, unfortunately frequently without selection on a molecular basis of disease types more prone to respond to these agents. *Aims.* On the basis of our previous findings, we think that HDAC inhibitors could be remarkably effective in the treatment of some forms of acute myeloid leukemias, i.e. CBF-AML. *Methods.* We analysed the effects of the short chain fatty acid derivative valproic acid (VPA 2mM) on the proteome of AML1-ETO-positive, AML blast cells. We also analysed the differences in response to VPA shown by AML1/ETO-inducible U937-A/E-9/14/18 cells, with and without expression of AML1/ETO protein. VPA is suitable for oral administration and is the HDACi most easily available for clinical use in Europe. Total cell protein extracts of treated and untreated AML1-ETO-positive cells were separated by two dimensional electrophoresis (2DE) on non-linear pH gradient 3-10. The 2D gels were analysed by adequate software (Image Master TM Platinum), for spot detection and quantification. 2D gels were virtually superimposed and aligned for proteomic comparison. *Results.* Significant differences both qualitative and quantitative in protein spots appeared between treated and untreated cells. Moreover, spot differences were increasing throughout the period of culture (24-72 hours) in the presence of VPA and subsequent histone acetylation (determined by western blot), inhibition of proliferation (cell cycle analysis, MTT test) and apoptosis (annexin -V expression). MALDI-TOF MS analysis (PMF and/or MS/MS experiments) of the most representative protein spots indicated that the correspondent identified proteins may be divided into functional categories: DNA binding/signal transduction proteins, metabolic enzymes, heat shock proteins and chaperones. Proteins induced by VPA were: enolase-1, laminin -binding protein Siah-interacting protein (SIP). VPA significantly enhanced the expression of the following proteins: nucleophosmin, EEF1D protein, stathmin 1, activator of HSP-90 ATPasi, tumor rejection antigen (gp 96). VPA inhibited the expression of cofilin-1 and calreticulin precursor variant , as well as that of proteasome subunit alpha and beta. *Conclusions.* There is growing evidence of the importance of nucleus-cytoplasm trafficking in neoplastic cells. Therefore, the identification of a significant modulation by VPA of proteins regulating cell trafficking and of chaperone proteins indicates a fundamental role of this agent in affecting the function of AML1/ETO positive leukemic cells. Moreover, common proteins with different spot distribution were identified, leading to the hypothesis of the presence of different isoforms or post translational modifications of the same protein. The effects of VPA, like other HDACi, appeared significant in AML1-ETO-positive cells where the stability of DNMT/HDAC repressor complex binding to DNA was reduced, strongly suggesting to consider therapy with HDACi for AML molecular subtypes, such as CBF-AML, in which chromatin-organizing proteins are involved in the pathogenesis of disease. Thus, the treatment with HDACi, possibly in combination with hypomethylating agents, as we also observed, may specifically influence the natural history of leukaemia by interfering with the molecular mechanisms of leukaemogenesis.

0514

PROTEOMIC MODIFICATIONS INDUCED BY AZACITIDINE IN AML1/ETO POSITIVE LEUKEMIA CELLS

V. Santini,[1] F. Buchi,[2] G. Ferrari,[2] E. Spinelli,[2] T. Lunghi,[2] A. Gozzini,[2] A. Bosi[2]

[1]University of Florence, FIRENZE; [2]AOU Careggi, University of Florence, FIRENZE, Italy

Background. Alterations in chromatin organization are a common mechanism in leukemogenesis. Many drugs affecting epigenetic modulation of gene expression have been proposed in clinics, mostly without selection on a molecular basis of disease types more prone to respond to these agents. On the basis of our previous findings, we think that some forms of acute myeloid leukemias, i.e. CBF-AMLs, could be specifically sensitive to DNMT inhibitors AZA has been shown to prolong survival in MDS and has been used also in AML therapy. *Aims.* We analysed the effects of the hypomethylating agent azacitidine (AZA 1uM) on the proteome of AML1-ETO-positive, AML blast cells. We also analysed the differences in response to AZA shown by AML1/ETO-inducible U937-A/E-9/14/18 cells, with and without expression of AML1/ETO protein. *Methods.* Total cell protein extracts of treated and untreated AML1-ETO-positive cells were separated by two dimensional electrophoresis (2DE) on non-linear pH gradient 3-10. The 2D gels were analysed by adequate software (Image Master TM Platinum), for spot detection and quantification. 2D gels were virtually superimposed and aligned for proteomic comparison. Methylation of p15 promoter (methylation specific-PCR), histone acetylation (Western blot), inhibition of proliferation (cell cycle analysis,) and apoptosis (annexin-V expression) were evaluated in parallel. *Results.* AZA inhibited cell proliferation and induced significantly higher levels of apoptosis in AML1-ETO expressing cells. At proteome analysis, significant differences both qualitative and quantitative in protein spots appeared between AZA treated (24-72 hours) and untreated cells. Total spots number was 620 in untreated cell lysates, and 486 in AZA treated. 400 spots were matching, and MS identified 31 proteins with different level of expression in treated *vs* untreated cells. MALDI-TOF MS analysis (PMF and/or MS/MS experiments) of the most representative protein spots indicated that the correspondent identified proteins could be divided into 4 functional categories: DNA binding/signal transduction proteins, metabolic enzymes, heat shock proteins and chaperones, structural proteins. Proteins induced by AZA were: calmodulin and Cypa /Hvgpia. AZA significantly enhanced the expression of the following proteins: NM23a , hPCNA, both DNA binding proteins and the structural protein muscle Z-line. AZA inhibited the expression of ER60 protease, VCP protein, proline-4-hydroxylase. Heat shock proteins like HSP90, 60, 70 gp96, but also tubulin and enolase 1 had different spot distribution in AZA treated lysates *vs* untreated, indicating post translational modifications induced by the hypomethylating agent. Western blots with specific antibodies for single identified proteins were performed to confirm quantitative and qualitative results. *Conclusions.* We observed a significant modulation by AZA of proteins regulating cell trafficking and of chaperone proteins. The role of these proteins in maintaining the transformed phenotype in AML1/ETO positive leukemic cells has to be ascertained, because indeed the characterization of their activity could be important in identifying markers of sensitivity to DNMTinhbitors.

Acute myeloid leukemia - Clinical II

0515

SAFETY AND EFFICACY EXPERIENCE OF SNS-595 IN RELAPSED/REFRACTORY ACUTE LEUKEMIA PATIENTS = 60 YEARS OLD COMPARED TO < 60 YEARS OLD: RESULTS OF A PHASE 1 STUDY

C Michelson,[1] J. Lancet,[2] H. Kantarjian,[3] F. Ravandi,[3] F. Giles,[4] G. Michelson,[1] J. Karp[5]

[1]Sunesis Pharmaceuticals Inc, SOUTH SAN FRANCISCO; [2]H Lee Moffitt Cancer Center, TAMPA; [3]M. D. Anderson Cancer Center, HOUSTON; [4]UT-San Antonio, SAN ANTONIO; [5]Johns Hopkins Sidney Kimmel Cancer Center, BALTIMORE, USA

SNS-595 is a novel naphthyridine analog, a subclass of quinolones not previously used for the treatment of cancer. SNS-595 has a specific, saturable interaction with DNA and is a topoisomerase II poison, causing replication-dependent site-selective double strand DNA damage, irreversible G2 arrest and rapid apoptosis. SNS-595 is not a substrate for P-glycoprotein, thereby evading a common drug-resistance mechanism, and has low potential for CYP450-mediated drug-drug interactions. SNS-595 is in phase 1 and 2 clinical trials in acute myeloid leukemia and ovarian cancer, with clinical responses in these indications as well as in NSCLC and SCLC. A phase 1 dose escalation study was recently completed (Proc ASH 2007) and the MTD on the weekly schedule (qw) was 72 mg/m^2 and on the twice weekly schedule (biw) was 40 mg/m^2. A review of the safety and efficacy data by age group is presented. *Methods.* SNS-595 was administered as a slow IV push on days 1, 8, 15 (qw) or days 1, 4, 8, 11 (biw). Patients were assessed at least weekly for safety and hematologic recovery. *Results.* Across both schedules, 70 patients (pts) were enrolled and are evaluable. 27 pts were < 60 years old (yo) (median=43 yo; range: 21-59 yo) and 43 pts were ≥60 yo (median=69 yo, range: 60-85 yo). Pt demographics were comparable between the two treatment schedules: most pts were males (63%) and white (74%), ECOG PS=0 or 1 (91%), and had relapsed or refractory AML (83%). For the 27 pts who were < 60 yo, the most common grade 3 or higher AEs were: febrile neutropenia (31%), thrombocytopenia (19%), neutropenia (15%), and stomatitis (15%). Similarly, among the 43 pts who were ≥60 yo, the most common grade 3 or higher AEs were: febrile neutropenia (31%), neutropenia (24%), thrombocytopenia (21%), and stomatitis (12%). The DLT for both schedules was oral mucositis with similar incidence of stomatitis among those < 60 yo and ≥60 yo. There were 5 total CR/CRps across both schedules: 4 CR/CRps in the qw schedule (2 were in pts < 60 yo, 2 were in pts ≥60 yo); and 1 CRp in a pt on the biw schedule ≥60). In addition, there was one CRi in the ≥60 yo group. All the CR/CRps in the qw schedule had previously failed anthracyclines and cytarabine and had intermediate or unfavorable cytogenetics. Linear pharmacokinetics was observed across both patient age groups and antileukemic activity was associated with time above a 1 μM threshold. *Conclusions.* SNS-595 appears to be generally well tolerated in pts with advanced leukemias in pts ≥60 yo and <60 yo. Complete remissions have been observed in both age groups. Given this safety profile, a single agent study of SNS-595 as front line treatment for AML pts ≥60 yo is underway as is a combination study with cytarabine for relapsed/refractory AML in pts ≥18 yo.

0516

EXTRA-MEDULLARY MYELOID TUMOR: A RETROSPECTIVE CLINICOPATHOLOGICAL STUDY OF 32 CASES.

T. Todd, M. Besser, J. Craig, R. Marcus

Cambridge University Hospitals NHS Foundation Trust, CAMBRIDGE, UK

Background. Extra-medullary myeloid tumour (EMMT, also granulocytic sarcoma, myeloid sarcoma or chloroma) is characterized by collections of immature myeloid cells outside the bone marrow. High rates of misdiagnosis have been shown in multiple studies in the previous century although no studies have examined the impact of this on outcome. Current UK guidelines recommend treatment of EMMT in the same fashion as acute myeloid leukaemia but a recent European study (clinical follow up of 67 patients) suggests EMMT has a very poor outcome (long term survival rate 11%) and recommends allogeneic stem cell transplantation as first line therapy in all patients. *Aims*. To assess the current rate of, and reasons for, misdiagnosis, the possible impact of this on patient outcome and survival, and survival in patients not treated with allogeneic transplant. *Methods*. Cases of extramedullary myeloid tumour (EMMT) diagnosed at our institution between 01/01/1997 and 15/06/07 were identified by search of an electronic database. Case notes and pathology for these patients were reviewed. *Results*. 32 patients were identified. 15 (47%) had pre-existing or simultaneously diagnosed myeloid malignancy and 17 (53%) were *de novo* isolated EMMT. Misdiagnosis occurred only in the latter group (10/17, 59%) most commonly as lymphoma (6/10) and sarcoma (2/10). The initial pathology report highlighted difficulty of diagnosis in all cases. Nine of nine EMMT cases tested were strongly positive for MIB1, 6 of 6 for bcl-2, 3 of 3 for vimentin and 2 of 18 for CD79a. All the bcl-2 positive cases were misdiagnosed as Non-Hodgkin's lymphoma and 2 vimentin positive cases as sarcoma. No misdiagnosed case was initially tested for chloroacetate esterase or myeloperoxidase, though in 9/10 subsequent testing showed one or both to be positive. Overall survival for all patients from presentation was 14 months. Patients with lymphadenopathy (n=17) did significantly worse (median survival 8 months *vs* 27 months; log rank test $p<0.01$). There were no long term survivors in whom the mass was >10 cm at diagnosis (n=7) and only 1 long term survivor from 6 patients initially given non-AML chemotherapy. This patient switched to AML therapy within 7 days. 9 (28%, 7 adults, 2 children) cases were long term survivors (median follow up 1640 days). Two of these received local excision and radiotherapy only. The others received intensive AML chemotherapy, and one received allogeneic stem cell transplant. *Conclusions*. Despite older studies highlighting this issue, isolated *de novo* EMMT continues to present considerable diagnostic difficulty. Expression of markers such as vimentin or bcl-2 which are often seen in other malignancies contributes to this. Staining for myeloperoxidase and chloroacetate esterase in cases of diagnostic difficulty of solid tumours or lymphoma is suggested. Lymphadenopathy, mass size >10cm and incorrect initial therapy seem to adversely affect overall survival. Our series, with almost identical median follow up to the study quoted above, has a much higher long term survival rate despite only one patient receiving allogeneic stem cell transplantation. Our data do not support a need for allogeneic transplant as routine first line therapy.

0517

SEQUENTIAL TREATMENT WITH LOW DOSES OF RETINOIC ACID (LOATRA)±VALPROIC ACID(VPA) FOLLOWED BY LOW DOSES ARAC(LODAC) IN ELDERLY ACUTE MYELOID LEUKAEMIA(AML) PRELIMINARY CLINICAL AND BIOLOGICAL RESULTS

S. Fenu,[1] C. Nobile,[2] C. Nervi,[3] A. Chierichini,[1] P. Anticoli-Borza,[1] B. Anaclerico,[1] V. Bongarzoni,[1] R. Bruno,[1] S. Cortese,[1] P. Iacovino,[1] F. Pauselli,[1] C. Tozzi,[1] G. Cimino,[4] L. Annino[1]

[1]AZ. Osp. S.Giovanni-Addolorata, ROME; [2]Campus biomedico university, ROMA; [3]San raffaele Bio-medical Park Foundation, ROMA; [4]Department of cellular Biotechnolgy and hematology university La sapienza, ROMA, Italy

Background . Recent reports indicated the biological therapeutic potentiality and the clinical feasibility of epigenetic treatments with VPA+ATRA or ATRA alone in AML, and suggested their capability in sensitizing leukemic cells to chemotherapy. Therefore, we designed a single centre induction schedule with LoDAC preceded by VPA+LoATRA or LoATRA alone sequential administration to treat AML patients aged >65years or not eligible for intensive therapy. *Aims.*The aims of this study were to evaluate the clinical efficacy (in term of response rate and toxicity) and the biological changes occurring in leukemic blasts in relation to haematological response. *Methods.* From September 2006 to December 2007 we enrolled 14 consecutive AML patients (median age 66years, range: 44-81); of these 7 were *de novo* AML, 2 secondary AML and 5 were in ≥1 relapses. At treatment onset BM blasts ranged from 30% to 81%. As cytogenetics 6 patients had normal karyotype, 4 a complex one and 4 presented other abnormalities (Dup1, t(2;13), t(8;21), -y). Induction schedule consisted of VPA (in escalating dosage from 10 mg/kg/die to 30 mg/Kg/die) d1-55, LoATRA 25 mg/m^2 d7-55; LoDAC 40 mg t.d./die s.c. d10-16 and d45-51. Therapy was repeated every 20 days up to 3 cycles. Five patients (2=*de novo*; 3=relapsed) received VPA+LoATRA+LoDAC whereas 9 patients (5=*de novo*; 2=sAML ;2=relapsed) received LoATRA+LoDAC because of concomitant dysmetabolic(6) and psychiatric diseases(3). PB and BM samples for biological studies (morphology, cytochemistry, immunophenotype, cell cycle and apoptosis, histone acetylation status, gene expression analysis) were sequentially collected at days 0,7,14,28,35,55. Response was evaluated after the first cycle according to IWG criteria. *Results.*Haematological response was observed in 8 patients: 5 achieved complete remissions, 2 partial remissions and 1 minor haematological improvement. Of the remaining 6 pts: 1 died during induction and 5 were no responders. Thus the Overall Response Rate(ORR) was 50%. In the 8 responder patients,

PB recovery (Hb>9g/dL;PMN>1000/m3and PLTS>50.000/m^3) and BM blasts clearance occurred at d42 (range35-50) and at d35 (range25-50), respectively. No patient had extra-haematological toxicities WHO grade 3 or 4, and in all cases treatment was applied on out-patient basis. Preliminary results of biological assays showed a significant difference in responder patients *vs* no responders occurring before LoDAC administration: 1)increasing rate of S phase cells (p=0.02 at d+7 and p=0.03 at d+14); 2)cytochemical changes (increasing MPO/CAE expression); 3)progressive decreasing of early myeloid markers (HLA-DR, CD34, CD117) expression and a concomitantly increasing of late myeloid (CD11b, CD15 or CD14) differentiation markers (Figure1). As of January 2008 of the 8 responder patients, 1 is in 1stcontinuous CR and 2 in PR for +6,+10,+10 months, 5 of them relapsed in a median time of 6 months (range 5-9), two of them achieved a 2nd CR.Seven out of the 14 patients are still alive; median OS was 8 months (range 2-14). *Conclusions*. Sequential VPA and/or LoATRA+LoDAC are feasible, well-tolerated and safe treatment that enabled a 50% of responses. These treatments also induce AML blast phenotypical changes that may increase their sensitivity to LoDAC. However a larger number of cases are required to confirm these results.

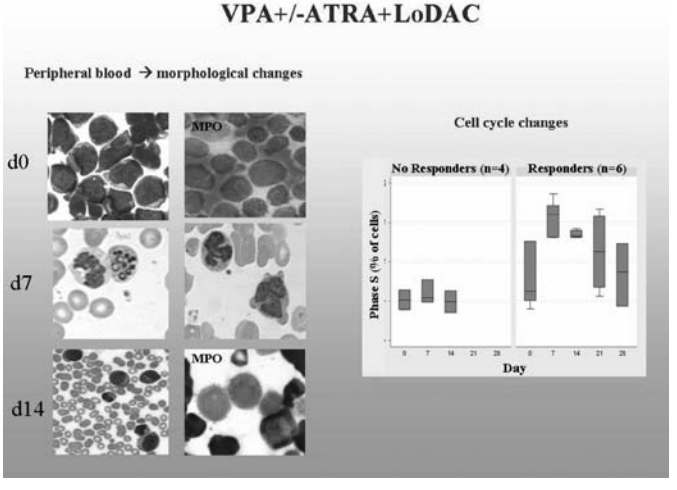

Figure 1.

0518

IN VITRO CHEMOSENSITIVITY OF 'NON LEUKEMIC' CFU-GM, IN AML PATIENTS, IS CORRELATED WITH CD34$^+$ MOBILIZATION AND MAY IDENTIFY GROUPS WITH DIFFERENT DISEASE FREE SURVIVAL

G.S. Sortino, G.M. Milone, G.A. Avola, G.L. Leotta, A.S. Strano, M.G. Camuglia, M. Poidomani, S. Coppoletta, A. Triolo, S. Toscano, M.P. Azzaro

Hematology, CATANIA, Italy

Background. An high number of CD34$^+$ cells in P.B. during mobilization has been associated in AML patients in CR to a high relapse rate and to greater amount of minimal residual disease (Keating, Feller 2003). A different pharmacokinetics of chemotherapy drugs administered during induction or an intrinsic chemoresistence of normal bone marrow precursors have been hypothesized as possible explanations for the observed association between mobilization of non leukemic CD34$^+$ cells and leukaemia residual disease. *Methods.* With this background we assessed in a group of AML in CR the *in vitro* chemosensitivity of non leukemic BM cells to Maphosphamide and Etoposide and correlated it to mobilization strength as well as to DFS. 37 patients affected by AML have been prospectively studied, all were treated using a same induction and consolidation chemotherapeutic regimen. Sensitivity to Maphosphamide and to Etoposide of CFU-GM, BFU-E, CFU-E, CFU-GEMM obtained from bone marrow in 1st CR was studied 2-4 weeks after PBSC mobilization. *Results.* Chemosensitivity of CFU-GM to ASTA-Z as well as to Etoposide and of CFU-GEMM to ASTA-Z, expressed as residual colony growth in comparison to untreated cells, was significantly correlated with peak of CD34$^+$ cells in P.B. during mobilization (R=0.639, p=0.0001 at 75 mcg/mL of ASTA-Z).To study relationship between chemosensitivity of non leukemic CFU-GM and survival we splitted patients in the 3 groups according to their chemosensitivity in respect to normal controls. Survival at 6 months was 0% for hyposensitive group, 60% in normosensitive group and 78% in hypersensitive group (log rank: 0.064). The survival of hyposensitive patients was significantly lower than the remaining patients (log rank: 0.01). Sensitivity of CFU-GM to 100 mcg/mL to ASTA-Z was found important for DFS also when studied in Cox proportional hazard model (Likehood ratio p=0.03). In a stepwise selection, sensitivity to ASTA-Z but not CD34$^+$ peak during mobilization was selected as important for DFS, moreover ASTA-Z sensitivity of normal non leukemic CFU-GM was found important for DFS also in group of AML pts having at diagnosis a normal cytogenetic. *Conclusions.* We have found that in AML patients sensitivity of normal non leukemic CFU-GM to maphosphamide and to Etoposide is highly variable and significantly correlated to CD34$^+$ cells peak reached during mobilization. Chemosensitivity of normal non leukemic CFU-GM was also found to be related to DFS.

0519

LEUKEMIA-FREE SURVIVAL (LFS) AS A SURROGATE FOR OVERALL SURVIVAL (OS) IN AML PATIENTS IN REMISSION (CR): A TRIAL OF A NOVEL IMMUNOTHERAPY WITH HISTAMINE DIHYDROCHLORIDE PLUS LOW-DOSE IL-2 (HDC/IL-2)

M.E. Buyse,[1] P. Squifflet,[1] S.E. Allard,[2] D. Bhagwat,[2] J.M. Rowe[3]

[1]*International Drug Development Institute (IDDI), LOUVAIN-LA-NEUVE, Belgium;* [2]*EpiCept Corporation, TARRYTOWN, NY, USA;* [3]*Rambam Medical Center, HAIFA, Israel*

Background. The goal of cancer therapies is to cure disease and extend OS. However, due to the number of patients and length of follow-up required, OS as a clinical trial endpoint is often impractical. Disease-free survival has been shown to be a valid surrogate for OS in clinical trials of novel therapies for some solid tumors (Buyse *et al.* Biostatistics, 2000 and JCO, 2007). LFS is a well-accepted endpoint in trials of remission maintenance therapies in AML. In such trials, the OS endpoint is particularly exposed to confounding factors that include different post-relapse therapies and unrelated deaths, particularly in older patients. *Aims.* To investigate whether LFS is an acceptable surrogate for OS in AML patients in CR by re-examining data from a clinical trial of remission maintenance immunotherapy with HDC/IL-2 *vs* no active therapy. *Methods.* A randomized multinational trial of 320 adults with AML compared HDC/IL-2 self-administered for up to 18 months to standard-of-care (no treatment) (Brune *et al.* Blood, 2006). A significant benefit of HDC/IL-2 was demonstrated for the primary endpoint of LFS for all patients and for patients in first CR (n=261) (log-rank test p= 0.008 and p= 0.011, respectively). OS was a secondary endpoint, and the corresponding log-rank test P-values were 0.21 and 0.16, respectively. Whether LFS is an acceptable surrogate for OS was assessed by performing weighted linear regression analyses (WLRA) between LFS and OS, and between the estimated effects of HDC/IL-2 on LFS and OS. Coefficients of determination (R^2) quantified the proportion of variance explained by the regression.

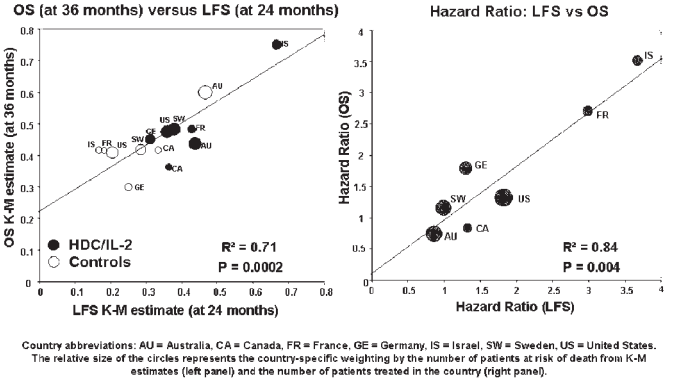

Figure 1.

Results. Country-specific Kaplan-Meier (K-M) estimates of 24-month LFS were highly correlated with 36-month OS in both treated and untreated groups (R^2 = 0.71; p=0.0002) (left panel in Figure 1). The WLRA equation was OS(36) = 0.22 + 0.70 x LFS(24). Similar correlations were found between 24-month LFS and 48-month or 60-month OS. Country-specific hazard ratios (HR), reflecting the effect of HDC/IL-2 on LFS and OS, were also highly correlated (R^2 = 0.84; p=0.004) (right panel in Figure 1) with a WLRA equation HR(OS) = 0.10 + 0.86 x HR(LFS). *Conclu-*

sions. Our analyses confirm that in AML, LFS is strongly associated with OS. Additionally, in this trial of HDC/IL-2 immunotherapy for remission maintenance, the treatment effect on LFS was strongly associated with the treatment effect on OS. Taken together, these observations support the claim that LFS may be a valid surrogate for OS in AML patients in CR.

0520

5-AZACYTINE IMPROVES SURVIVAL IN RELAPSED/REFRACTORY AML, RESULTS OF A MONOCENTRIC ANALYSIS OF 21 CASES

S. Ayari, T. Guillaume, P. Chevallier, E. Brissot, T. Gastinne, M. Mothy, J.L. Harousseau, J.D. Delaunay

University Hospital, NANTES, France

Refractory or relapsed AML have very poor prognosis with a survival of few weeks. 5-Azacytidine, a DNA hypomethylating agent with cytotoxic activity, results in clinical response in high risk MDS and AML. *Patients and Methods*. Since August 2006, 21 patients with refractory/relapsed AML (10F/11M) median age 66 (range 27-80) years received 75mg/m^2 5-azacytidine sc for 7days every 28 days. Relapsed and refractory AML were present in 11 (52%) and 10 (48%) patients respectively, secondary AML in 14 (66%) pts. Median number of chemotherapy cycles prior to 5- azacytidine was 3 (range 1-9). First line therapy was intensive chemotherapy in 12 (57%) patients, three of them received allogeneic transplantation. Low dose cytarabine and other alternative therapy had been received by 5(23%) and 4(19%) cases respectively. Unfavourable and intermediate cytogenetics were observed in 11(52 %) and 10 (48%) pts respectively. Median WBC prior to treatment was 4 (range 1.1-111)×10^9/L. Transfusion dependent anemia and thrombocytopenia were present in 19 (90%) and 14 (66%) pts respectively. Hematologic response was assessed according to the international working group criteria for AML first at 3 cycles of 5-azacytidine and then at 6 cycles or at the end of treatment. Because of early death, two patients (one died from pneumonia and the other from progression of disease) were not evaluable for response. *Results*. Median number of cycles received was 3 (2-14) with a total of 96 treatment cycles performed. Four (21%) pts have achieved response: 1 complete remission and 3 partial responses. Thirteen (68%) pts were in stable disease while 2 (10.5%) progressed. The median number of courses to achieve response was 3 (range 3-6). Tolerance of treatment was acceptable. Infections, including 6 bacteraemia episodes and 3 pneumonias, were the most common non-hematologic complications requiring admission to the hospital with a median days of hospitalisation of 15 (3-34). Median number of erythoid units and platelet units transfused per cycle of treatment was 5 (2-8) and 4 (0-7) respectively. The median follow-up of 11 months. Despite a low rate of response, one year overall survival was 41% (CI 18-62%) with a median survival of 5.8 months for all patients. Durable remission and low toxicity allowed two patients (1CR and 1PR) to proceed to non ablative allogeneic stem cell transplantation with continuous CR at 3 and 8 months following transplantation. The median survival was only 5.4 months in the unfavourable cytogenetics group whereas was not reached in the intermediate cytogenetics one. *Conclusions*. In this report, 5-azacytidine appears to improve overall survival of patients with refractory/relapsed AML despite a low response rate. In addition, pts with intermediate cytogenetics had better outcome.

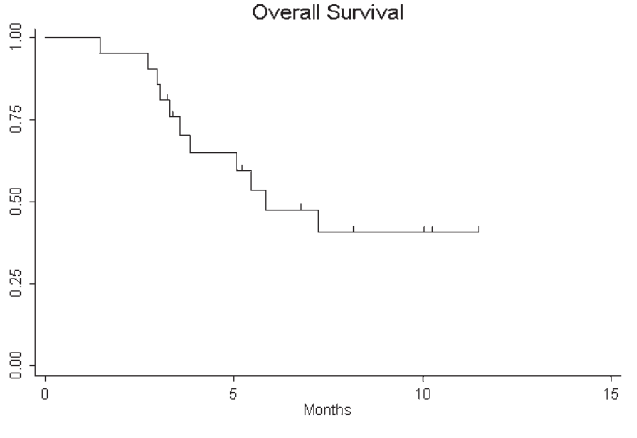

Figure 1.

0521

THE INCIDENCE AND SURVIVAL OF ACUTE DE NOVO LEUKEMIAS IN ESTONIA AND IN A WELL-DEFINED REGION OF WESTERN SWEDEN DURING 1997-2001: A SURVEY OF PATIENTS AGED 16-64 YEARS

L. Wennstrom,[1] E. Holmberg,[1] D. Stockelberg,[1] S. Safai-Kutti,[1] K. Vaht,[1] E. Luik,[2] H. Everaus,[2] K. Palk,[3] M. Varik,[3] I. Viigimaa,[3] J. Kutti[1]

[1]Sahlgrenska University Hospital, GÖTEBORG, Sweden; [2]Tartu University Clinics, TARTU, Estonia; [3]Regional Hospital, TALLINN, Estonia

Estonia regained its independence in 1991 after having been occupied by the Soviet Union for 5 decades. In view of political/socio-economic differences in between Estonia and a neighbouring country, a well-defined Region of Western Sweden, in a recent study (Leuk Lymphoma 2004; 45:915-921) we retrospectively compared the incidence and survival of *de novo* acute leukemia (AL) patients aged 16-64 years over three 5-year periods (1982-1996) in the two countries. Estonia and the so-called Western Swedish Health Care Region are well comparable area-wise (45,000 km^2 and 27,000, respectively) as to population (1.38 million inhabitants and 1.65, respectively). The age standardized incidence rate regarding total *de novo* AL was slightly, but not significantly, lower in Estonia than in Western Sweden (1.49/100,000/year for Estonia and 1,76 for Sweden, respectively). However, the survival data for the two countries were highly different (p<0.001). Thus, the relative survival for the total group of *de novo* AL in Estonia at 1 year was 20.7% and at 5 years 3.6%, respectively. The corresponding figures for the Swedish patients were considerably higher, 65.2% and 29.4%, respectively. Further, the 5-year survival significantly (p<0.05) increased for the Swedish patients over the 3 consecutive 5-year periods. No such improvement was recorded for the Estonian patients. In view of the dismal outcome for Estonian patients we decided to prospectively compare the results for incidence and outcome of *de novo* AL between the two countries over forthcoming 5-year periods. Herein we report on the results of the 5-year period comprising 1997-2001. All hospital records were carefully reviewed and only *de novo* AL were identified. The current report deals only with patients aged 16-64 years. The patients were categorized into 3 groups: those with unequivocal (1) acute myeloblastic leukemia (AML), (2) acute lymphoblastic leukemia (ALL), and (3) non-classifiable, undifferentiated or biphenotypic acute leukemia (uAL).The total number of *de novo* AL encountered in the Estonian population aged 16-64 years was 83 (43 males and 40 females), the corresponding figure for the Swedish population being 125 (65 males and 60 females). As regards Estonia the subgroups were: AML n=58, ALL n=22, and uAL n=3. The corresponding figures for Sweden were: AML n=95, ALL n=28, and uAL n=2. In Estonia, the yearly age standardized incidence per 100,000 inhabitants for *de novo* AL was 1.77 (1.38-2.16); for Western Sweden the figure was 2.17 (1.78-2.57). As compared to our previous study (cf. above) the survival data for the two countries were still highly different. Thus, in Estonia the relative survival at 1 year was 52.3% (40.9-62.4%) and at 5 years 16.4% (9.3-25.4%), respectively. The corresponding figures for the Swedish patients were considerably higher, 75.5% (66.9-82.2%) and 36.9% (28.4-45.5%), respectively. However, whereas in the current study the survival at 5 years for *de novo* AL in Estonia had improved significantly, there was no change as regards relative survival at 5 years for the Swedish patients.

0522

COMPLEX REARRANGEMENTS RESULTING IN LOSS OF BOTH 20Q AND 17P IN AML

J. Julie,[1] J.D. Howard,[1] M. Valgañón,[1] C. Grace,[1] H. Mazzulo,[1] M. Griffith,[2] P. Nacheva[1]

[1]Royal Free & UC Medical School, LONDON; [2]University of Oxford, OXFORD, UK

Background. Deletions of the long arm of chromosome 20 (del 20q) are a recurring abnormality associated with haematological malignancy (Mitelman 1995, Sandberg 1991, Heim and Mitelman,1995, Asimakopoulos & Green, 1996). The 20q deletions are seen in approximately 10% of the cases with PV and IMF, 4% of cases with myelodyspalstic syndrome (MDS) (Fenaux *et al.*, 1996), 1-2% of patients with acute myeloid leukaemia (AML) as well as other myeloproliferative disorders (MPD) (Bench *et al.* 2000). Deletions of chromosome 20q are well recognised in myeloid disorders and have been seen alone or as part of a complex karyotype (Johansson *et al.*, 1993, Schoch *et al.* 2002). Loss of 17p, via deletion, unbalanced translocation and isochromosome formation have been associated with the *17p-syndrome* (Lai *et al.* 1995, Soenen

et al. 1998). However these two aberrations are thought to occur independently and not usually seen with in the same karyotype (MacKinnon *et al.* 2007). *Results.* Here we present 9 patients characterised by a *classical* del(20)(q11.2) accompanied by cryptic (masked) loss of 17p within a complex karyotype. Molecular cytogenetic investigations revealed in 7/9 patients an unbalanced t(17;20)(p11;q11.2) with loss of the 17p11-pter genomic region. The remaining two cases showed more complex, three-way rearrangements albeit resulting in the same genomic loss. While the loss of TP53 appears to be consistent in these patients the break point on 20q is variable within the band 20q11. *Conclusions.* Importantly, the simultaneous loss of 17p and 20q due to the t(17;20) was identified in cases with clinically overt AML, most commonly preceded by MPD or MDS.

0523

A PHASE I STUDY OF TIPIFARNIB AND BORTEZOMIB IN THE TREATMENT OF POOR RISK ADULT ACUTE MYELOID LEUKEMIA

S. Paolini, E. Ottaviani, B. Lama, F. De Rosa, C. Laterza, P. Giannoulia, I. Iacobucci, C. Papayannidis, S. Parisi, P.P. Piccaluga, F. Salmi, M. Baccarani, G. Martinelli

Istituto di Ematologia e Oncologia Medica L. e A. Seràgnoli, BOLOGNA, Italy

Background. Outcome of older adults with acute myeloid leukemia (AML) is poor due to both disease and host-related factors. Tipifarnib (Zarnestra,) and bortezomib (Velcade) are new, promising targeted-treatments for hematological malignancies that might improve current results. In particular, tipifarnib is an oral, non-peptidomimetic farnesyltransferase inhibitor which has been shown to be effective in acute myeloid leukemias, allowing complete remission (CR) rates ranging from 8 to 22%, when used as single agent. Bortezomib is a proteasome inhibitor approved for multiple myeloma therapy, that determined hematological improvements in acute leukemia patients in phase I study. Interestingly, *ex vivo*, tipifarnib and bortezomib were proved to be synergistic in AML cell lines, above all overcoming environment-mediated resistance. *Aims.* We designed a phase I study aiming to investigate the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of ascending doses of bortezomib in association with standard dose of tipifarnib in patients with AML aged >18 years and unfit for conventional chemotherapy, or aged >60 years and in first or subsequent relapse. *Methods*. Bortezomib was administered as weekly infusion for three consecutive weeks (days 1, 8, 15) in a 28 days cycle, starting from 0.7 mg/m^2 and increasing by 0.3 mg/m^2, to cohorts including a minimum of 3 patients until the DLT was reached in one out of six patients. Tipifarnib was administered at the daily dose of 600 mg BID for 21 consecutive days in a 28 days cycle. Dose reduction of tipifarnib was scheduled in case of grade III/IV extra-hematological toxicity. *Results*. From April to June 2007 a total of 12 patient were enrolled; 11/12 actually received at least one dose of treatment and were then evaluable. Five patients have been treated with bortezomib at the dosage of 0.7 mg/m^2 without reporting DLT. The adverse events were nausea (n=1) and skin rash (n=1), both of grade II; the first occurred within the first 15 days of treatment and was considered tipifarnib-related, resolving with transient drugs interruption. The latter occurred 21 days after the first course and slowly resolved by adopting specific measures; in this case the treatment was definitively stopped. Six patient have been treated with bortezomib at the dosage of 1.0 mg/m^2. In the first cohort (n=3), DLT was reached, due to grade III central neurological toxicity. In particular, mental confusion occurred in one patient after ten days of therapy and resolved 36 hours after treatment withdrawal. Other adverse events were grade II skin rash (n=1) and grade I diarrhea (n=1), not requiring for dose modification. In consideration of this DLT another cohort of three patient was treated at the same dosage of bortezomib without any adverse event. As concern treatment response, one patient receiving bortezomib 1.0 mg/m^2 obtained a CR, while no response was documented in other cases. *Conclusions*. We conclude that the MTD of bortezomib administered once a week for three consecutive weeks every 28 days in association to standard dose of tipifarnib is 1.0 mg/m^2. A phase II study has been then planned and is currently ongoing.

0524

INCIDENCE AND CHARACTERISTICS OF CD56+ ACUTE MYELOID LEUKEMIA IN ADULTS AND CHILDREN: CD56 EXPRESSION IS HIGHER INCIDENCE AND STRONG ASSOCIATION WITH CNS INVOLVEMENT IN CHILDHOOD ACUTE MYELOID LEUKEMIA

H.R. Lee, I.H. Kim, S.S. Yoon, S.Y. Park, B.K. Kim, H.J. Kang, H.Y. Shin, H.S. Ahn, H.K. Kim, M.H. Park, H.I. Cho, D.S. Lee

Seoul National University College of Medicine, SEOUL, South-Korea

Background. CD56 is the membrane-bound glycoprotein, an isoform of the neural cell adhesion molecule (NCAM) and CD56 is used as a natural killer (NK) cell marker in immunophenotyping of leukemia. Several studies reported the association of CD56 expression and poor prognosis in acute myeloid leukemia (AML), however, most of these studies were confined to adult AML. *Aims.* To investigate the clinical difference between CD56+AML and CD56-AML in adults and children, we examined the incidence of CD56+AML and analyzed several clinical and biological characteristics (age, sex, WBC count, and median blast count in peripheral blood), cytogenetic features, immunophenotypic features, and clinical prognosis of CD56+AML and CD56-AML. *Methods.* 213 patients (177 adults, 36 children) with *de novo* AML in Seoul National University Hospital were enrolled. FAB subtypes were 4 M0 (2.3%), 26 M1, (12.2%), 78 M2 (36.6%), 27 M3 (12.8%), 43 M4 (20.2%), 15 M5 (7.1%), 14 M6 (6.6%), and 5 M7 (2.4%). Immunophenotyping was performed by immunohistochemical stain or multiparameter flow cytometry. The association between CD56 expression and variables (age, sex, WBC count, median blast count, cytogenetic features, extramedullary involvement, disease free survival, and overall survival) was analyzed by the fisher's exact test, chi-square test and T test, and Kaplan-Meier method. All statistical calculation was performed using SPSS system. *Results.* CD56 was detected in 6 of 177 adults (3.4%) and in 9 (25.0%) of children, showing significantly higher in children group (p<0.001). The CD56 expression was significantly frequent in M7 subtype (p=0.003). The other clinical characteristics, such as sex, WBC count, median blast count in peripheral blood, were not significantly associated with CD56 expression. The frequency of central nervous system (CNS) involvement was significantly higher in CD56+AML (p=0.025), although the frequency of overall extramedullary involvement did not differ between CD56+AML (3/15, 20.0%) and CD56-AML (27/198, 13.6%). Three patients in CD56+ AML presented extramedullary involvement at initial diagnosis or relapse. They were all children, and the involved sites were all CNS (Table 1). Among cytogenetic changes, AML1/ETO rearrangement was significantly associated with CD56 expression (p=0.024). When CD56 expression was analyzed by age group, AML1/ETO rearrangement was not associated with CD56 expression in children group (p=1.000). However, AML1/ETO rearrangement was significantly associated with CD56 expression in adults group (p=0.002). Aberrant expression of one or more lymphoid markers was observed in 42.9% (6/15 CD56+AML) and 45.2% (80/198 CD56-AML), showing no difference between CD56+AML and CD56-AML. Median disease free survival was 7 months in CD56+AML and 19 months in CD56-AML, showing no significant difference in two groups (p=0.247). Median overall survival was not reached in CD56+AML and 25 months in CD56-AML (p=0.164). *Conclusions.* The incidence of CD56+ AML was higher in childhood AML. We consider that CD56+ AML have unique biologic behavior, showing strong association with CNS involvement in childhood AML and AML1/ETO rearrangement in adult AML. To our knowledge, this is the first report on the higher incidence of CD56 expression in childhood AML compared to adult AML.

Table 1. Extramedullary involvement of CD56+ vs CD56- AML.

Total (n=213)	CD56+ n=15 (7.0%)	CD56- n=198 (93.0%)	*p*
Extramedullary involvement	3 (20.0%)	27 (13.6%)	0.450
Adult	0	19	
Child	3	8	
CSF involvement	3 (20.0%)	7 (3.5%)	0.025
Adult	0	4	
Child	3	3	

* chi-square test or fisher s exact test (level of significance: p<0.05)

0525

MOLECULAR MONITORING OF BAALC AS A MINIMAL RESIDUAL DISEASE MARKER IN PATIENTS WITH NEWLY DIAGNOSED ACUTE LEUKEMIA

Y. Najima, K. Ohashi, T. Yamashita, H. Akiyama, H. Sakamaki

Tokyo Metropolitan Komagome Hospital, TOKYO, Japan

Background. Although expression of the gene BAALC (brain and acute leukemia, cytoplasmic) predicts outcome in acute leukemia (AL), there is little information about clinical implication of molecular monitoring of transcripts of this gene as an assessment of minimal residual disease (MRD). *Aims.* To assess the clinical role of molecular monitoring BAALC gene expression in AL without well-known MRD markers. *Methods.* A real-time quantitative reverse transcriptase-polymerase chain reaction (RQ-PCR) was used to quantify BAALC gene expression in 21 patients (pts) with newly diagnosed AL. Bone marrow (BM) samples, obtained pretreatment and post chemotherapy, were serially analyzed to quantify these transcripts. Peripheral blood (PB) samples were also obtained to evaluate the correlation between BM and PB samples. These transcripts levels were compared with that of Wilms' tumor gene (WT1) in 14 pts without well-known MRD markers. In 7 pts with PML/RARα, AML/ETO or BCR/ABL carrying AL, these specific translocation markers were also monitored in parallel. GAPDH transcripts were used as an internal control and the ratio BAALC was thus estimated for evaluating the kinetics of residual clones. *Results.* BAALC expression levels showed clear correlation between BM and PB samples (r=0.945), and was seen in PB of healthy controls with a median expression level of 2.89x10exp-4/GAPDH. While, AL patients showed increasing BAALC transcript levels with 3 logs higher than the background level, and following induction chemotherapy the number of BAALC transcript was reduced along with pts obtaining hematological remission. In one patient with chemotherapy refractory AL, allogeneic bone marrow transplantation caused a significant reduction of the BAALC transcripts near the cutoff level. Comparative monitoring of MRD by RQ-PCR for WT1 and specific translocation markers demonstrated that BAALC had a closed kinetics to WT1 but not to specific translocation markers. *Conclusions.* The quantitation of the BAALC gene expression made it possible to assess MRD in pts with AL. To our knowledge, this is the first report concerning the use of BAALC mRNA expression for MRD monitoring.

0526

LOW DOSE GENTUZUMAB OZOGAMICIN, AFTER INTENSIVE INDUCTION IN OLDER AML PATIENTS: RESULTS IN 13 PATIENTS IN A SINGLE CENTRE INSTITUTION

A. Poloni,[1] S. Trappolini,[1] B. Costantini,[1] D. Capelli,[1] E. Troiani,[1] G. Mancini,[1] G. Discepoli,[2] M. Montanari,[1] G. Gini,[1] I. Scortechini,[1] P. Leoni,[1] A. Olivieri[1]

[1]Clinica di Ematologia, ANCONA; [2]Istituto di Citogenetica, ANCONA, Italy

Background. The optimal post-remission treatment for elderly patients with acute myeloid leukemia (AML) is presently unknown. Gemtuzumab ozogamicin (GO) is a humanized anti-CD33 monoclonal antibody conjugated to calicheamicin, that is rapidly internalized after binding to CD33. So GO is a more selective agent for acute myeloid leukemia (AML), because the CD33 antigen is expressed on AML, while it is not expressed on normal hematopoietic stem cells and non hematopoietic tissues. However, some studies indicated that this agent was often ineffective in patients with refractory AML cells via various mechanisms even though these preliminary data have been obtained during the induction phase of therapy. *Aims.* We evaluate the efficacy of low dose GO as post-remission approach after complete remission (CR), in order to eliminate the minimal residual disease in elderly patients enrolled in a pilot prospective study. *Methods*. We enrolled in an intensive induction protocol 78 elderly AML patients, eligible for aggressive chemotherapy. Fifthy-seven (73%) achieved CR; among these 48 (84%) received first consolidation with intensive chemotherapy and were planned to receive a further consolidation with ASCT or low dose GO in case of PBSC collection failure: 21 patients successfully achieved PBSC collection and 19 undergo ASCT: in this subset we observed 4 TRD (21%) with 9 patients relapsing and 6 patients still alive in CR with a median follow-up of 60 months (range, 7-100). Thertheen patients, that failed PBSC mobilization, have been furtherly consolidated with GO at the dose of 3 mg/m^2 for three times, monthly, with a median follow-up was 23 months (range, 6-62). *Results.* WHO grade III/IV adverse events included hematological toxicity (n = 13), hypertransaminasemia (n = 1), and anaphylactic syndrome (n=3). There were no major adverse events. Two patients relapsed and eventually died after 7 and 13 months. One patient relapsed 29 months after, but achieved a second complete remission after a reinduction regimen; GO was added again for three times and now he is in complete remission after 5 months. Eleven patients out of 13 are alive and 12 in continuous complete remission. *Conclusions.* Our preliminary data indicate a potential role for GO in the consolidation therapy in old patiens with AML in CR after intensive induction and first consolidation chemotherapy. After the first consolidation course patients allocated to the transplantation arm showed an high transplant related mortality and a very high relapse rate. Conversely we observed a superior OS and LFS in those patients who received consolidation with GO compared with those who received consolidation with ASCT (p=0.003). These preliminary data encourage the use of low dose GO as consolidation therapy of MRD in older patients with AML; it remains to establish if the PBSC collection failure after CR represents an independent favourable prognostic factor in this subset of patients.

Chronic lymphocytic leukemia - Prognostic factors

0527

HIGH CD54 CELL SURFACE EXPRESSION ON B CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA IS AN INDEPENDENT PREDICTOR OF POOR OUTCOME

P. Bulian,[1] M.I. Del Principe,[2] A. Zucchetto,[1] L. Maurillo,[2] R. Bomben,[1] F. Buccisano,[2] M. Dal Bo,[1] F. Luciano,[2] F.M. Rossi,[1] A. Venditti,[2] M. Degan,[1] S. Amadori,[2] V. Gattei,[1] G. Del Poeta[2]

[1]IRCCS CRO, AVIANO; [2]Dipartimento di Ematologia, Ospedale S.Eugenio, Universita' Tor Vergata, ROMA, Italy

Background. The intercellular adhesion molecule-1 (ICAM-1, CD54) is expressed in B Chronic Lymphocytic Leukemia (B-CLL) at lower intensity compared to other chronic leukemic B-cell disorders. Higher expression was found in patients with marked lymphadenopathy and/or splenomegaly, with Binet stages B and C, and with rapid lymphocyte doubling time (LDT). Preliminary reports investigating the prognostic relevance of CD54 in B-CLL suggested that patients expressing CD54 in more than 30% of the neoplastic component experienced shorter time to treatment (TTT) and overall survival (Hjalmar, Eur J Haematol, 2002), although so far no one has tested the independence of CD54 as prognosticator in the context of other prognostic factors. Aims. To test the independent contribution of CD54 to prognosis estimation as measured by TTT, we performed a multivariate analysis, adjusting for modified Rai stage (mod.Rai), beta-2 microglobulin, LDT, CD38 and ZAP-70 expression. Methods. All variable were measured at diagnosis or prior to start of protocol therapies. Patients treatments were established following the National Cancer Institute Working Group criteria. Expression of CD38 and CD54 was analysed by three-colour immunofluorescence with CD19 and CD5. Irrelevant antibodies were used to determine *Background.* Expression was reported as percent of $CD5^+CD19^+$ CLL cells above *Background.* Flow-cytometry detection of ZAP-70 was performed in four colour by using a PerCP-Cy5.5 CD19, APC CD5, PE CD3/CD56 and Alexa-488 ZAP-70. ZAP-70 expression was reported as percent of CD19+ CLL cells expressing the protein above a marker set at the left tail of ZAP-70 fluorescence distribution of T cells. Results. CD54 expression in 231 B-CLL cases showed a skewed distribution ranging from 2% to 99%, with a median of 43%. Sixty-six percent of patients expressed CD54 above 30%. Median CD38 expression was 9%, with 37% of patients above 30%. Median ZAP-70 expression was 17%, with 41% patients above 20%. Modified Rai stage was I in 37% of patients, II in 61% and III in 3%. Median beta-2 microglobulin was 1.97 g/L, ranging from 1 to 10 g/L. A LDT shorter than 12 months was present in 13% of patients. Fifty-three percent of patients were treated, with a median TTT of 64 months (54-90). In univariate analysis all factors were significant at $p<0.05$, the hazard ratio (HR) was 4.44 for LDT<12 months, 4.30 for a mod.Rai stage>= 2, 3.06 for CD54>=30%, 2.38 for CD38>=30, 2.07 for ZAP-70>=20 and 1.55 for 1 gr/L increment of beta-2 microglobulin. After multivariate analysis the HR for LDT was 2.59 ($p=0.002$), for mod.Rai 3.06 ($p<0.0001$), for CD54 2.08 ($p=0.0064$), for CD38 1.48 ($p=0.07$), for ZAP-70 1.43 ($p=0.09$), for beta-2 microglobulin 1.26 ($p=0.0026$). Conclusion. Surface membrane expression of CD54 on B-CLL cells has a prognostic power greater than CD38 and ZAP-70 expression, even after adjustment for Rai stage, lymphocyte doubling time and beta-2 microglobulin concentration.

0528

MICRORNA-34A IS SIGNIFICANTLY DOWN-REGULATED IN B-CLL PATIENTS WITH P53 ABNORMALITIES

M. Mraz,[1] J. Kotaskova,[2] B. Tichy,[2] K. Malinova,[2] M. Trbusek,[2] Y. Brychtova,[2] J. Mayer,[2] S. Pospisilova[2]

[1]Medical Faculty MU and University Hospital Brno, BRNO; [2]Medical Faculty MU and University Hospital Brno, Department of Hematooncology, BRNO, Czech Republic

Background. MicroRNAs (miRNAs) are small RNA molecules acting as post-transcriptional regulators of gene expression. They are dynamically regulated during cell differentiation, proliferation and apoptosis. Many studies have shown that miRNAs are aberrantly expressed in cancer cells, suggesting that they might act as a novel class of oncogenes or tumor suppressors. A possibility to use microRNA signatures for a specific cancer classification has also been demonstrated. *Aims*. The aim of this study was to identify miRNAs, which could have a potential role in B-CLL pathogenesis and might be useful as prognostic markers. We primarily focused on the identification of microRNAs abnormally expressed in a B-CLL subtype with poor prognosis harbouring a deletion/mutation of the TP53 gene. *Methods*. Peripheral blood samples from 30 B-CLL patients were collected (del/mut TP53 n=13: unmut IgVH n=8; mut IgVH n=3, polyclonal n=2; wt TP53 n=17: mut IgVH n=9; unmut IgVH n=8). B-CLL lymphocytes were separated by RosetteSep™ Human B Cell Enrichment antibody cocktail (obtained purity was >90% of $CD5^+19^+$ B-CLL cells). Real-time PCR (ABI TaqMan MicroRNA Assays) was used to detect the expression of 35 microRNAs, which were chosen based on target prediction, using the software miRanda, TargetsScan and literature data. The obtained values were correlated with functional status of the p53 (deletion/mutation of TP53 or wt TP53) and other known standard prognostic markers, such as mutation status of IgVH, del13q, expression of ZAP70 and expression of CD38. *Results*. We observed a statistically significant down-regulation of miR-34a in the TP53-abnormal samples (p=0,00015; non-parametric Mann-Whitney U test). This most differently expressed microRNA was ~15-fold down-regulated in B-CLL cells with TP53 abnormalities compared to the samples with wt TP53. MiR-34a has been recently reported to be directly regulated by p53 protein *in vitro* and these data show, to our knowledge for the first time, that this microRNA is abnormally expressed in leukemic patients and its expression is directly dependent on the p53 functional status. We also detected the down-regulation of miR-34a in p53 abnormal cells in tumor cell lines with different p53 status. In addition, we also identified several other microRNAs, whose expression correlates with B-CLL prognosis, for instance miR-18a expression is related to the IgVH mutation status and is significantly lower (p=0,025) in samples harbouring the unmutated IgVH. *Summary and Conclusions*. Our results show that microRNAs expression varies in the B-CLL prognostic subtypes. B-CLL patients with a deletion/mutation of the TP53 gene display significantly lower expression of miR-34a targeting anti-apoptotic gene Bcl2. Consequently, this miRNA could potentially play an important role in the pathogenesis of chronic lymphocytic leukemia subtypes.

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0529

INFERIOR OVERALL SURVIVAL IN CLL PATIENTS WITH TP53 MUTATIONS AND MDM2 SNP309 POLYMORPHISM

R.H. Linderholm,[1] K. Willander,[2] K. Karlsson,[3] P. Söderkvist[2]

[1]Linköping University Hospital, LINKÖPING; [2]Dept Clinical and Experimental Medicine, Linköping University Hospital, LINKÖPING; [3]Dept of Haematology, Lund University Hospital, LUND, Sweden

Background. Patients with CLL harbouring 17p deletions, the chromosomal location of TP53, have a dismal prognosis, indicating the importance of a functional p53. There are several ways to inactivate p53 where deletions and gene mutations are obvious causes. The MDM2 protein impair p53 activity and an increased expression of MDM2 is implicated as a susceptibility factor for different forms of cancer. A single nucleotide polymorphism (SNP) at nucleotide +309 (T>G) in intron 1, the promotor region of the MDM2 gene, is associated with an increased expression of the protein and subsequently impaired p53 function. *Aims.* In the present work we have focused on the clinical impact of the MDM2 SNP309 polymorphism and TP53 mutations in CLL.

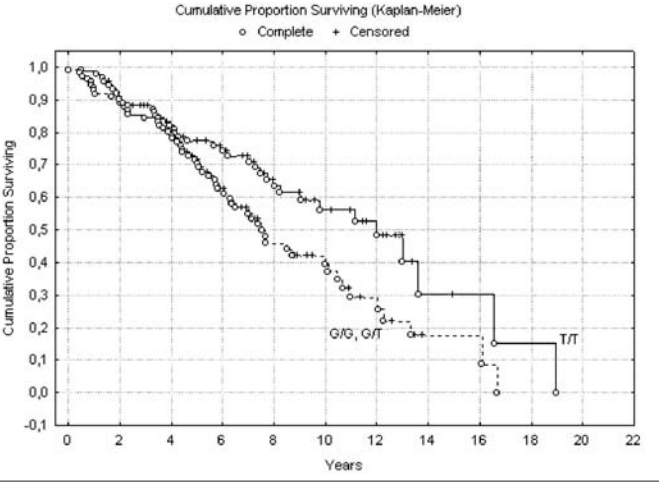

Figure 1. OAS according to MDM2 SNP309 status

Methods. In 213 patients with B CLL, we have investigated MDM2 SNP309, TP53 mutations in exon 5-8, and the mutational status of the IgVH gene and correlated with clinical parameters, such as, Binet stage, age of onset, time from diagnosis to first treatment, time from first to second treatment and overall survival (OAS). *Results.* There were 148 males and 65 females with a median age of 62,8 years. At diagnosis the number of patients in Binet stages A, B, and C were 101, 43, and 50, respectively. In 103 of 154 (67 %) patients, an unmutated (<98% homology with germline) IgVH gene was found. The median (range) follow up time was 6,6 (1,6-15,0) years. The OAS among 95 non-surviving patients was 4,7 (0-19,0) years. In total, 8,1 % out of 211 patients examined were homozygous for the G-allele of SNP309 (G/G) and 48,3 % were heterozygous (G/T). Somatic mutations of the TP53 was demonstrated in 19/213 (8,9 %) patients. Patients with MDM2 SNP309 G/G or G/T genotypes had a signficantly shorter survival (log rank test, p=0.016), as compared with patients with T/T genotype (Figure 1). The difference was approximately two years in median OAS. Patients with somatic mutations of the TP53 disclosed an even shorter OAS, as previously shown. *Conclusions*. CLL patients with mutations in the TP53 and/or 17p deletions have a short OAS. The SNP309 polymorphism of the MDM2 gene seems to add prognostic information in CLL possibly by an attenuation of the p53 pathway. Trials with MDM2 antagonists might be of particular interest in this subset of patients.

0530

GENE EXPRESSION PROFILE OF CLL PATIENTS TREATED WITH DNA-DAMAGING AGENTS IDENTIFIES BIOLOGICAL PATHWAYS IMPLICATED IN RESPONSE ACHIEVEMENT AND RELAPSE

N. Villamor,[1] F. Bosch,[1] A. Ferrer,[2] M. Aymerich,[1] A. Muntañola,[1] P. Jares,[1] D. Colomer,[1] E. Montserrat,[1] E. Campo[1]

[1]Hospital Clínic, BARCELONA; [2]Laboratori de citologia hematològica. Pathology Department, Hospital del Mar, BARCELONA, Spain

Background. P53 abnormalities are the only well established biomarker for poor response to therapy in patients with CLL treated with DNA damaging agents. In addition, other markers such as CD38 and ZAP-70 expression or IgVH mutational status have been associated with a shorter duration of the response. The mechanisms underlying the variability of the response in patients with CLL have not been fully investigated. *Aims.* To identify biological factors associated to response to treatment in patients with CLL by means of an extensive analysis of genomic expression. *Methods.* Magnetically purified CLL cells from 22 previously untreated patients receiving fludarabine, cyclophosphamide and mitoxantrone (FCM) were hybridized to oligonucleotide HGU133 Plus 2.0 arrays (Affymetrix). Gene expression measures were normalized using RMA methodology from the Affy package (Bioconductor project). Unsupervised and supervised analyses were performed with dChip v2.6. Patients achieving a MDR-negative status or a complete response (CR) were compared to other responses. Also relapsing patients were compared to continuous CR. *Results.* Fourteen patients achieved CR (8 MDR-negative and 6 MDR-positive), six patients achieved partial response and two failed to respond. The supervised analysis identified 268 genes differentially expressed between patients achieving CR and the remaining patients. Among these genes we found transporters (ABCA7, SLC16A7, SLC26A2. TMC6), transcription regulators (HDAC6, IFI16, ILF3, VEZF1), tumor suppressor genes (LATS2, MTSS1, RAP1A), metabolism of nucleotides (ARHGEF1, GNAI2), cytoeskeleton (MACF1, MICAL1, TUBAC3) or apoptosis regulators genes (BCL2, HRK, CDKN2A, PRKCE, CARD11 PDCD4). When comparing patients achieving MRD-negative CR and those obtaining other degrees of response 105 genes differentially expressed were identified. Interestingly, six genes (including MTSS1, GLT8D1, IFI16, MAP4K1) were found differentially expressed when comparing CR *vs* non-CR patients and MRD-negative CR *vs* other responses. Among patients who obtained CR, 64 genes were differentially expressed in MDR-negative and MRD-positive CR cases, 15 of them in common with the 105 found between MDR-negative CR and the rest of responses. These common genes included MAP4K1, DGKQ, MTSS1, TARBP1, GPSM3, UGT8, KTN1, PICALM and AMICA1 that are involved in apoptosis, guanosine metabolism, movement of cells and microfilaments, and adhesion and invasion of cells. At diagnosis, overexpression of ZAP70, TNFRSF1B, IL27RA, LAT2, or underexpression of PDCD4, GMNN, HSPA4, ABCA1 or NFKBIE were associated to relapse, among 159 differentially expressed between relapsing and non-relapsing patients. *Summary*. Response to treatment in patients with CLL seems to imply, besides classical apoptosis and cell cycle regulation, other multiple cellulars pathways. Interestingly, among these other pathways are included genes regulating transport, cytoeskeleton, adhesion, transcription or metabolism of nucleotides.

0531

CLL PATIENT COMPARISON ACCORDING TO ZAP70 MRNA LEVEL: NEW PROGNOSTIC FACTORS, DIFFERENCES IN MICRORNA EXPRESSION AND DISTINCT INTERACTION CAPACITIES WITH THE MICROENVIRONMENT

B. Stamatopoulos, H.B. Haibe-Kains, N. Meuleman, D. Bron, P. Martiat, L. Lagneaux

Laboratory of Experimental Hematology, Institut Jules Bordet, Université Libre de Bruxelles (ULB), Brussels, Belgium

Background. Gene expression profile is a powerful tool to better understand the biology, the clinical outcome and the molecular mechanism implicated in chronic lymphocytic leukemia (CLL). This disease presents an extremely variable clinical course with overall survival times ranging from months to decades. Therefore a plethora of prognostic factors which classified patients in poor or good behaviour have been investigated. Zeta-associated protein 70 (ZAP70) is one of the most promising prognostic factors to predict CLL evolution. Furthermore, we previously described a quantitative real-time PCR (qPCR) method to measure ZAP70 and demonstrated its prognostic power (Stamatopoulos *et al.*, Clin. Chem., 2007). *Aims.* In this study, we compared gene expression profile of patients expressing high *vs* low ZAP70 mRNA level in order to find genes not only associated with prognosis but also with cell biology. We also confirmed some microRNA differentially expressed between these two groups and linked them to treatment-free survival (TFS) and overall survival (OS). *Methods.* ZAP70 was evaluated by qPCR in a cohort of 108 patients ; two groups of 7 patients were chosen in the top-20 of patients expressing high and low level of ZAP70 mRNA and their gene expression profiles were compared using Affymetrix technology. Selected genes were verified by qPCR in an extended patient cohort (n=85) with a median follow-up of 72 months. Adhesion/migratory capacities into a stromal microenvironment or in response to conditioned medium were also evaluated. Finally, we investigated the differential expression of some microRNA by qPCR in a cohort of 61 patients with a median follow-up of 74 months. *Results.* 43 probe sets were differentially expressed with a FDR<10%, 135 with a p<0.001 and 932 with a p<0.05 with a fold change of >1.5 (increase or decrease). Several of these genes were TFS and/or OS significant predictors: PDE8A and FCRL family genes were downregulated in ZAP70$^+$ patients and can predict TFS and OS; ITGA4 mRNA was upregulated in ZAP70$^+$ patients and can significantly predict OS. Moreover pathway analysis reveals an over-representation of adhesion/migration genes. CXCR4/SDF1-alpha pathway was one of them. We observed a downregulation of CXCR4 in stromal-adherent cells only in ZAP70$^+$ patients indicating that only these patient cells can respond to microenvironment stimulus. Furthermore, ZAP70$^+$ patient cells can significantly better adhere to fibronectin and ZAP70$^+$ cells have better migratory capacities in response to conditioned medium. MicroRNA comparison confirmed the differential expression of miR-29c and miR-223 and we showed for the first time that these two microRNA had a TFS and OS individual prognostic power. *Conclusions.* This study identifies new prognostic factors (genes and microRNA) and shows the better adhesion/migratory capacities of ZAP70$^+$ cells in their microenvironment explaining their better survival and the aggressiveness of the disease.

0532

QUANTITATIVE GENE EXPRESSION ANALYSES OF SURROGATE MARKERS FOR GENETIC RISK GROUPS AND SURVIVAL IN CLL

D. Kienle,[1] A. Benner,[2] C. Läufle,[1] D. Winkler,[1] A. Bühler,[1] A. Habermann,[1] T. Zenz,[1] R. Dalla-Favera,[3] P. Lichter,[2] H. Döhner,[1] S. Stilgenbauer[1]

[1]University of Ulm, ULM, Germany; [2]German Cancer Research Center, HEIDELBERG, Germany; [3]Columbia University, NEW YORK, USA

Background. In chronic lymphocytic leukemia (CLL), a number of gene expression surrogate markers for genetic features such as VH mutation status have been described. Their detailed relation to the most important genetic subgroups, i.e. VH mutation status, V3-21 usage, del11q22-q23 (11q-), and del17p13 (17p-), and their value as prognostic markers in the context with established prognostic factors is largely unknown. *Methods.* Transcript levels of 18 candidate genes (ADAM29, ATM,

CLLU1, DMD, GLO1, HCSL1, KIAA0977, LPL, MGC9913, PCDH9, PEG10, SEPT10, TCF7, TCL1, TP53, VIM, ZAP70, ZNF2) were determined by real-time quantitative RT-PCR (RQ-PCR) in CD19-purified as well as unpurified patients samples. Their predictive value regarding individual genetic subgroups, an integrated genetic risk model, treatment free (TFS) and overall survival times (OS) was investigated in 151 CD19 purified patients samples including multivariate analyses. In a subset of 55 cases ZAP-70 expression was determined by FACS for comparison with RQ-PCR data. *Results.* The strongest association with the VH mutation status was observed for LPL (correct prediction of 84% of cases) and ZAP70 (84%), followed by TCF7 (74%), a marker being overexpressed in VH mutated CLL. Additional significant associations were observed between the 11q- subgroup and ATM (downregulation), and between the 17p- subgroup and TCL1, TCF7, ZNF2, and ADAM29 (all downregulated). Patients at genetic risk (VH unmutated or V3-21 usage or 11q- or 17p-) were best assigned by ZAP70, whereby a determination by RQ-PCR yielded better results compared to the FACS method. When assessing a hierarchical risk model integrating the relevant genetic subgroups (risk 17p- > 11q- > VH unmutated or V3-21 usage > VH mutated) high misclassification rates occurred with any individual marker (42% for ZAP70), which was mainly due to the impossibility of separating cases with 11q- and 17p- from VH unmutated cases without these abnormalities. This was improved using a marker combination (misclassification rate 30% for a combination of LPL, TCF7, ZAP70, ZNF2, and ATM). Still, a discrimination of 11q- or 17p- from VH unmutated patients without these abnormalities was achieved in only approx. 50% of 11q- and 17p- cases. In multivariate analyses including all candidate genes, LPL was the strongest OS predictor, whereas TFS was best predicted by ZAP70 and TCF7. When genetic factors were included in multivariate OS and TFS analyses, the surrogate markers lost their independent prognostic significance. *Conclusions.* Screening for patients at genetic risk can be performed using the individual markers ZAP70 or LPL. Usage of a marker combination reduces misclassifications especially regarding the highest risk groups 11q- and 17p-. However, a reliable distinction of these risk groups is not achieved and the prognostic impact of the surrogate markers remains inferior compared to the established genetic factors.

0533

A DISTINCTIVE TRASCRIPTIONAL PROFILE CHARACTERIZES THE CHROMOSOME 17P LOSS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

S. Fabris,[1] L. Mosca,[1] K. Todoerti,[1] G. Cutrona,[2] S. Matis,[2] M. Colombo,[2] L. Agnelli,[1] M. Lionetti,[1] D. Intini,[1] M. Gentile,[3] M. Mauro,[4] V. Callea,[5] G. Festini,[6] S. Molica,[7] G. Lambertenghi-Deliliers,[1] F. Morabito,[3] M. Ferrarini,[8] A. Neri[1]

[1]Università di Milano, Fondazione IRCCS Policlinico, MILANO; [2]Istituto Nazionale per la Ricerca sul Cancro, IST, GENOVA; [3]Azienda Ospedaliera di Cosenza, COSENZA; [4]Azienda Ospedaliera S. Martino, GENOVA; [5]Azienda Ospedaliera, Reggio Calabria, REGGIO CALABRIA; [6]Azienda Ospedaliero-Universitaria, Ospedali Riuniti di Trieste, TRIESTE; [7]Azienda Ospedaliera Pugliese- Ciaccio, CATANZARO; [8]Università degli Studi di Genova, GENOVA, Italy

Background. Distinct genetic abnormalities such as TP53 deletion at 17p13.1 have been identified as having an adverse prognostic relevance in B-cell chronic lymphocytic leukemia (B-CLL). Conventional cytogenetic studies have shown that TP53 deletion in B-CLL is associated predominantly with 17p loss (17p-) resulting from different complex chromosomal rearrangements such as unbalanced translocations or isochromosome 17q formation. *Aims*. The purpose of this study was to characterize a deletion-mapping of chromosome 17p in a subset of 17p- B-CLLs using genome-wide DNA profiling and Fluorescence in-situ hybridization (FISH) analyses. Additionally, gene expression profiling analysis was performed to identify specific transcriptional patterns or altered molecular pathways associated with 17p aberrations, which may have biological and clinical relevance in 17p- B-CLL. *Methods*. A panel of 71 untreated Binet A B-CLLs (18 of which carrying a TP53 monoallelic deletion) was characterized for the most recurrent genomic aberrations (trisomy 12 and deletions of 13q14, 11q22.3 and 17p13) and for the major prognostic markers. The genomic profile of chromosome 17p was investigated with GeneChip® Human Mapping 50K Xba 240 arrays in 12/18 17p- B-CLLs. Inferred copy numbers were derived from a Hidden Markov Model (HMM) based algorithm implemented in CNAT 4.0.1 software (Affymetrix). FISH probes covering a region of approximately 6 Mb in 17p11.2-p12 was selected to validate the array results. The transcriptional profiles of the 60 B-CLLs (7 carrying 17p-) have been generated on Affymetrix GeneChip® U133A arrays. The identified transcriptional fingerprints of the 17p- cases was validated on an independent dataset of 100 B-CLL cases (Haslinger *et al.*, 2004) using a Multi-class Prediction Analysis. Polymerase chain reaction was used to define the mutational status of the TP53. *Results*. Genome-wide DNA analysis of TP53 -deleted samples showed 17p loss in 11/12 cases, with distinct deletion patterns scattered along the 17p11.2-p12 region. FISH analysis confirmed these findings and revealed 17p loss in a small fraction of leukemic cells in the remaining TP53 -deleted case. In addition, FISH indicated 17p loss in the 6/18 cases not investigated by SNP. Mutations in exons 5 to 9 of the remaining TP53 allele were found in 9/12 deleted samples. Gene expression profiling of 60 B-CLLs, including 7 patients with 17p loss, identified 40 differentially expressed genes in 17p- *vs* 17p normal samples, 35 of which were down-regulated in 17p- tumors: the majority (30/35) of these transcripts, including putative tumor suppressor genes (GABARAP, GPS2 and OVCA1) mapped to 17p, indicating a remarkable gene dosage effect. *Conclusions*. Our study confirms and extends previous observations indicating that TP53 deletion in B-CLL patients is associated with the loss of chromosome 17p. Gene expression profiling showed that chromosome 17p loss is associated with significant down-regulation of genes located on 17p, indicating a gene-dosage effect. The potential coordinated loss of tumor suppressor genes on 17p other than TP53 , may represent an important mechanism for the negative clinical outcome associated with this lesion in B-CLL and warrants further investigation.

0534

INTEGRATIVE GENOMIC ANALYSIS OF TRISOMY 12 ABNORMALITY IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

L. Mosca,[1] K. Todoerti,[1] S. Fabris,[1] G. Cutrona,[2] S. Matis,[2] M. Colombo,[2] L. Agnelli,[1] M. Lionetti,[1] M. Gentile,[3] M. Spriano,[4] V. Callea,[5] G. Festini,[6] S. Molica,[7] G. Lambertenghi-Deliliers,[1] F. Morabito,[3] M. Ferrarini,[8] A. Neri[1]

[1]Università di Milano, Fondazione IRCCS Policlinico, MILANO; [2]Istituto Nazionale per la Ricerca sul Cancro, IST, GENOVA; [3]Azienda Ospedaliera di Cosenza, COSENZA; [4]Azienda Ospedaliera S. Martino, GENOVA; [5]Azienda Ospedaliera, Reggio Calabria, REGGIO CALABRIA; [6]Azienda Ospedaliero-Universitaria, Ospedali Riuniti di Trieste, TRIESTE; [7]Azienda Ospedaliera Pugliese- Ciaccio, CATANZARO; [8]Università degli Studi di Genova, GENOVA, Italy

Background. B-cell chronic lymphocytic leukemia (B-CLL) is a genetically heterogeneous disease with a high variable clinical course. Trisomy 12 (+12) is frequently associated with the disease (approximately 20%) and it has been reported to be correlated with an intermediate prognosis. Despite the recent remarkable progresses in the understanding the molecular pathogenesis of B-CLL, the biological significance of trisomy 12 remains to be fully elucidated. *Aims.* To identify signalling pathways and additional lesions associated with tumorigenesis in +12 B-CLLs. Methods. A panel of 80 Binet A untreated B-CLLs including 18 patients with trisomy 12 was investigated. This series was characterized for the most recurrent genomic aberrations (deletions of 13q14, 11q22.3 and 17p13) and for the major prognostic markers. Gene expression profiles were generated on Affymetrix GeneChip® U133A arrays. The identified transcriptional fingerprint of +12 was generated and then validated on an independent dataset of B-CLL cases (Haslinger *et al.*, 2004) by a Multi -class Prediction Analysis. Furthermore, genome-wide profiling data were generated by means of Affymetrix GeneChip® Human Mapping 250K Nsp SNP arrays in a subset of 45/80 B-CLLs including 9 patients with +12. Inferred copy numbers were derived from a Hidden Markov Model (HMM) based algorithm implemented in CNAT 4.0.1 software (Affymetrix). Results. The transcriptional analysis revealed 140 genes as the best classifier for the +12 B-CLLs. 92 out of the 118 genes up-regulated in +12 patients mapped on chromosome 12, most of which (86%) on the long arm. The remaining 26 genes and the 22 genes down-regulated in +12 samples showed different chromosomal localizations. The transcriptional fingerprint validation on an independent cohort of 100 B-CLL patients showed a global classification rate of 92.5%. A functional analysis of the deregulated genes revealed the involvement in transcriptional regulation, DNA, mRNA and protein metabolism. Many genes (ANAPC5, CCT2, CCT4, CDK2AP1, CDKN1B, CHFR, MCRS1, PCTK2) were also related to regulation of cell-cycle, cell death (ATXN1, CD63, DNM1L, DYRK2, HRK, OPTN, TEGT) and immune response (CKLF, CD58, CTLA4, HDAC9, IL21R) as well as to different cellular metabolic processes (AMPD3, CS, LDHB, NUDT4). Consistent with FISH data,

genome-wide DNA analysis showed that +12 is never found to be associated with the other most recurrent aberrations of B-CLL. Moreover, SNPs analysis identified, among the +12 patients, other recurrent copy number variations, such as gains of 14q32, 15q11, 17q21 and losses of 11p15, 14q11, 14q32 and 15q11: however, this correlation did not reach a statistically significant level. Conclusions. Trisomy 12 appears to affect gene expression in B-CLL not only by a dosage effect but also by influencing the expression of genes located on different chromosomes leading to the deregulation of multiple cellular functions. None of the copy number alterations identified by SNP genomic analysis appeared to be tightly correlated with +12.

0535

ANALYSIS OF TP53 MUTATION IN A LARGE COHORT OF CLL PATIENTS BEFORE FIRST-LINE TREATMENT: ANALYSIS OF THE GENETIC PROFILE WITHIN THE CLL4 TRIAL (F *vs* FC) OF THE GCLLSG

T. Zenz,[1] T. Denzel,[1] S. Häbe,[1] D. Winkler,[1] A. Bühler,[1] R. Busch,[2] B. Eichhorst,[3] M. Hallek,[3] H. Döhner,[1] S. Stilgenbauer[1]

[1]University of Ulm, ULM; [2]Institute of Medical Statistics and Epidemiology, Technical University, MUNICH; [3]University of Cologne, COLOGNE, Germany

CLL patients with 17p deletion have a dismal prognosis after treatment. The exact prognostic role of TP53 mutations in the absence of 17p deletion and any differential impact of the mutation in cases with 17p deletion (*vs* sole deletion) is currently unclear. *Aims.* To assess the incidence, profile and prognostic impact of TP53 mutations as assessed by sensitive DHPLC followed by direct sequencing in a well characterized and homogeneous patient population from a prospective trial (CLL4 trial GCLLSG). *Methods.* We studied 342 of 375 CLL4 trial patients with available material. The population is well characterized and detailed genetic characteristics are available (FISH, VH-Status). We used DHPLC to detect TP53 mutations in the coding exons (2-11). Aberrant profiles were confirmed by sequencing including the use of fragment collection in cases with low grade mutations. *Results.* We found an overall incidence of TP53 mutations of 8.2 % (28/342 patients). The mutations were exclusively located in the DNA binding domain. We observed 2 splice site mutations, 3 deletions, 2 insertions but the majority of mutations were missense mutations in exons 5-8. Two patients had 2 different mutations. Fourteen of 16 patients with 17p deletions also had a TP53 mutation (87.5 %). Interestingly, both patients with a 17p deletion where no TP53 mutation was identified, showed a low proportion of 17p- cells (19-21%) suggesting that detection limits of the technique might explain this finding. We found TP53 mutations in the absence of 17p deletions in 4.3% (14/326). The proportion of mutated allele in cases without 17p deletion ranged from 10 to 90 percent. In two patients with follow-up samples we found evidence of clonal evolution at the time of relapse. The genetic profile of cases with TP53 mutation showed a high incidence of 17p deletions (13/28 cases), but also included cases with 11q deletion 3/28, 13 deletion as the sole abnormality (5/28) and normal karyotype (4/28). The majority of cases with TP53 mutations had an unmutated VH status (n=21)(75%), while 6 of 28 had a mutated VH status (V3-21: n=1). Correlation with baseline parameters and clinical outcome is currently being performed and will be presented. *Conclusions*. In this first line treatment trial population, TP53 mutations without 17p deletion occurred in 4.3% (14/326) of patients and the majority of cases with 17p deletion also have TP53 mutations. The demonstration of clonal evolution in cases with TP53 mutation without 17p deletion after F-based therapy points to the biological and clinical significance of TP53 mutations in CLL.

0536

SCREENING FOR COPY-NUMBER ALTERATIONS AND LOSS-OF-HETEROZYGOSITY IN CHRONIC LYMPHOCYTIC LEUKEMIA - A COMPARATIVE STUDY OF FOUR DIFFERENTLY DESIGNED, HIGH RESOLUTION MICROARRAY PLATFORMS

R. Gunnarsson,[1] J. Staaf,[2] M. Jansson,[3] A.M. Ottesen,[4] H. Göransson,[5] U. Liljedahl,[6] U. Ralfkiær,[7] M. Mansouri,[3] A.M. Buhl,[8] K. Ekström Smedby,[9] H. Hjalgrim,[10] A.C. Syvänen,[6] A. Borg,[2] A. Isaksson,[5] J. Jurlander,[8] G. Juliusson,[11] R. Rosenquist[3]

[1]Lund University, LUND, Sweden; [2]Department of Oncology, SCIBLU Genomics, Lund University, LUND, Sweden; [3]Department of Genetics and Pathology, Uppsala University, UPPSALA, Sweden; [4]Department of Growth and Reproduction, Juliane Marie Centre, Rigshospitalet, COPENHAGEN, Denmark; [5]Department of Medical Sciences, Uppsala University, UPPSALA, Sweden; [6]Department of Medical Sciences, Molecular Medicine, Uppsala University, UPPSALA, Sweden; [7]Danish Cancer Society, Laboratory of Cancer Genomics, COPENHAGEN, Denmark; [8]Department of Hematology, Leukemia Laboratory, Rigshospitalet, COPENHAGEN, Denmark; [9]Department of Medicine, Clinical Epidemiology Unit, Karolinska Institutet, STOCKHOLM, Sweden; [10]Department of Epidemiology Research, Statens Serum Institut, COPENHAGEN, Denmark; [11]Stem Cell Center, Hematology and Transplantation, Lund University, LUND, Sweden

Background and Aims. Screening for gene copy-number alterations (CNAs) has improved by applying genome-wide microarrays, where SNP-arrays also allow analysis of loss-of-heterozygozity (LOH). Few comparative studies of high-resolution platforms have thus far been performed and there is a current need to compare platforms in order to understand the pros and cons with the differently designed arrays. Here we investigated chronic lymphocytic leukemia (CLL) samples by applying four differently designed microarrays for evaluation of: 1) baseline variation and ratio response to FISH-validated genomic aberrations, 2) detection of known recurrent and novel CNAs and concordance between platforms, and 3) detection of LOH with the Illumina and Affymetrix SNP-arrays. *Materials and Methods*. We screened 10 CLL samples using four different high-resolution platforms: BAC-arrays (32K), oligonucleotide-arrays (185K, Agilent), and two SNP-arrays (250K, Affymetrix and 317K, Illumina). Baseline variation and copy-number ratio response was calculated on normalized array-data. Analysis of CNAs and LOH was performed applying the Bio Array Software Environment (BASE) and dChip software, respectively. *Results*. Evaluation of baseline variation and copy-number ratio response showed the best performance for the Agilent platform and confirmed the robustness of BAC-arrays. Accordingly, these platforms demonstrated a higher degree of platform-specific CNAs. The SNP-arrays displayed higher technical variation, although this was compensated by high density of elements. Cross-platform comparison revealed 29 concordantly detected CNAs, including FISH validated known recurrent alterations, which confirmed that all platforms are powerful tools when screening for large aberrations. However, detection of 32 additional regions present in 2-3 platforms illustrated a discrepancy in detection of small CNAs, which often involved reported copy-number variations. LOH-analysis using dChip revealed concordance of mainly large regions, but showed numerous, small non-overlapping regions and LOH escaping detection. Affymetrix detected a higher degree of CNAs compared to Illumina, while the latter showed a lower noise level and higher detection rate in the LOH analysis. *Conclusions*. Usage of high resolution microarrays will improve the possibility to detect new recurrent microevents in CLL leading to identification of new important subgroups potentially refining the prognostic hierarchy established by FISH. If only copy-number data is preferred, oligonucleotide-arrays such as Agilent provide a high sensitivity of this type of analysis. If LOH-analysis is desirable, SNP-arrays are the preferred choice but, parallel improvement of analysis tools is required for an in-depth analysis of allelic imbalances.

0537

CYTOGENETIC ABNORMALITIES DEL(17P) AND DEL(11Q) IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): A PROSPECTIVE STUDY ON 156 YOUNG PATIENTS IN DIFFERENT PHASES OF THE DISEASE

I. Del Giudice, F.R. Mauro, M. Nanni, M.S. De Propris, S. Santangelo, M. Marinelli, N. Peragine, F. Mancini, D. Armiento, L. Quattrocchi, A. Guarini, R. Foa'

University La Sapienza, ROMA, Italy

Background. In chronic lymphocytic leukemia (CLL), the presence of del(17p) is associated to chemotherapy resistance and poor prognosis. p53 function can be impaired by del(11q)/ATM mutations, which also confer an unfavorable outcome. In previously published series, the incidence of these abnormalities ranges from 10% to 32% for del(11q) and 3% to 27% for del(17p), depending on the stage of the disease and whether or not treatment was administered. *Aims*. To assess the incidence of del(17p)/del(11q) in CLL patients in different phases of their disease, the correlation of these abnormalities with other biologic parameters and their impact on treatment requirement. *Methods*. From November 2002 to December 2007, 156 young CLL patients (<60 years-old) were included in this study. Three different cohorts were prospectively evaluated: 79 cases at diagnosis with stable disease for at least 12 months (group 1), 53 at first progression (group 2), 24 at progression after one or more therapies (group 3). Cytogenetic abnormalities, ZAP-70 and CD38 expression, as well as IgVH mutational status were analyzed. *Results*. The distribution of unfavorable prognostic markers in the 3 different groups is summarized in the Table 1. The incidence of del(17p) (cut off >20% cells) raised progressively from group 1, to groups 2 and 3 (0%, 6% and 26%, respectively; $p<0.001$). The incidence of del(11q) (cut-off >10%) increased significantly from group 1 to 2 (5% and 16%; p 0.04), though it was not higher in group 3 (5%). The proportion of unmutated IgVH (<98% homology) cases also increased progressively in the 3 groups, 24% in group 1, 52% in group 2 and 71% in group 3 ($p<0.001$). The same trend was found for CD38 >7% ($p<0.001$) and ZAP-70 >10% expression (p 0.03). Patients with del(17p) or del(11q) almost exclusively showed unmutated IgVH ($p<0.001$) and were mostly CD38$^+$ (p 0.003) and ZAP-70+ (p 0.01). Focusing on previously untreated patients with progressive disease (group 2), they required treatment after a median time of 39 months (range 1-144) from diagnosis. Cases with del(17p) or del (11q) had a treatment-free interval (TFI) that was on average half of that of cases without these abnormalities (23 *vs* 40 m), as well as cases with IgVH unmutated *vs* mutated (18 *vs* 50 m), CD38 pos *vs* neg (21 *vs* 40 m), ZAP-70 pos *vs* neg (26 *vs* 41 m). *Conclusions*. Del(17p) and del(11q) occur in CLL cases with other adverse biologic features, as unmutated IgVH, ZAP-70+ and CD38$^+$, and show a rapidly progressive course. They represent a small subgroup of cases in the early phases of disease, which progressively raises with more advanced disease. Thus, their incidence, as well as that of other biologic factors with adverse prognostic impact, changes considerably when patients in different phases of the disease are investigated. This has to be considered in the design of clinical trials. Moreover, reassessment of chromosomal abnormalities during the course of CLL is warranted, especially after treatment.

Table 1.

	CLL diagnosis		CLL 1st progression			CLL Previously treated/refractory		
	N° cases	%	N° cases	%	p	N° cases	%	p
IgVH mutated (≥98%)	59/78	76%	25/52	48%	.001	7/24	29%	.001
IgVH unmutated (<98%)	19/78	24%	27/52	52%		17/24	71%	
CD38 ≥7%	11/79	14%	23/52	44%	.001	17/24	71%	.001
ZAP-70 ≥10%	26/74	35%	28/50	56%	.02	10/17	59%	.03
del(17p) >20%	0/78	0%	3/51	6%	.03	5/19	26%	.001
del(11q) >10%	4/78	5%	8/51	16%	.04	1/19	5%	.09
del(17p)+ del(11q) pooled	4/78	5%	11/51	22%	.004	6/19	32%	.019

0538

SPONTANEOUS CLINICAL REMISSION IN CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICAL AND BIOLOGIC FEATURES OF 9 CASES

I. Del Giudice, F.R. Mauro, S. Chiaretti, S. Tavolaro, S. Santangelo, M. Marinelli, R. Maggio, M.S. De Propris, F. Mancini, L. Quattrocchi, D. Armiento, A. Guarini, R. Foa'

University "La Sapienza", ROME, Italy

Background. Spontaneous clinical remission in chronic lymphocytic leukemia (CLL) is an exceptionally rare phenomenon, the biologic features of which have not been reported. *Aims and Methods*. Aiming at a better definition of the biologic profile of such cases, we report on 9 patients with CLL who underwent a spontaneous clinical remission of the disease over a median follow-up of 11 years (range 3-28). At diagnosis, all cases were Binet stage A, the median lymphocyte count was 11.8×10^9/L (range 8.7-27.6) and the proportion of CLL cells in the peripheral blood (PB) was 78% (range 65-82%). At the time of the study, the median lymphocyte count was 3.16×10^9/L (range 1.3-4.9) and the proportion of CLL cells in the PB was 44% (range 5-53%). *Results*. At the time of regression, CD38 expression was less than 7% in all cases. PCR analysis of the variable IgVH region showed a mutated status in all 7 cases evaluated, with a mean mutation frequency of 7.2%±2.8 (median 7.4%, range 2.7-10.7%). IgVH usage was restricted to the VH3 family in all cases but 1, with the following gene distribution: VH3-30 in 2 patients, VH3-07 in 1, VH3-15 in 1, VH3-33 in 1, VH3-72 in 1, VH4-34 in 1. The light chain variable region genes, mutated in 6/8 cases, were: Vκ4-1 in 3 patients, Vκ2-30 in 1, Vκ3-15 in 1, Vλ1-47 in 1, Vλ1-51 in 1 and Vλ2-11 in 1. The Vκ4-1 cases had highly homologous LCDR3 amino acid sequences. Microarray analysis revealed a distinctive genomic profile for such cases with an over-representation of BCR-related genes, ribosomal genes, regulators of signal transduction and transcription. No significant differences were observed between CLL in spontaneous remission and healthy individuals in the absolute number of activated T lymphocytes producing IFN-γ, TNF-α and IL-4, whilst a significant lower amount of TNF-α producing T cells was found in spontaneous remission, stable CLL and healthy individuals compared to progressive CLL. *Conclusions*. We confirm that in CLL spontaneous regressions up to a state of protracted clinical remission can occur despite the persistence of the neoplastic clone at microsopic levels. Biologic features of these cases include negative CD38, mutated VH3-30 and Vk4-1 usage with an identical LCDR3 common motif, as well as a peculiar gene profile, suggesting that the activation of BCR-related and translation-associated genes is the most significant change occurring in the regressing leukemic clone.

0539

P53 EVALUATION IN CLL BY P53 ARRAY: A SIMPLE, SENSITIVE AND SPECIFIC METHOD THAT UNRAVELS A HIGH PERCENTAGE OF POLYMORPHISMS AND MUTATIONS

S. Chiaretti,[1] S. Tavolaro,[1] M. Messina*,[1] M. Marinelli,[1] I. Del Giudice,[1] F.R. Mauro,[1] S. Santangelo,[1] N. Peragine,[1] S. Truong,[2] M.S. De Propris,[1] M. Nanni,[1] A. Guarini,[1] R. Foà[1]

[1]*University La Sapienza, ROMA, Italy;* [2]*Roche Molecular Systems, PLEASANTON, USA*

Background. Chronic lymphocytic leukemia (CLL) is an heterogeneous disease. Several biologic parameters are relevant prognostic markers; among these, p53 mutations are relatively rare events, usually detected in the late stages of disease, highly predictive of unfavorable outcome and associated with Ritcher's syndrome. *Aims*. To evaluate the specificity, sensitivity and advantages of the AmpliChip p53 Test array - a product currently under development at Roche Molecular Systems, Inc - and to correlate the results with the most relevant clinico-biological parameters. *Patients and Methods.* Forty-eight untreated CLL patients were evaluated; 35 had progressive disease: 16 were IgVH germline and 19 mutated. Thirteen had a stable disease and were IgVH mutated. Concordance between IgVH status and ZAP70 expression was 77%; CD38 proved positive in 78.6% IgVH germline patients and in 16% IgVH mutated cases. TP53 sequencing revealed 2 mutations, 2 polymorphisms and 1 heterozygosis within progressive cases, and 1 polymorphism within stable patients; p53 protein accumulation was identified in 2 cases. Finally, FISH analysis revealed 13q deletions in 64% patients, no aberrations in 20% and trisomy 12, 11q and 17p deletions in 12%. The p53 array allows sequencing of exons 2-11: 50 ng of genomic DNA are amplified in two separate PCR reactions. Subsequently, PCR products are fragmented, hybridized, stained and scanned. The whole procedure requires roughly 8 hours. Each array queries 1268 nucleotide positions that are investigated by individual probesets that contain five probes: 1 for wild-type sequence, 3 for possible substitutions and 1 for deletion. Each probe contains multiple copies of an oligonucleotide sequence. The mutation detection algorithm detects single base pair substitutions and deletions. *Results*. The p53 array detected 7 mutations (14.5%), involving exon 5 in 2 patients (with 1 case harboring 2 different mutations), exon 8 in 2, exon 9 and exon 7 in 1, respectively. P53 array also detected the following polymorphisms: codon 72 in 17 patients (35%), codon 213 in 4 (8%) and codon 36 in 2 (4%). There was no significant association between p53 mutations and IgVH mutational status, ZAP-70 and CD38expression, and cytogenetic findings. From a clinical standpoint, mutations were only detected in progressive cases and there was not a statistical association between codon 72 polymorphisms and clinical behavior (Fisher exact test = 0.32). Although the numbers are small, similar conclusions can be drawn for codon 213. Comparison with TP53 sequencing highlighted a higher sensitivity of the AmpliChip p53 Test array: in fact, the mutations that were identified by sequencing were also detected by p53 array, while 3 additional mutations, at exon 5, 7 and 9 were not detected by TP53 sequencing: this discrepancy will be further investigated. *Conclusions*. The p53 array represent a highly reliable and non-time consuming tool for mutational analysis of TP53. Using this new approach, our results corroborate that p53 mutations occur exclusively in progressive patients and are not associated with other prognostic parameters. Furthermore, p53 array highlights a high percentage of codon 72 polymorphisms in CLL patients, regardless of the clinical behavior, whose role needs to be further investigated.

** Equal contribution*

0540

PRESENCE OF HETEROZYGOUS ATM DELETION MAY NOT BE CRITICAL FOR THE RESPONSE OF CLL CELLS TO FLUDARABINE

M. Trbusek,[1] S. Cejkova,[1] L. Rocnova,[1] D. Potesil,[2] J. Smardova,[1] J. Malcikova,[1] D. Zezulkova,[1] M. Doubek,[1] Y. Brychtova,[1] S. Pospisilova,[1] J. Mayer[1]

[1]*University Hospital Brno, BRNO;* [2]*Masaryk University, Faculty of Science, BRNO, Czech Republic*

Background. Abnormalities of the TP53 or ATM, two co-operating tumor-suppressor genes, significantly deteriorate prognosis and treatment options in CLL patients. Although their aberrations seem to be mutually exclusive in CLL cells, the inactivation of the former gene leads to a much shorter survival and a higher drug resistance. Association between the ATM mutation and resistance of CLL cells to fludarabine has recently been reported and a similar observation has also been noted in cases manifesting a low level of Atm protein. *Aims.* (i) to assess an *in vitro* effect of fludarabine on CLL cells with heterozygous ATM deletion in comparison to TP53-abnormal and wild-type cases (ii) to monitor the activation of the p53 pathway in the corresponding cells. *Methods.* Interphase FISH was used for a determination of the TP53 and ATM deletions. Functional FASAY analysis coupled to sequencing was employed to supplement the screening also for the TP53 mutations. Metabolic WST-1 assay monitored a cell viability after fludarabine administration (25; 6.3; 1.6 and 0,4 μg/mL). Western blot analysis was used to detect the p53 stabilization and real-time RT-PCR was employed to monitor the activation of the p53-downstream targets, PUMA, BAX and p21. *Results.* A representative series of 59 CLL cultures was used. Fludarabine provided a clear concentration-dependent curve after 48 h cultivation in most of the samples, with the exception of some strongly resistant cases (harboring abnormal TP53). The sensitivity to fludarabine was assessed at the concentration of 1.6 μg/mL, since it had provided the most significant differences among individual samples. The TP53-abnormal samples (n=20) were remarkably more resistant to this drug than the remaining CLL (p=0.012; Mann-Whitney U-test), whilst there were no significant differences between the ATM-deleted (n=21) and wt (n=18) subgroups (p=0.71). All ATM-deleted (n=6) and wt (n=4) samples tested by the Western blot showed a clear stabilization of the p53 protein after fludarabine exposure. On the contrary, two of the three tested, ATM-deleted cases, did not induce the p53 after the administration of doxorubicin, a known activator of Atm kinase through the dsDNA breaks. Q-RT-PCR analysis confirmed the induction of the p53-downstream target genes, i.e. PUMA, BAX and p21, in virtually all tested ATM-deleted (n=7) and wt (n=6) samples. The TP53-abnormal samples were devoid of any induction, what again supported a view that a clear borderline distinguishes the TP53-abnormal samples from the remaining CLL, as it regards the response to fludarabine. *Summary and Conclusions.* Presence of ATM deletion in CLL cells should not be considered a priori as a marker of fludarabine resistance. The Atm kinase does not seem to be critical for the activation of the p53 pathway after fludarabine administration.

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0541

CYTOTOXIC EFFECT OF SDX-308, ANALOGUE OF R-ENANTIOMER OF ETODOLAC, IN COMBINATION WITH PURINE ANALOGUES OR MONOCLONAL ANTIBODIES ON *EX VIVO* B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

P. Robak, T. Robak, P. Smolewski, A. Linke, B. Cebula

Medical University of Lodz, LODZ, Poland

Background. Non-steroidal anti-inflammatory drugs (NSAIDs) were recently found to inhibits proliferation and invasive growth or inducing cell apoptosis in several tumors, including some hematologic malignancies. One of those agents, non-cyclooxygenase 2-inhibiting R-enantiomer of etodolac (SDX-101) was found to be very active against chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) cells. The indole-pyran analogue of SDX-101, SDX-308 (CEP-18082), showed even more potent cytotoxicity than SDX-101 against MM cells and inhibits orthoclase formation and activity of mature osteoclasts. The *Aims.* In this report we demonstrate activity SDX-308 in combinations with purine analogues or monoclonal antibodies against CLL cells. Material and *Methods.* Cytotoxic effects of SDX-308 alone and in combinations with purine analoques, fludarabine (FA) or cladribine (2-chlodeoxyadenozine, 2-CDA), and monoclonal antibodies, anti-CD52 (campath, alemtuzumab, ALT) or anti-CD20 (rituximab, RTX) was assessed *ex vivo* in samples obtained from 50 untreated CLL patients. *Results.* SDX-308 was found to be very active against CLL cells, inducing apoptosis-based cytotoxicity. In preliminary experiments, SDX-308 was evaluated in concentrations 3,4-340 μg/mL, with 50% cytoxicity (IC 50) gained at the dose of 34 μg/mL after 24 hours of incubation. Based on these data, SDX-308 in concentrations 8,5-25,5 μg/mL have been chosen for further experiments. All combinations of SDX-308 occurred to provide either synergistic or additive cytotoxic effects. Namely, 24 hour incubation with 25,5 μg/mL of SDX-308 used alone induced mean control-compensated cytotoxic index (CCI) - 22.3%. In these settings FA (1 μg/mL) and 2-CdA (50 μg/mL) induced mean CCIs 4.2% and 5.5%, respectively. RTX and ALT, in cells initially cross-linked with secondary IgG antibodies, induced mean CCIs 3.2% and 9.3%, respectively. Mean CCI in response to combination of 25,5 μg/mL SDX-308 with FA was 45.1% (*vs* effects of single agents - $p<0.001$). Mean CCI for 2-CdA was 45.9% and for SDX-308+RIT (for both -$p<0.001$). Finally, SDX-308 combined with ALT exerted mean CCI 46.9% ($p<0.005$). The main mechanism of SDX-308 action was apoptosis. The agent triggered significant activation of caspase-3 (*vs* untreated controls - $p=0.003$), upregulating Bax protein expression. Moreover, SDX-308 induced overexpression of p73 protein, whereas expression of p53 was not significantly triggered. All combinations upregulated expression of proapototic Bax ($p<0.05$) in CLL cells, increasing the Bax/Bcl-2 ratio. Further investigations of detailed mechanisms of these interactions are currently ongoing in our institution. Conclusion: These data indicate that combinations of SDX-308 with 2-CdA, FA and both monoclonal antibodies, RIT or ALT, exert synergistic or additive, proapototic effects against CLL cells. This suggest feasibility of such treatment in this disease.

Chronic myeloid leukemia - Clinical II

0542

THE VARIANCE IN PATIENT-REPORTED NONADHERENCE WITH IMATINIB TREATMENT IN CHRONIC MYELOID LEUKEMIA IS ATTRIBUTABLE TO BOTH PHYSICIANS AND PATIENTS - RESULTS FROM THE ADAGIO STUDY

L. Abraham,[1] L. Noens,[2] R. De Bock,[3] G. Verhoef,[4] P. Zachee,[5] Z. Berneman,[6] Ph. Martiat,[7] Ph. Mineur,[8] K. Van Eygen,[9] M.-A. Van Lierde,[10] K. MacDonald,[1] S. De Geest,[11] T. Albrecht,[12] I. Abraham[13]

[1]Matrix45, EARLYSVILLE, USA; [2]UZ Gent, GENT, Belgium; [3]ZNA Middelheim, ANTWERPEN, Belgium; [4]UZ Gasthuisberg, LEUVEN, Belgium; [5]ZNA Stuivenberg, ANTWERPEN, Belgium; [6]UZA, ANTWERPEN, Belgium; [7]Institut Jules Bordet, BRUXELLES, Belgium; [8]Hopital St. Joseph, GILLY, Belgium; [9]AZ Groeninge, KORTRIJK, Belgium; [10]Novartis Pharma, VILVOORDE, Belgium; [11]University of Basel, BASEL, Switzerland; [12]Matrix45 & University of Virginia, EARLYSVILLE, VA, USA; [13]Matrix45 & University of Arizona & University of Pennsylvania, EARLYSVILLE, VA, USA

Background. Imatinib therapy for chronic myeloid leukemia is a long-term treatment potentially compromised by patient nonadherence. Nonadherence is often seen as a patient problem. Studies tend to ignore the statistically hierarchical and partially dependent relationship between physicians and patients. A given physician may treat several patients enrolled in a study, and thus these patients share common treatment practices as a result of being *nested* under a given physician. Quantifying the proportions of variance attributable to patients and physicians may reveal the impact of class effects. *Aims*. To quantify the relative proportions of variance in patient-reported nonadherence with imatinib treatment for chronic myeloid leukemia that are attributable to physicians before any patient determinants are considered. *Methods*. The ADAGIO study1 is a prospective, 90-day observational, open-label, multicenter study of patients with chronic myeloid leukemia (CML) and treated with imatinib. Sample of 169 evaluable pts who had been on imatinib for a minimum 30 days at enrollment and treated by 46 physicians. Patients rated their nonadherence on a visual analog scale (0-100) as part of routine clinical practice. Hierarchical (multilevel) modeling was used to examine the fixed effect of physician on patient-reported nonadherence and to calculate the associated intraclass correlation coefficient (ICC), which quantifies the relative proportion of variance attributed to the hierarchically higher class (in this case physicians). *Results*. A statistically significant model for the fixed effect of physician on patient-reported nonadherence was retained ($p<0.0001$) with intercept (patients' average nonadherence rating on scale 0-100) of 4.59 (95%CI 3.16 to 6.01). The ICC associated with the class effect for clinicians was 0.346. *Summary and conclusions*. In a fixed effects analysis, 34.6% of the variance in patient self-reported nonadherence was accounted for by the class effect of clinician. Conversely, the remaining 65.4% of variance remains attributable to patients. This shows the significant effect that treating physicians may have on patients' medication behavior. Instead of routinely attributing all causes of nonadherence to patients, physicians should be cognizant of their potential role if they observe their patients to be nonadherent. Further research is needed to define and explicate both physician- and patient-related determinants of nonadherence with imatinib therapy for chronic myeloid leukemia.

0543

IMATINIB PLASMA LEVELS CORRELATE WITH MOLECULAR RESPONSE IN CML PATIENTS

E. Faber, D. Friedecky, J. Tomkova, S. Rozmanova, P. Rohon, I. Skoumalova, T. Adam, M. Jarosova, K. Indrak

University Hospital, OLOMOUC, Czech Republic

Background. Examination of imatinib plasma levels are recommended in the CML patients with suboptimal response or failure. However, there are reports suggesting that imatinib plasma through levels correlate both with cytogenetic and molecular response of the patients and may be used for routine management of the treatment. *Aims.* In order to analyse the impact of imatinib plasma levels on treatment results we have introduced the examination of imatinib plasma levels into our laboratory follow-up of CML patients in 2007. All patients have signed informed consent before blood sampling. *Methods.* High performance capillary electrophoresis for determination of imatinib was used. Separation of drug

and desmethylated metabolite in methanol deproteinated plasma was performed in 50 mmol/L citrate buffer adjusted with γ-amino-n-butyric acid to pH 3.6. Limit of detection of the method was 10 nmol/L and recovery and imprecision were better then 86.7 % and 4.68 %, respectively. *Results.* We report here the results of the first comparison of plasma drug levels in the CML patients with optimal (ratio of BCR-ABL/ABL lower than 0.06%) and suboptimal (ratio of BCR-ABL/ABL higher than 0.06%) molecular response. Total 115 samples from 76 CML patients were examined. However, only 18 samples from patients with major and better molecular response and 17 samples from patients with suboptimal molecular response were chosen for the final comparison. The actual sample was eligible for analysis providing patient had been treated with standard dose 400mg daily for at least one year, there was no other evident cause of the suboptimal response (any presence of additional cytogenetic abnormalities or BCR/ABL mutation) and the sample was taken between 24±6 hours after the ingestion of the drug. Other medication, the actual duration of the therapy (providing it was longer than one year), time interval between the dose and the meal ingestion, weight, body-mass index, body surface area or co-morbidity of the patients were not taken into the account. All patients except the two in the suboptimal group have achieved the complete cytogenetic response and in addition to that other two patients in the suboptimal group have progressed, while there were any such patients in the optimal group. The results of drug level measurements showed considerable variability between the patients. However, the imatinib plasma levels in the optimal group median 2.93 μmol/L (range of 1.33-5.61) were found significantly higher that in the suboptimal group 1.75 μmol/L (range of 1.04 - 3.41) $p<0.001$, t-test. Moreover, patients with negativity in quantitative RT-PCR testing showed a trend for even higher drug plasma levels: median 3.54 μmol/L (range of 1.60-5.61) $p<0.007$, t-test. *Conclusions.* Imatinib plasma levels measured according to our methodology are in CML patients associated with molecular response to therapy. Further studies are needed in order to establish the exact role of imatinib plasma levels measurement in the management of CML patients.

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0544

ANALYSIS OF BCR-ABL MUTATIONS IN MEXICAN PATIENTS WITH IMATINIB-RESISTANT OR -INTOLERANT PHILADELPHIA-CHROMOSOME POSITIVE CHRONIC MYELOID LEUKEMIA (PH+ CML) TREATED WITH NILOTINIB

J.L. Ayala,[1] R. Hurtado,[2] N. Delgado,[3] P. Vargas,[2] L. Meillon,[3] N. Tapia,[4] M. Alvarado,[5] J. De Diego,[5] P. Azaola,[6] M. Gonzalez,[4] Y. Lugo,[7] R. Hernandez,[8] J.J. Kassack,[9] S. Cleto,[10] M. Nambo,[10] G. Reyes,[11] O. Cantu,[12] A. Aguayo,[13] I. Mucius,[14] A. Herrera,[14] K. Nacho[14]

[1]Instituto Mexicano del Seguro Social, MEXICO CITY; [2]Hospital Angeles del Pedregal, MEXICO CITY; [3]Hospital de Especialidades, CMN Siglo XXI,IMSS, MEXICO CITY; [4]IMSS, MEXICALI, BC; [5]CMN 20 de Noviembre ISSSTE, MEXICO CITY; [6]PEMEX, MEXICO CITY; [7]HGR 1 Carlos Sanchez McGregor Navarro,IMSS, MEXICO CITY; [8]UMAE 25, IMSS, MONTERREY, NUEVO LEON; [9]Hospital General de Mexico, Secretaria de Salud, MEXICO CITY; [10]Hospital de Oncologia, CMN Siglo XXI, IMSS, MEXICO CITY; [11]UMAE T1, IMSS, LEON, GTO; [12]Hospital Universitario, MONTERREY, NUEVO LEON; [13]Instituto Nacional de Ciencias Médicas y Nutricion Salvador Zubirán, MEXICO CITY; [14]Novartis Oncología, MEXICO CITY, Mexico

Background. Nilotinib, a potent and highly selective BCR-ABL inhibitor, has been approved in several countries including the US, Mexico, and Europe for the treatment of patients (pts) with Ph+ CML in chronic phase (CP) and accelerated phase (AP) who are resistant or intolerant to prior therapy including imatinib. *Aims.* To describe the baseline mutational analysis in Mexican imatinib-resistant or-intolerant CML pts before starting treatment with nilotinib. *Methods.* The study population included adult pts with imatinib-resistant or -intolerant Ph+ CML, most were in advanced phases of the disease. Physical exam, EKG, bone marrow aspiration, karyotyping and BCR-ABL mutation screening were performed in all pts before starting nilotinib. All pts signed an informed consent. Nilotinib was administered orally at a dose of 400mg twice daily (BID). *Results.* In Mexico, between October 2006 and June 2007, 53 pts were included in the nilotinib compassionate use program (CUP). The median age was 41.7 (22-68) years; 19 (44%) were men. Most pts (20/53; 47%) had accelerated phase (AP), 15 (35%) had chronic phase(CP) and 8 (19%) had blastic crisis (BC). The median duration since CML diagnosis was 73.8 (14-183) months. The median duration of prior imatinib use was 27.6 months. 37 (86%) pts were imatinib-resistant. For this analysis, only 44 (100%) pts had available data. Before starting nilotinib, a plasma-based ABL mutational analysis was performed. The ABL kinase domain was amplified in the semi-nested PCR followed by direct sequencing using ABI/prism Big-Dye terminator cycle sequencing kit on automated capillary DNA sequencer (Quest Diagnostics, ABL Kinase domain mutation): 29 (66%) pts did not have detectable mutations and 15 (34%) pts had BCR-ABL mutations at baseline. The mutational types detected were: 1 (2.3%) pt each with F359[V,F], Y253H, G250E, E355G and E255K respectively; 2 (4.5%) pts with F317L; 2 (4.5%) pts with M351T; 3 (6.8%) pts with F486S and 3 (6.8%) pts with T315I. The 12 month follow-up analysis showed that 1 pt with F359[V,F] lost the mutation. 1 pt without baseline mutation developed a new E255[K,E] mutation (pt also had progressive disease). 12 pts remain without any mutations. There are some pending mutational reports that will be available at the time of the meeting. The overall hematology response to nilotinib was 79%. The Table 1, summarizes the baseline mutational status and the current pt status. *Conclusions.* Resistance to imatinib in CML has an heterogeneous pattern. 34% of Mexican pts included in this program had detectable mutations. Nilotinib is an effective treatment option for pts with imatinib-resistant Ph+ CML regardless of the presence or absence of BCR-ABL mutations. Additional follow-up is warranted in these pts.

Table 1. Baseline mutations and most recent status.

Baseline mutational status	*Status on 15 Feb 2008*	*N (%)*
Mutation detected	Alive	8 (18%)
Without detectable mutation	Alive	22 (50%)
Mutation detected	Dead	7* (16%)
Without detectable mutation	Dead	7 (16%)
Total		44 (100%)

*3 (6.8%) pf pts had T315I mutation at baseline

0545

MANAGEMENT OF CHRONIC MYELOID LEUKAEMIA IN CLINICAL PRACTICE IN FRANCE: RESULTS OF THE FRENCH COHORT OF THE UNMET NEEDS IN CML (UNIC) STUDY

M. Michallet,[1] F. Maloisel,[2] C. Chaleteix,[3] M. Hacini,[4] F. Huguet,[5] A. Oukessou,[6] B. Bregman,[6] A. Guerci[7]

[1]Hôpital Edouard Herriot, LYON; [2]Hôpital Civil, STRASBOURG; [3]Hôpital Hôtel-Dieu, CLERMONT FERRAND; [4]Centre Hospitalier, CHAMBERY; [5]CHU Purpan, TOULOUSE; [6]Bristol-Myers Squibb, RUEIL-MALMAISON; [7]CHU Brabois, VANDOEUVRE LES NANCY, France

Background. The prevalence of chronic myeloid leukaemia (CML) and its treatment with imatinib in France have been reported in two studies (Tardieu S, *et al.*, 2005; Corm S, *et al.*, 2006), but few data exist on current practical CML management patterns or extent of imatinib resistance and/or intolerance. *Aims.* The Unmet Needs in CML (UNIC) study, conducted across eight European countries including France, aimed to: estimate proportions of (i) patients ever treated with imatinib and (ii) imatinib-treated patients who have experienced imatinib resistance and/or intolerance (primary objectives); and assess disease management patterns, including imatinib dosing, and proportions of patients tested for treatment response. Here, we present a restricted analysis of the French cohort. *Methods.* UNIC was a cross-sectional study, with retrospective chart review of patients currently treated for CML (recruited September 2006-March 2007). A registry was collected of potentially eligible patients - those aged ≥18 years and treated for CML at participating centres. Case Report Forms (CRFs) were completed for eligible patients until the recruitment target was reached. Data were collected at most recent visit and retrospectively through clinical chart review. *Results.* Of the 1266 French patients, CRFs were completed for 767 CML patients. Analysis was restricted to 654 chronic phase (CP) CML patients, of whom 627 (96%) had received imatinib at some point during follow-up. In total, 22.8% of CP-CML patients in the French cohort discontinued imatinib therapy (Table 1). By last observation, 44.2% of imatinib-treated CP-CML patients needed a change in imatinib dose; 35.2% had a dose increase and 27.6% a decrease (patients could have both). A patient was defined as imatinib resistant if reported as such by the physician in the medical chart, and intolerant to imatinib (or other concurrent treatment) if toxicity led to

a change in imatinib use, as reported in the medical chart. Rates of reported imatinib resistance or intolerance in the French CP-CML cohort were similar to those in the total European population (Table 1): 38.3% of CP-CML patients in the cohort from France were reported as imatinib resistant and/or intolerant at some time. Of CP-CML patients, 39 (6.2%) discontinued imatinib due to toxicity. In the French CP-CML cohort, median (Q1-Q3) daily dose of imatinib was 400 (397-410) mg in the total sample (N=627), 390 (322-442) mg in patients who had ever experienced imatinib resistance/intolerance (N=240), and 400 (400-400) mg in those who had never experienced imatinib resistance/intolerance (N=387). With respect to disease monitoring, 70% (424/604) of CP-CML patients had < 4 PCR analyses to assess molecular response in the last 12 months. Furthermore, 46% (40/93) of imatinib-resistant CP-CML patients had not been assessed for mutations since diagnosis. *Summary and Conclusions.* In this large observational study of CML patients in France, nearly all patients were exposed to imatinib therapy. Nearly half needed a change in imatinib dose, 38.3% experienced imatinib resistance and/or intolerance and 22.8% discontinued imatinib according to physician criteria. Molecular monitoring of disease appeared to be used less often in clinical practice in France than according to recommendations.

Table 1.

Imatinib-treated patients, n/N (%)	French chronic phase CML cohort	Total European chronic phase CML sample
Discontinued imatinib during the follow-up period	143/627 (22.8)	289/1441 (20.1)
At least one change in imatinib dose by last observation	263/595 (44.2)	677/1370 (49.4)
Imatinib dose increase	209/594 (35.2)	563/1365 (41.2)
Imatinib dose decrease	163/591 (27.6)	441/1352 (32.6)
Imatinib resistance / intolerance reported in the medical chart		
Resistant	93/627 (14.8)	206/1441 (14.3)
Intolerant	197/627 (31.4)	552/1441 (38.3)
Resistant and/or intolerant	240/627 (38.3)	643/1441 (44.6)
Resistant and intolerant	50/627 (8.0)	115/1441 (8.0)

0546

MULTICENTER OPEN LABEL STUDY OF SUBCUTANEOUS (SC) OMACETAXINE (OMA) IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS (PTS) THAT ARE RESISTANT OR INTOLERANT TO TWO OR MORE TYROSINE KINASE INHIBITORS (TKIS)

J. Cortes,[1] M. Wetzler,[2] L. Akard,[3] J.H. Lipton,[4] A.C. Benichou,[5] A.R. Craig,[6] E. Humphriss,[6] H. Kantarjian[1]

[1]MD Anderson Cancer Center, HOUSTON, USA; [2]Roswell Park Cancer Institute, BUFFALO, USA; [3]Indiana Blood and Marrow Transplantation Center, BEECH GROVE, USA; [4]Princess Margaret Hospital, TORONTO, Canada; [5]Stragen France, LYON, France; [6]ChemGenex, MENLO PARK, USA

Background. Omacetaxine mepesuccinate (semi-synthetic homoharringtonine, HHT) is clinically active against Ph^+ CML, with a mechanism of action independent of tyrosine kinase (TK) inhibition. The development of TKI resistance and intolerance is an emerging problem. Patients who have failed multiple TKIs may benefit from an alternative therapy for CML. The development of BCR-ABL mutations may result in increased resistance to currently available TKIs. *Aims.* We are evaluating the safety/efficacy of SC OMA in CML Pts that have failed or are intolerant to 2 or more TKIs and do not harbor the T315I BCR-ABL KD mutation. *Methods.* Adult pts with CML following failure or intolerance to at least 2 TKIs are eligible. All enrolled patients are provided informed consent prior to commencing OMA treatment. Induction schedule: 1.25 mg/m^2 SC twice daily (BID) for 14 days every 28 days until complete hematologic response (CHR) or hematologic improvement (HI); patients may receive maintenance OMA therapy with 1.25 mg/m^2 BID for 7 days every 28 days, for up to 24 mos. *Results.* To date, 18 Pts have been enrolled, 9 in chronic phase (CP), 7 in accelerated phase (AP) and 2 in myeloid blast phase (BP). Patients have received a median of 2 prior TKIs (range 2-4). Four patients have entered the trial with BCR-ABL mutations identified; one of these patients had multiple mutations (G250E/V299L); the others harboring the N331S, G250E and F317L mutations, respectively. Median age: 45 yrs (29-67), median disease duration: 26 mo. (16-36). Response data are available for 4 CP patients, 2 AP patients and 4 BP patients. After median follow-up on therapy of 3 mo. (1-6), overall hematologic response rate: CP 75% (3/4, 2 CHR, 1 HI) with one minor cytogenetic response, AP 100% (2/2, both CHR) with one patient achieving a partial cytogenetic response and then discontinuing therapy to receive bone marrow transplant, and BP 50% (2/4, 1 return to chronic phase, 1 HI). Median time to response is 1 mo. (range 1-2 mo); median duration of response is 6 mo. (range 1-9 m.o). Preliminary safety data are available (N=7). OMA therapy has been well-tolerated with myelosuppression the primary toxicity observed, (managed with adjustments to the number of dosing days per cycle). Incidence of treatment emergent grade 3/4 events: neutropenia 57%, thrombocytopenia 14% and anemia 14%. *Conclusions.* OMA therapy in CML Pts who have failed or are intolerant to multiple tyrosine kinase inhibitors has been well tolerated and has resulted in cytogenetic and hematologic responses.

0547

LONG-TERM SURVIVAL IN CHRONIC MYELOCYTIC LEUKEMIA AFTER A FIRST PRIMARY MALIGNANCY

D. Pulte,[1] A. Gondos,[2] H. Brenner[2]

[1]Weill Cornell Medical College, NEW YORK, USA; [2]German Cancer Research Center, HEIDELBERG, Germany

Background. Within the past 10-15 years, advances in therapy of chronic myelocytic leukemia (CML), including stem cell transplant (SCT) and tyrosine kinase inhibitors (TKI), have entered clinical practice, resulting in strong improvements in survival rates for patients with CML. The risk of CML is increased after a number of malignancies, including breast and thyroid cancer and lymphoid malignancies. Few studies of survival in patients with CML occurring after a prior malignancy are available.

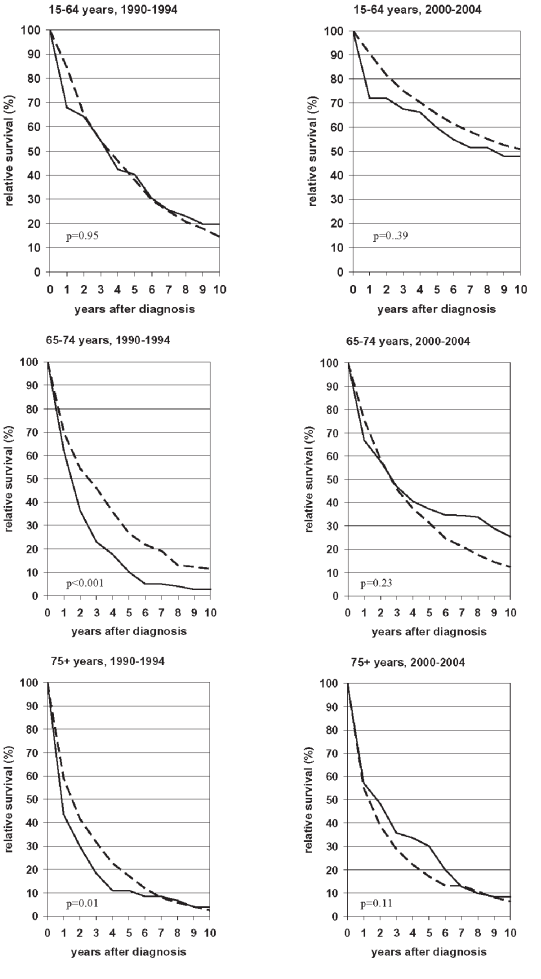

Figure 1. Ten-year relative survival curves according to calendar period and age group for CML patients with a prior malignancy (solid curves). Survival curves for patients with primary CML are given for comparison (dashed curves).

Aims. To examine survival on a population level of patients diagnosed with CML after a prior malignancy. *Methods.* We estimated trends in age specific 5- and 10-year relative survival of patients with secondary CML in the United States from 1990-1994 to 2000-2004 using the Surveillance, Epidemiology, and End Results Program database. Period analysis was employed to disclose recent developments with minimum delay. *Results.* Eight hundred eighty-three patients diagnosed with CML after a previous malignancy during the period of interest were identified. The most common preceding cancers were prostate (22.3% of preceding malignancies overall), breast (17%), and colon/rectum (15.1%). Cervical cancer was also relatively common among younger patients (9.9% among patients aged 15-64). Overall, 5-year relative survival increased from 17.6% to 37.7% (p<0.0001), and 10-year relative survival increased from 7.6% to 23.8% (p<0.0001) between 1990-94 and 2000-04. Patients experienced particularly strong improvements in 10-year relative survival (from 19.8 to 48.0%) in age group <65 and in 5-year survival (from 10.2 to 37.5%) in age group 65-74. Even in age group 75+, 5-year relative survival increased from 11.0 to 30.2%. In 1990-94, patients aged 65-74 and 75+ who were diagnosed with CML after a prior malignancy had a distinct survival disadvantage compared with patients diagnosed with CML without a prior malignancy. By 2000-04, this survival disadvantage was overcome and tentatively reversed (Figure 1). *Conclusions.* Survival for patients with CML diagnosed after a prior malignancy improved greatly in all age groups between 1990-94 and 2000-04. The survival disadvantage for patients aged 65+ with prior malignancy observed in 1990-94was absent and possibly reversed in 2000-04. This change in survival may be due to the availability of less toxic treatments such as TKI, which, unlike SCT, can be used even in patients with relatively poor health, as may be more common in older patients who have survived a prior malignancy.

0548

SEQUENTIAL MOLECULAR MONITORING OF PATIENTS WITH CHRONIC PHASE MYELOGENOUS LEUKEMIA DURING IMATINIB MESYLATE TREATMENT. CLINICAL SIGNIFICANCE AND PREDICTIVE VALUE

H Kamel,[1] Y El-Nahas,[1] M Abdel Moaty,[1] R Abdel Fattah,[1] M El Emary,[1] W. ElMetnawy[2]

[1]*National Cancer Institute, Cairo University, CAIRO, Egypt;* [2]*Cairo University, medical School, CAIRO, Egypt*

Background and Purpose. To determine the value of regular sequential determinations of bcr-abl transcripts by RT Q-PCR in identifying the pattern of long term response to IM in Egyptian patients with chronic phase CML, correlation with hematologic and cytogenetic response. *Patients and Methods.* Seventy five Egyptian patients with CP CML , treated with a daily oral dose of IM 400 or 600mg were followed for a median follow up (FU) period of 20.6 months (range 6-43 months) . RT Q-PCR was performed at regular intervals every 3 months during the whole FU period ,cytogenetic analysis by conventional karyotyping or FISH was performed every 3 or 6 months. *Results.* The median Bcr-Abl/Abl ratio before the start of therapy was 12.3 (range: 0.23-20). Analyses of the results showed that 70 patients (93%) had hematologic response, 53 patients (70%) achieved partial and complete cytogenetic response (PCR &CCR) by 12 months. Among these 53 patients only 36 patients (48%) who achieved major molecular response (3 log reduction from initial Bcr-Abl/Abl ratio), and Bcr-Abl transcripts became undetectable in 19 (25%) of them through 24 months follow up period with more than 4.5 log reduction from initial Bcr-Abl/Abl ratio upon consequetive measurements. Four patients (5%) attained major molecular response (3 log reduction) after 18 months and were considered late responders. A suboptimal response was observed in 13 patients (17%) who maintained 2 log reduction with partial cytogenetic response along 18-43 months follow up, and five of them demonstrated a reincrease of Bcr-Abl transcripts as detected by Q-RT PCR .Only one of the 5 patient could gain major molecular response upon increasing the dose of IM to 600 mg. Primary resistance was observed in 18 (24%) of the 75 patients who have not demonstrated any molecular or cytogenetic response since the start of treatment. *Study of the abl kinase mutations.* T 315 I, P 311 L , E 255 K and the M 351 T was performed by AS PCR in 12 patients. The M 351 T mutation was found in 3 cases out of 7 with primary resistance, the same mutation was also found in one out of 3 patients with suboptimal response. One patient relapsed during treatment did not have any of the studied mutations but had clonal evolution with acquisition of a new chromosomal abnormality. *Conclusions*. Molecular monitoring of bcr-abl transcripts during IM treatment of patients with CP CML is a reliable predictive method of the course of the disease. The inferior response to IM in this sample of Egyptian population may be either attributed to the limited sample size or more likely, to differences in biology from the western population.

0549

MULTICENTER EXPERIENCE WITH IMATINIB MESYLATE IN 202 NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA (CML) PATIENTS

A.M. Carella,[1] E. Orlandi,[1] M. Lazzarino,[1] M. Annunziata,[1] F. Ferrara,[1] E. Pungolino,[1] E. Morra,[1] C. Baratè,[1] M. Petrini,[1] M. Miglino,[2] M. Gobbi[2]

[1]*Hematology Division, GENOA;* [2]*Institute of Hematology, GENOA, Italy*

Background. Imatinib mesylate (IM) is a powerful and selective p210 Bcr-Abl tyrosine kinase inhibitor that has been demonstrated to be effective for the treatment of CML. *Aims.* An Italian multicenter study was performed to investigate the safety, efficacy, tolerance and compliance of IM in newly diagnosed CML patients. *Methods.* From February 2000 through February 2007, we collected 339 CML patients in different phases of disease and previously treated with Interferon-alpha and/or other therapies. Two hundred and two untreated Ph+ CML patients received Imatinib at diagnosis at a dose of 400 mg orally per day; 119 were male and 83 female with a median age of 50 (range, 17-84) years. Sokal risk: low 39,6%, intermediate 44,6% and high 15,8%. *Results.* Complete hematological remission was achieved in 94% of patients. The estimated rate of complete cytogenetic remission (CCR) was 78,7% after 12 months; with a median follow up of 38 months the rate of CCR raised to 86,8%. Levels of Bcr-Abl transcripts had fallen by at least 3 log in 52% of cytogenetic remitters patients at 12 months; after a median follow up of 38 months, 26% of these patients achieved complete molecular remission (CMR). The most commonly reported adverse events after IM were edema (including peripheral and periorbital edema) (50%), muscle cramps (37,7%), diarrhea (40,7%), fatigue (38,8%); moreover in 8 (5,2%) patients grade 3-4 events were observed consisting of pancitopenia and/or elevated liver enzymes. Eight (5,2%) patients died: 2 patients for progressive disease after allografting, 2 patients of cardiac infarction, 1 patient of severe necrotic fascitis, 1 patient of metastatic colon cancer and 2 patients of blastic crisis. In summary, at 84 months 194/202 (96%) patients are alive and 175 (87%) of them are still receiving IM. Nineteen (13%) patients discontinued Imatinib: 7 patients for adverse events grade 3-4 and 12 patients for progressive disease. *Conclusions.* These data are comparable to those of IRIS study and confirm the safety and efficacy of IM in newly diagnosed patients.

0550

NILOTINIB IS ACTIVE IN IMATINIB RESISTANT AND INTOLERANT CHRONIC MYELOID LEUKEMIA (CML) PATIENTS

M.K.M. Koren-Michowitz,[1] P. Le Coutre,[2] J. Duyster,[3] C. Scheid,[4] J.M. Rowe,[5] N. Goldschmidt,[6] E. Ribakovsky,[1] A. Nagler[1]

[1]*Chaim Sheba Medical Center, RAMAT GAN, Israel;* [2]*Campus Virchow Klinikum Charité, BERLIN, Germany;* [3]*Dept. of Internal Medicine III, Technical University of Munich, MUNICH, Germany;* [4]*Klinik I für Innere Medizin Universität zu Köln, KÖLN, Germany;* [5]*Rambam Medical Center, HAIFA, Israel;* [6]*Hadassah - Hebrew University Medical Center, JERUSALEM, Israel*

Background. Nilotinib is active in imatinib resistant pts and in those with imatinib intolerance. Here, we report the results of 63 CML pts treated with niltoinib within 2 phase 2 trials (CAMN107AIL01, Israel and ENACT, Germany). *Pts and Methods.* Mean age of the pts was 56 years (range 25-77), the mean disease duration was 64 months (range 2-55) and the mean number of prior therapies was 2.18 including imatinib. Forty pts had chronic phase (CP), 12 had blast crisis (BC) and 11 accelerated phase (AP) CML. Forty-three pts were imatinib resistant and 20 intolerant to imatinib. Thirteen pts were treated with a second generation TKI prior to nilotinib (dasatinib [11] INNO406 [1] SKI606 [1]). Fifteen pts (28% with available data) had 16 ABL KD mutations at trial entry including F359V (2), M244V (2), Y253H, L387M, E355K, T277A, T315I, G250E (2), F317L, L248V, T315I, Q252H, E279K. *Results.* After a median follow up of 12 months (range 2.5-28) CHR was achieved in 24 pts without a CHR at baseline (57%), 56% in CP, 67% in AP and 42% in BC (1 additional BC pt has returned to CP). CCyR was newly achieved in 40% of pts, 40% in CP, 20% in AP and 42% in BC and MMolR was achieved in 27% pts without a MMolR at baseline, 28% in CP, 11% in AP and 25% in BC. Best response according to baseline features is presented in Table 1. Nilotinib therapy was discontinued in 26 pts (50% of pts with available data) due to disease progression (N=11), side effects (N=9), referral for Allo SCT (N=3) and other reasons (N=3). The mean duration of treatment was 7.5 months (range 0.4- 24). Grade 3-4 hematological toxicity was seen in 32% of pts, 39% in advanced phase disease and 27% in CP (p=0.3). The most common non-

hematological adverse events were rash (N=10), fever / infection (N=8), bone pain (N=5), elevation of liver enzymes (N=5), indirect hyperbilirubinemia (N=8) and asymptomatic hyperglycemia (N=6). Grade 3-4 non-hematological toxicity was observed in 19% necessitating drug withdrawal in 6. There were no cases with pleural effusions. *Conclusions.* Nilotinib therapy can result in cytogenetic and molecular responses in CML pts including those in advanced stage, those harboring ABL KD mutations and those failing prior 2nd generation other TKIs.

Table 1.

	CHR	PCyR	CCyR	MMolR	No response/ progression
Disease Phase (N)					
CP no baseline CHR (23)	6	1	2	3	11
CP baseline CHR (8)	2	1	3	2	
CP baseline PCR (4)			1	3	
CP baseline CCR (4)			1	3	
CP baseline MMolR (3)				3	
AP (9)	3	1	1	1	3
BC (12)	2*	1	2	3	4
Prior 2nd generation TKI (N)					
CP no baseline CHR (4)	1				3
CP baseline CHR (1)			1		
CP baseline PCR (1)			1	1	
CP baseline CCR (2)				2	
CP baseline MMolR (1)				1	
AP (2)				1	1
BC (1)			1		
IM Intolerant (20)	4*	1	4	7	4
IM Resistant (43)	10	4	6	8	15
ABL KD mutations (15)	3	2	2	2	6
No ABL KD mutations (39)	8*	3	8	11	9

* 1 return to CP

0551

NONADHERENCE WITH IMATINIB TREATMENT IN CHRONIC MYELOID LEUKEMIA IS A FUNCTION OF DISEASE, HEALTH, KNOWLEDGE, AND SOCIAL FACTORS - RESULTS FROM THE ADAGIO STUDY

L. Abraham,[1] L. Noens,[2] R. De Bock,[3] G. Verhoef,[4] P. Zachee,[5] Z. Berneman,[6] Ph. Martiat,[7] Ph. Mineur,[8] K. Van Eygen,[9] M.-A. Van Lierde,[10] K. MacDonald,[1] S. De Geest,[11] T. Albrecht,[12] I. Abraham[12]

[1]Matrix45, EARLYSVILLE, USA; [2]UZ Gent, GENT, Belgium; [3]ZNA Middelheim, ANTWERPEN, Belgium; [4]UZ Gasthuisberg, LEUVEN, Belgium; [5]ZNA Stuivenberg, ANTWERPEN, Belgium; [6]UZA, ANTWERPEN, Belgium; [7]Institut Jules Bordet, BRUXELLES, Belgium; [8]Hopital St. Joseph, GILLY, Belgium; [9]AZ Groeninge, KORTRIJK, Belgium; [10]Novartis Pharma, VILVOORDE, Belgium; [11]University of Basel, BASEL, Switzerland; [12]Matrix45 & University of Virginia, EARLYSVILLE, VA, USA; [13]Matrix45 & University of Arizona & University of Pennsylvania, EARLYSVILLE, VA, USA

Background. Imatinib therapy for chronic myeloid leukemia is a long-term treatment potentially compromised by patient nonadherence. There is little evidence of the determinants of patient nonadherence. Identifying determinants of nonadherence may assist in improving patients' medication behavior and thus treatment outcomes. *Aims*. To quantify the multivariate associations among two nonadherence measures and disease, health, knowledge, and social factors and to model their relative contribution to patient nonadherence. *Methods*. The ADAGIO study[1] is a prospective, 90-day observational, open-label, multicenter study of patients with chronic myeloid leukemia (CML) and treated with imatinib. Sample of 169 evaluable pts who had been on imatinib for a minimum 30 days at enrollment. Canonical correlation analysis was used to model the multivariate relationship between nonadherence and baseline determinants. Measurements included two vectors: (1) nonadherence at 90 days vector: Basel Assessment of Adherence Scale for patients (0/1 with 1=nonadherent); and percent of imatinib taken per pill count (subtracted from 100 to reflect nonadherence); (2) Determinants vector: age; months since CML diagnosis; months since initiation of imatinib treatment; knowledge of CML disease, treatment, and imatinib; general health (SF-8); and living status (alone Y/N). Nonadherence was defined as a two-element vector so as to increase measurement reliability and decrease the biases associated with various methods of adherence assessment used in routine clinical practice. *Results*. Two canonical correlations were generated: 0.389 (Bartlett's Chi-squared=25.572, p=0.012) and 0.213 (Bartlett's Chi-squared= 5.661, p=0.341); the second canonical correlation was deleted because it was not statistically significant. The canonical loadings (or structure coefficients) for the retained model were: age 0.951; months since CML diagnosis 0.203; months since initiation of imatinib treatment 0.143; general health 0.027; knowledge of disease, treatment, and imatinib -0.366; and living alone 0.121. *Summary and Conclusions*. The patient nonadherence vector was related to the determinants vector as follows: nonadherence increased as patients were older, had been diagnosed with CML for a longer period of time, and had been on imatinib treatment for a longer period time. Taken together, these three variables may refer to disease chronicity. Nonadherence increased if patients were in slightly better health at enrollment, perhaps reflecting some self-neglect behavior as patients felt less ill. Living alone was associated with higher nonadherence, and may suggest the importance of social support and daily assistance. These variables may be warning signs for nonadherence for clinicians to consider in practice, given the long-term nature of imatinib treatment. Better patient knowledge of disease and treatment, a clinically modifiable determinant, was associated with a decrease in nonadherence. In summary, clinicians should be aware of potential nonadherence in CML patients on imatinib who present with the following risk factors: older age, longer disease duration and imatinib treatment duration, poor knowledge of disease and treatment, living alone, and relatively good health.

Reference

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0552

FLOW CYTOMETRIC ANALYSIS OF ONCOGENIC SIGNAL TRANSDUCTION PATHWAYS IN PRIMARY LEUKAEMIC CELLS

A. Beel,[1] B. Wasag,[1] V. Van Duppen,[1] J. Cools,[1] D. Bron,[2] L. Michaux,[3] G. Verhoef,[1] P. Vandenberghe[1]

[1]KULeuven, LEUVEN; [2]Institut J.Bordet, BRUSSELS; [3]University Hospitals St-Luc, WOLUWE, Belgium

Background. The development of small molecule inhibitors is one of the most exciting developments in recent cancer treatment and established oncogenic tyrosine kinases as prime targets for therapy. As the efficiency of molecular inhibitors critically depends on target inhibition, and underlined by the emergence of molecular resistance, it becomes crucial to assess the activation status of oncogenic signalling. *Aims*. 1. to develop a flow cytometric alternative for measuring intracellular protein phosphorylation status in CML and Ph$^+$) ALL. 2. to predict or confirm sensitivity or resistance to TKI on functional grounds. *Methods*. Fresh cells were incubated with imatinib (IM) or dasatinib (DAS). After fixation and permeabilisation, they were incubated with phospho-epitope specific antibodies or whole protein antibodies, and counterstained with Alexa647-conjugated secondary antibodies. Experimental conditions were fine-tuned in BCR-ABL transfected BaF3 cells and validated with western blotting. PBL were obtained by venipuncture or therapeutic leukapheresis, and density centrifugation. Measurements were performed on a FacSCanto cytometer (Becton Dickinson). *Results*. In BCR-ABL transfected BaF3, P-CRKL was reduced by 54% and 38% by IM 1 and 10 µM respectively. T315I BCR-ABL BaF3 were completely resistant, consistent with the known resistance profile. Q252H BCR-ABL BaF3 showed an intermediate response. These changes in CRKL phosphorylation were confirmed by Western blot. We tested 16 CML patients in early chronic phase (ECP), 4 patients with CML in acceleration phase/blast crisis (AP/BC), 3 CML patients in complete cytogenetic remission (CCyR), 2 patients with T315I and 2 ALL patients with t(9;22)(q34;q11). The relevant cell population was identified by surface coimmunostaining. In ECP, incubation with IM at a concentration of 1µM induced a clear-cut decrease of CRKL phosphorylation in CD34$^+$ cells, neutrophils, but not in lymphocytes. Likewise, DAS induced a reduction in CRKL phosphorylation at a concentration of 10 nM. Total CRKL immunostaining was not altered by incubation with a TKI. In samples from patients with CML in CCyR, AML or reactive neutrophil leukocytosis, and from healty controls, CRKL phosphorylation remained unchanged. P-ERK and P-TYR decreased in some, but not all patients. Other downstream targets (P-CBL, P-FAK, P-STAT5,) did not reveal a response to TKI. In 2 patients with a T315I mutation, CRKL phosphorylation was entirely resistant to IM and DAS. In one, *in vitro* sensitivity to IM/DAS was restored after IM discontinuation. Sequencing and RT-PCR of the BCR-ABL kinase domain with allele-specific primers showed a concomitant decrease and later on disappearance of the T315I clone. A *de novo* Ph$^+$ ALL was sensitive to IM/DAS, while a relapsing Ph$^+$ ALL under imatinib was entirely resistant to IM but sensitive to DAS. Kinase

domain mutation analysis is pending here. *Conclusions*. This assay reliably measures the activation status of BCR-ABL, and allows a *signalling* diagnosis of CML in patient material. Moreover, it allows to explore and monitor clinical resistance to kinase inhibitors *in vitro*, and may assist in therapeutic choices. Given the limited dynamic measurement range, its potential to predict the long-term outcome under TKI is uncertain.

0553

COMPARISON OF ALLOGENEIC STEM CELL TRANSPLANTATION AND IMATINIB FOR CHRONIC MYELOID LEUKEMIA: RESULTS FROM CAMELIA REGISTRY

E. Faber,[1] J. Muzik,[2] V. Koza,[1] E. Demeckova,[1] J. Voglova,[1] L. Demitrovicova,[3] J. Chudej,[1] I. Markuljak,[4] E. Cmunt,[5] T. Kozak,[6] E. Tothova,[1] M. Jarosova,[1] L. Dusek,[7] K. Indrak[1]

[1]University Hospital, OLOMOUC, Czech Republic; [2]Institute Biostatistics and Analyses, BRNO, Czech Republic; [3]National Oncology Institute, BRATISLAVA, Slovakia; [4]FD Roosevelt Hospital, BANSKÁ BYSTRICA, Slovakia; [5]1st University Hospital, PRAGUE, Czech Republic; [6]University Hospital Kralovske Vinohrady, PRAGUE, Czech Republic; [7]Institute of Biostatistics and Analyses, BRNO, Czech Republic

Background. CAMELIA is an international population-based registry for patients with chronic myeloid leukemia (CML) treated at 10 major centers in the Czech Republic and Slovakia. *Aims.* To compare the results of imatinib therapy and allogeneic stem cell transplantation used as the first or second-line treament for CML patients in CAMELIA Registry. *Methods.* Patients were selected from the cohort of 242 women and 272 men with Ph-positive CML patients (median age 50; range 15-83) diagnosed after 2000. Comparison was performed as a retrospective study. Registry-procedures were fully in accord with country legal and ethic requirements. *Results.* Median duration of the patients' follow-up was 3.2 years (range: 0.1-7.9 years). Imatinib was used for the first and second-line therapy in 244 and 189 patients after median of 34 and 407 days (range 0-127 and 103-2660) from diagnosis, respectively. There were no statistically significant differences between both groups with respect to age, phase of CML and Sokal score, however, there were significantly more patients with high-risk Hasford score in the group treated with first-line imatinib (p<0.001). The rate of complete cytogenetic and major molecular response did not differ between the both groups, however, there were more patients progressing 40 *vs* 62 (16.4 *vs* 32.8%; p<0.001), more patients treated with second-generation tyrosine kinase inhibitors 13 *vs* 20 (5.3 *vs* 10.6%) or transplanted 15 *vs* 17 (6.2 *vs* 8.9%) in the second-line group. As the result both overall survival and progression free survival were better in the group treated with first-line imatinib (p=0.046 and 0.054; Figure 1).

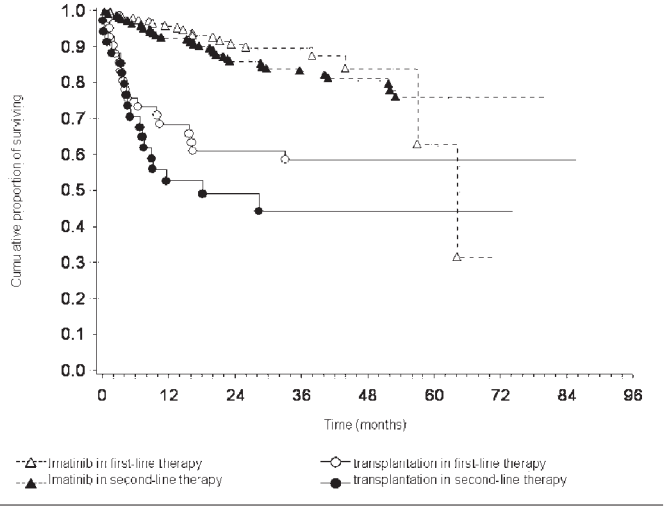

Figure 1.

Allogeneic stem cell transplantation was used as the first and second-line treatment modality in 41 and 34 patients aged 18-59 and 17-59 years (median 34 and 36) (p<0.001; Mann-Whitney test; in comparison with patients treated with imatinib). Most patients were transplanted from the related donor 43 (57.3%) after myeloablative conditioning 58 (77.3%) and using peripheral stem cell grafts 53 (70.7%). For primary and secondary transplantation median interval from diagnosis to transplant was 153 and 516 days, respectively. Complications of transplantation were the most frequent cause of death in both groups (16 and 7 patients, respectively), however, higher number of patients died after CML progression in the group where transplantation was performed as second-line treatment (1 and 8 patients, respectively). Both first and second-line imatinib therapy showed significantly better overall and event-free survival when compared with the transplantation used as the first or second-line treatment modality (p<0.001; Log-rank test Figure 1). *Conclusions.* Our analysis from the population-based registry of CML patients diagnosed after 2000 has confirmed previous reports from other studies and proved the benefit of early imatinib treatment after the diagnosis and also the clear superiority of imatinib over allogeneic stem cell transplantation in the first-line therapy of CML.

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0554

IMATINIB IN THE FIRST-LINE CML TREATMENT: AN ANALYSIS OF A COMPREHENSIVE NON-COMMERCIAL DATABASE OF ALL CONSECUTIVE PATIENTS IN A DEFINED POPULATION

J. Mayer,[1] H. Klamova,[2] D. Zackova,[1] M. Doubek,[1] P. Cetkovsky,[2] J. Rulcova,[2] K. Machova,[2] J. Moravcova,[2] D. Dvorakova,[1] T. Jurcek[1]

[1]University Hospital, BRNO; [2]Institute of Hematology and Blood Transfusion, PRAGUE, Czech Republic

Background. Imatinib has changed the paradigm of CML treatment. Moving imatinib into the first-line CML therapy is based predominantly on excellent results of IRIS trial. Population-based real-life data, however, are still scarce. *Aims*. To describe the efficacy and toxicity of imatinib in the first-line setting on an unselected population of CML patients. *Methods*. Data about CML patients in the first chronic phase from the region of about 6 million inhabitants who were treated with imatinib were collected into the database called INFINITY (tyrosine kinase Inhibitors iN the FIrst aNd followIng CML Treatment). One of the main goals was to include all consecutive patients. The database is quite detailed: there are 85 entry parameters and up to 106 parameters during the different visits in the follow-up. The BCR/ABL examination was centralized into 2 labs: one used B2M and the second one ABL as a control gene. Comparison of both methods (serial dilution of cell-line K562, and the blinded patients' samples) revealed that the results obtained from both laboratories were comparable up to 10% of BCR-ABL. The study so far is a voluntary activity with no company sponsorship. *Results*. 105 patients (pts) have been included (51 males and 54 females) aged 54 y (median; 20-77). 103 pts received initially hydroxyurea and 11 underwent leukapheresis. The Hasford score distribution was: low, 14%; intermediate, 52%; high, 34%. Median follow-up at the date of the analysis is 18 month (3-60). The median actually administered imatinib dose was 400 mg. After 12 months of therapy, the response rates were: complete hematological response 92%, complete cytogenetic response 70%, partial cytogenetic response 12%, complete molecular response 4%, and major molecular response 42%; twelve months BCR/ABL expression was 0.25% (median; 0%-31%). The improvement of ECOG performance status during the first year of therapy was striking: ECOG PS 0 increased from 25% to 79%, and PS 2 decreased from 25% to 4%. Non-hematological toxicity of any grade decreased from 80% (month 3) to 34% (month 12) with edema and cramps being the most prevalent. At 12 months, 16% of pts had hematological toxicity of any grade with anemia being dominating. In total, 14 patients stopped the treatment for various reasons: 5 pts underwent allogeneic hematopoietic stem cell transplantation, 6 pts changed the tyrosine kinase inhibitor for imatinib failure (dasatinib, n=5; nilotinib, n=1), and 3 patients were switched to dasatinib for intolerable non-hematological toxicity. There was only one death, however, linked to the alcohol intoxication. *Summary*. In this non-study population of CML patients, we can confirm, that imatinib therapy leads to the excellent disease control in a majority of patients in the first chronic phase, and these results are surprisingly well comparable to the results of IRIS trial.

0555

THERE ARE NOT ONLY POINT MUTATIONS BUT ALSO SPLICING DEFECTS IN BCR-ABL GENE IN CML PATIENTS TREATED BY IMATINIB

A.V. Misyurin, M.V. Suchkova, A.A. Krutov, E.V. Aksenova, V.V. Tikhonova, A.G. Turkina, N.D. Khoroshko

National Research Center for Hematology, MOSCOW, Russian Federation

Background. Emergence of BCR-ABL kinase domain mutations in CML patients is considered the major course of inadequate response or loss of response to treatment by tyrosine kinase inhibitors. It is recommended that if level of BCR-ABL transcripts in CML patients treated by imatinib appears to be rising and if repeat test shows that the level has risen by 0.5 or 1.0 log, the next step should be to search for such mutations. More than 50 different point mutations of BCR-ABL kinase domain and a splice BCR-ABL isoform associated with the L248V mutation are reported in CML patients with acquired resistance to imatinib. *Aims.* To search for mutations in BCR-ABL kinase domain in CML patients that prone to lose response for imatinib treatment according to data of RQ-PCR quantification. Patients and *Methods.* Chronic phase CML patients has been treated by imatinib and monitored by means of RQ-PCR (BCR-ABL/ABL)x100%). At even weak signs of BCR-ABL level increasing, the mutational analysis has been performed by means of direct PCR fragment sequencing. We have used original primers that enable to search for mutations in the area spanning from a3 to a11 ABL exons of BCR-ABL gene (the whole BCR-ABL kinase domain and flanking regions). *Results.* Sequencing has been performed for 117 CML patients that were suspected to acquire imatinib resistance according to BCR-ABL expression quantification. 33 point mutations of 13 different types have been detected, among them 730A>G(Met244Val)- (2/33-6%), 742C>G(Leu248Val)- (4/33-12%), 749G>A(Gly250Glu)- (8/33-24%), 756G>T(Gln252His)- (1/33-3%), 763G>A (Glu255Lys)-(2/33-6%), 764A>T (Glu255Val)-(2/33-6%), 835G>A(Glu279Ala)-(1/33-3%), 944C>T(Thr315Ile)-(5/33-15%), 949T>C (Phe317Leu)-(1/33-3%), 949ë>G (Phe317Leu)-(1/33-3%), 1064A>C (Glu355Gly)-(1/33-3%), 1075T>G (Phe359Val)-(3/33-9%) and 1187A>G (His396Arg)-(2/33-6%). Interestingly, all 4 cases with 742C>G (Leu248Val) point mutation have additional abnormal variant of BCR-ABL gene as a splicing isoform lacking 81 bp of exon a4. The point mutation 742C>G exchange the motif CAAGCT for CAAGGT and the latter is a strong splice acceptor site. The same structure of acceptor splicing site is responsible for excision of intron sequence between exons a4 and a5. It seems that this cryptic splice acceptor site in the middle of 4a exon carrying 742C>G point mutation may compete with natural splice acceptor site from 4th intron. It gives rise to two BCR-ABL transcripts: one carrying a point mutation and the other one with 81 bp's deletion of the 3'end of exon 4a, without frameshift. We have also observed two novel cases of splicing defects in BCR-ABL gene. In one of the patients there has been found a 72 bp's deletion of the 5'end of exon a7. In this case there is also occurred no frameshift of the CDs. On the 3'end of this deletion there is a pyrimidine-rich region followed by AG dinucleotide. There is also GT sequence on 5'end of the remnant of exon a7. The motif AG/GT is believed a typical splice donor site and that is a possible explanation of the molecular mechanism that underlies this deletion. This deleted form of BCR-ABL gene has been the only one in this patient. After a temporary and slight increasing of BCR-ABL expression this patient regain major molecular response without escalation of imatinib dose. The last case of BCR-ABL splicing defects detected in CML patients was of arbitrary nature. In this patient with primary imatinib resistance mutational analysis has revealed an insertion of 35 bp (actttgataaccgtgaagaaagaacaagatagaag) between exons a8 and a9. This insertion adds 9 novel amino acids to the BCR-ABL protein encoded up to a8 exon and then there is incorporated a stop-codon giving rise to a truncated form of BCR-ABL protein. The imatinib has been exchanged for dasatinib for this patient; nevertheless, it gives rise to only partial molecular response (1 log). It turned out that this insertion sequence is originated from the middle of 8th intron. Conclusion: The most common BCR-ABL molecular defects connected to imatinib resistance are point mutations of BCR-ABL kinase domain. However, there may be less frequent structural abnormalities of other nature that should be also considered. Here we report 3 types of splice BCR-ABL isoforms, two of them are novel. The other that was reported earlier, namely, 81 bp's deletion of a4 exons seems to always accompany 742C>G point mutation.

0556

HAS SIMULTANEOUS PREGNANCY NEGATIVELY INFLUENCED PROGRESSION AND TREATMENT RESPONSE IN CML?

H. Klamova, J. Moravcova, K. Machova, J. Brezinova, M. Markova

Institute of Hematology and Blood Transfusion, PRAGUE, Czech Republic

Background. Although it is not very frequent to make a simultaneous diagnosis of chronic myeloid leukaemia during pregnancy, it occurs. The incidence of CML and concurrent pregnancy is estimated 1/ 75 000. The treatment of pregnant CML patients is not easy but it is possible at the present time. It contains risk for the embryonic development on one hand, on the other one an untreated CML can jeopardise both the mother and the unborn baby. No standard treatment proposal has been introduced so far. *Aims.* Evaluation of possibilities and the results of CML treatment in simultaneous pregnancy and in the period after physiologic delivery. Patients and *Methods.* From the total number of 298 patients with CML treated in our department, 84 women were in childbearing age. Of them 7 (8%) became pregnant. The pregnancy confirmation was either made together with diagnosis of CML, or during CML treatment. Two patients decided abortion, 5 women decided to continue pregnancy, which was closely monitored by obstetricians. In these 5 patients (age 29-36 years) the history of the CML disease during pregnancy and 6-57 months after childbirth is followed up. The achievement of haematologic (CHR), cytogenetic (CgR) and molecular (MMoR) response was evaluated together with quality of life. Informed consent was obtained. *Results.* In 4 patients the diagnosis of CML was set together with pregnancy confirmation, lasting 9-14 weeks. In one patient, who had been already treated with imatinib for 10 months, the pregnancy was confirmed in the 21.week of its duration. During first trimester leukodepletion was the only treatment, when needed. In the 2.and 3.trimester all the patients were treated with interferon (INF) and eventually leukodepletion. All mothers gave birth to completely healthy new-borns in 38.-39.week of pregnancy, as expected. The haematological remission was achieved in all 5 patients in 3 months after INF initiation, which lasted in 4 patients during the whole pregnancy. One patient lost the haematological remission 3 weeks before expected delivery, which was solved by INF dosage augmentation together with leukodepletion. After the delivery all the patients started to be treated with imatinib 400 mg/day. In 2 of them complete cytogenetic remission (CCgR) was achieved 6 and 12 months after the imatinib initiation, one patient has major cytogenetic response in 6 months of treatment. Other 2 patients did not have optimal response to imatinib, in one of them Y253H mutation was found. After switching to 2nd generation thyrosine kinase inhibitor (dasatinib), CCgR was achieved 6 and 12 months of treatment. Major molecular response was achieved in 4 patients. The quality of life is very good (PS ECOG =0) in all 5 patients 10 to 86 months from diagnosis. *Conclusions*. We conclude that in our patients simultaneous pregnancy did not negatively influenced further response to CML treatment. CML diagnosis in pregnancy is not conclusive reason for abortion, though it is always high-risk process. The treatment of CML in pregnant women is effective. Close collaboration of haematologist and obstetrician is necessary.

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0557

THE OCCURRENCE OF PLEURAL/PERICARDIAL EFFUSIONS IN PH+ CML PATIENTS FAILING PRIOR TYROSINE KINASE INHIBITORS (TKI) BEFORE STARTING NILOTINIB - ANALYSIS OF DATA FROM COMPASSIONATE USE PROGRAM

P. le Coutre,[1] E. ODwyer,[2] L. Mendes,[3] C. Woodman[3]

[1]*Charité, BERLIN, Germany;* [2]*University College Hospital, GALWAY, Ireland;* [3]*Novartis Oncology, FLORHAM PARK, NJ, USA*

Background. There are now three TKIs approved for the treatment of patients with Ph+ CML (imatinib, nilotinib and dasatinib). Although each of these drugs inhibits Bcr-Abl, their safety profiles are different, especially regarding fluid retention events. *Aims.* Evaluate the occurrence of pleural/pericardial effusions in resistant or intolerant CML patients who had failed either imatinib therapy only or imatinib and dasatinib therapy. *Methods.* Between June, 2006 and January 2008, pts with Ph+ CML (CP, AP, BC) were evaluated for compassionate use approval of nilotinib for the treatment of resistance or intolerance to either imatinib therapy alone or imatinib followed by dasatinib. The majority of pts (98%) had not previously received nilotinib therapy. Resistance and intolerance as well as CML phase were defined using similar criteria as that previously reported in the nilotinib pivotal phase I/II registration study. At the time of medical review for compassionate use approval safety information including the presence of or the history of pleural/pericardial effusions was collected along with dosing information for imatinib and dasatinib. *Results.* A total of 621 pts were evaluated for nilotinib through the compassionate use program. The median age was 52 yrs (range, 12-88); 16 pts (2%) were < 18 yrs of age. 491 (79%) pts had failed imatinib alone (78%, resistant; 22%, intolerant) and 130 (21%) pts had failed imatinib and dasatinib therapy (53%, resistant; 47%, intolerant). For the complete dataset , 359 (58%) pts had CP, 152 (24%) pts had AP, and 110 (18%) pts had BC. For the pts treated with imatinib only, 4 pts (<1%) had pleural effusions alone (n=1) or in combination with pericardial effusions (n=3). For the pts treated with imatinib followed by dasatinib, 38 pts (29%) had pleural effusions alone (n=34) while 4 pts (3%) had pleural and pericardial effusions. Of the pts with dasatinib-associated effusions; 15% occurred on daily doses > 140 mg, 35% on doses of 140 mg, 24% on doses of 100 mg and 24% on doses of < 100 mg. There were 9 additional pts who developed effusions in which the dasatinib dose at the time of the effusion was not available. Of the 24 pts with effusions on dasatinib daily doses of 140 mg, 15 pts discontinued therapy due to an effusion and 5 pts had effusions persist despite a reduction in dasatinib dose. One pediatric pt (age 12 yr) developed a pleural effusion on a dasatinib dose of 100mg QD. None of the pts who developed a pleural/pericardial effusion on dasatinib had a history of pleural/pericardial effusion on imatinib. *Conclusions.* This large dataset supports earlier reports that pleural/pericardial effusions occur commonly in CML pts treated with dasatinib and that the occurrence of effusions with dasatinib may occur even at daily doses <140 mg, and in some cases with daily doses <100 mg.

0558

USEFULNESS OF *IN VITRO* SENSITIVITY TESTING OF LEUKEMIC CELLS TO KINASE INHIBITORS FOR THE MANAGEMENT OF TREATMENT WITH IMATINIB AND DASATINIB IN CML PATIENTS

R. Solna,[1] J. Veselovska,[1] S. Rozmanova,[2] E. Faber,[2] M. Jarosova,[2] M. Holzerova,[2] K. Indrak,[2] V. Divoky[1]

[1]*Department of Biology, Faculty of Medicine, Palacky University, OLOMOUC;* [2]*Department of Hemato-oncology, University Hospital, OLOMOUC, Czech Republic*

Background. Second generation Abl tyrosine kinase inhibitors (TKIs), such as dasatinib (DAS) or nilotinib, are currently available for the treatment of patients with chronic myeloid leukemia (CML) resistant or intolerant to imatinib (IM) therapy. The efficacy of TKIs can be evaluated *in vitro* by degree of inhibition of phosphorylation of selected signaling molecules downstream Bcr-Abl after incubation of leukocytes with the drug. *Aims.* To evaluate functional assay that enables the clinicians to predict therapy responses and the degree of resistance/sensitivity of Bcr-Abl-positive leukemia patients to TKIs. *Methods*. Quantitative real time RT-PCR (Q-RT-PCR) was performed to monitor the level of BCR-ABL transcripts. BCR-ABL mutational status was assessed using sequencing of the RT-PCR products. The *in vitro* test of sensitivity to TKIs was based on detection of inhibition of phosphorylation of Crkl and Phospho-Src Family kinases (SFK, Tyr416). The pellet of leukocytes was washed with Optimem I (Gibco) and incubated in RPMI (Sigma) with10% fetal bovine serum (Gibco) and with or without 10 μM IM or 250 nM dasatinib at 37°C/5% CO2 for 1 hour. After cultivation the cells were washed two times with ice-cold PBS and lysed in IP buffer with phosphatase and protease inhibitors. Protein lysates were subjected to electrophoresis SDS-PAGE and western blot with immunodetection with primary antibody anti-Crkl 32H4 (Cell Signaling), Phospho-Src Family (Tyr416) Antibody (Cell Signaling), and with secondary antibody (Stabilized Goat Anti-Mouse/Anti-Rabbit HRP-conjugated, PIERCE) and detection substrates (Super Signal West Dura/Femto Chemiluminiscence Substrate, PIERCE). *Results*. Sixty patients were analyzed using the test, some of them repeatedly. The extent of inhibition of phosphorylation of Crkl and SFK in this assay correlated with the clinical status and the numbers of BCR-ABL-positive cells assessed by FISH and/or Q-RT-PCR. The patients' cells with Y253H and T315I mutations showed a complete or partial failure to inhibit Bcr-Abl TK by IM, based on the ratio of mutant and wild-type clones. DAS in these patients did not inhibit Bcr-Abl TK, but completely inhibited SFK, suggesting, that in these patients SFK were not activated downstream Bcr-Abl. Patient with E255K mutation showed resistance to IM but sensitivity to DAS, which correlated with his *in vivo* responses to these drugs. Some IM-resistant patients lacking mutation of BCR-ABL proved sensitivity to higher dosage of IM or to DAS in this assay, which lead to appropriate therapeutical intervention. In one patient, BCR-ABL clone was eradicated on IM therapy; however, a Ph-negative myeloproliferative disorder associated with a high activation of SFK persisted, suggesting activation of an alternative signaling pathway not inhibited by IM and independent of Bcr-Abl. The patient developed resistance to DAS, which corresponded with a failure to inhibit SFK in *in vitro* phosphorylation assay. *Conclusions*. *In vitro* test of sensitivity of Bcr-Abl-leukemia cells to TKIs allows direct evaluation of the Bcr-Abl protein inhibition and reflects both the (possible) mutational status of Bcr-Abl as well as other mechanisms of resistance intrinsic to the cell. It could serve as a simple test for indication of these inhibitors in a patient-specific manner.

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0559

DASATINIB PHARMACOKINETICS AND EXPOSURE-RESPONSE (E-R): RELATIONSHIPS TO EFFICACY AND SAFETY IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE (CML-CP)

C. Nicaise,[1] X. Wang,[1] A. Roy,[1] M. Pfister,[1] T.T. Chen,[1] E. Bleickardt,[1] A. Hochhaus,[2] N.P. Shah,[3] F.E. Nicolini,[4] R.E. Clark,[5] G. Saglio,[6] H. Kantarjian[7]

[1]Bristol-Myers Squibb, WALLINGFORD, CONNECTICUT, USA; [2]Universität Heidelberg, MANNHEIM, Germany; [3]University of California San Francisco School of Medicine, SAN FRANCISCO, USA; [4]Hôpital Edouard Herriot, LYON, France; [5]Royal Liverpool University Hospital, LIVERPOOL, UK; [6]University of Torino, ORBASSANO-TORINO, Italy; [7]MD Anderson Cancer Center, HOUSTON, TEXAS, USA

Background. Dasatinib is 325-fold more potent than imatinib and 16-fold more potent than nilotinib against BCR-ABL *in vitro*. In a randomized, phase III, dose-optimization study in patients with CML-CP (CA180-034), a 100 mg QD schedule was associated with similar efficacy but greater tolerability (less frequent pleural effusion, cytopenia, and dose reduction/interruption) than three other dosing regimens (50 mg BID, 140 mg QD, 70 mg BID). *Aims.* To characterize the relationship between dasatinib pharmacokinetic exposure, efficacy (major cytogenetic response [MCyR]), and tolerability (pleural effusion) with data from the four dosing regimens evaluated in study CA180-034. *Methods.* Patients (n=670) were randomized to dasatinib 100 or 140 mg/d, and to a QD or BID schedule, using a 2x2 factorial design. Exposure was characterized by a population-pharmacokinetic (PPK) model and was used to derive the individual estimates of steady-state concentration (Cavg), steady-state trough concentration (Cmin), and steady-state peak concentration (Cmax). Pleural effusion E-R was described using a Cox proportional-hazards model. Covariates assessed were age, gender, body weight (PPK only), and history of cardiac disease (E R only). MCyR E R was described using logistic regression.

Table 1.

	Dosing schedule			
	100 mg QD	*70 mg BID*	*50 mg BID*	*140 mg QD*
Mean C_{min} ng/mL (CV%)	2.69 (26)	6.9 (24)	5.13 (24)	3.86 (28)
Mean C_{max} ng/mL (CV%)	66.85 (56)	53.71 (46)	37.6 (48)	94.09 (55)
Mean C_{avg} ng/mL (CV%)	14.16 (20)	20.34 (17)	14.32 (18)	19.96 (18)
	C_{min} ng/mL			
		<2.5	2.5-<5.0	≥5.0
Pleural effusion (%)		4	14	25
Dose reduction (%)		26	45	59
Dose interruption (%)		51	64	75
Major cytogenetic response (%)		69	63	62

Results. Samples from 567 of the 670 randomized patients were available for the PPK analysis. Of the patients participating in the PPK analysis, 94 experienced pleural effusions, 271 required dose reduction, and 376 required dose interruption. The PPK analysis demonstrated that dasatinib exposure was independent of age, gender, or body weight, but was dependent on dosing schedule. E-R analysis showed that the risk of pleural effusion increased with Cmin (2.33-fold for each doubling of Cmin; $p<0.001$) and age (1.93-fold for each ten years of life; $p<0.001$). Cmax and Cavg were not found to be significant predictors of pleural effusion. Cmin also correlated with dose reduction or interruption, but not with efficacy, which was related to Cavg, duration of dose interruption, and age. *Conclusions.* Cmin appears to correlate strongly with dasatinib toxicity, but not with efficacy. The lowest Cmin is achieved with a 100 mg QD dosing schedule, previously shown to have the optimal benefit'risk assessment among the tested dosing schedules.

Cytogenetics and molecular diagnostics II

0560

A NOVEL MGB PROBE-BASED ASSESSMENT OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA CARRYING NPM1 (NUCLEOPHOSMIN) EXON 12 MUTATIONS

D. Dvorakova, D. Ohlidalova, J. Pospisilova, M. Lengerova, J. Mayer

University Hospital Brno, BRNO, Czech Republic

Background. Insertions into exon 12 of NPM1 gene represent the most frequent molecular aberration in the subgroup of patients with acute myeloid leukemia (AML) otherwise showing normal karyotype. These mutations appear to be stable and can be detected in the majority of patients at relapse. Therefore, NPM1 mutant may be used as a marker for quantification of minimal residual disease (MRD). *Aims*. As an alternative to published methods that employ mutation-specific primers (either forward or reverse) and common TaqMan probe, we propose here a novel approach for long-term follow-up of residual leukemia cells based on mutation-specific probe with minor groove binder (MGB) at the 3´end. *Methods*. We designed reverse and forward primers on the exon 12 that are common for wild-type and mutant alleles and three mutation-specific short MGB probes. MGB probes include VIC/MGB probes for mutant A and mutant B allele, and one FAM/MGB probe that is specific for wild-type allele of NPM1. This approach based on allelic discrimination is highly specific and we did not observed any cross reactivity in clinical samples. For rapid screening of A or B mutation that represents approximately 78 and 12% of NPM1 mutations, respectively, we used two independent TaqMan assays involving two sets of MGB probes: FAM/wild-type probe with VIC/mutation A probe or FAM/wild-type probe with VIC/mutation B probe. For absolute quantification of each individual allele, we used one corresponding MGB probe in separate assay. All assays were performed using 7300 Sequence Detector System (Applied Biosystems). For all NPM1 targets, the best amplifications were obtained using 62°C annealing temperature. For absolute quantification, standard curve with serial dilutions of a plasmid containing the target sequences was used. The mutation value was normalized on the number of albumin gene copies. *Results.* We determined that quantitative detection of mutations A and B is highly specific without any background caused by the amplification of wild-type genomic DNA. Sensitivity of the assay was tested using 10-fold serial dilutions of genomic DNA obtained from NPM1-mutated patient at diagnosis in DNA pool from peripheral blood leukocytes of six healthy donors. At least 10-4 sensitivity was reached in all cases. Furthermore, sensitivity of the assay was tested on 10-fold serial dilution of plasmid with type A mutation, type B mutation, and wild-type NPM1 sequences, respectively. Maximal reproducible sensitivity was 10 plasmid molecules. *Conclusions.* Here we propose highly sensitive and reproducible method that could be used for long-term follow-up of MRD and represents suitable alternative for the standard routine laboratory evaluation. Our method employing highly mutation-specific short MGB probes is based on allelic discrimination assays of NPM1 mutant alleles. We suggest that MGB probes that could be successfully employed for genotyping of single nucleotide polymorphisms represent efficient approach for specific detection of NPM1 mutations without any amplification of wild-type allele.

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0561

THE POOR PROGNOSIS ASSOCIATED WITH GAIN/AMPLIFICATION OF 1Q21 IN RELAPSED MULTIPLE MYELOMA PATIENTS MAY BE OVERCOME BY VELCADE BASED REGIMEN IN CONTRARY OF THALIDOMIDE BASED REGIMEN

P. Nemec,[1] H. Greslikova,[1] J. Smetana,[2] R. Zaoralova,[3] R. Kupska,[3] K. Berankova,[2] H. Filkova,[4] D. Kralova,[3] M. Krejci,[5] L. Pour,[5] L. Zahradova,[5] V. Sandecka,[5] Z. Adam,[5] P. Kuglik,[2] R. Hajek[5]

[1]University Research Centre - Czech Myeloma Group, BRNO; [2]Institute of Experimental Biology, Faculty of Science, Masaryk University, BRNO; [3]University Research Centre - Czech Myeloma Group, Masaryk University, BRNO; [4]Department of Experimental Biology, Faculty of Science, Masaryk University, BRNO; [5]Internal Haemato-oncology Clinic, Fac. Hospital, Fac. of Medicine, Masaryk Univ., BRNO, Czech Republic

Background. The presence of chromosomal aberrations detected by fluorescence *in situ* hybridisation (FISH) in plasma cells is considered to

be an important prognostic factor for patients with multiple myeloma (MM). However for relapsed patients, it is unknown whether or not the negative impact of these aberrations can be eliminated by treatment based on the new drugs like Velcade or thalidomide. *Aims.* This study is aimed at comparison of ability to overcome the negative prognostic impact of the most common cytogenetic aberrations by treatment regimens based on Velcade or thalidomide. *Methods.* Velcade group: A total of 40 patients (median age 65.1 years; median follow-up 10.4 months; median of previous therapy lines 2 (range 0-3, 37.5% in first and 45.0% pts. in second relapse) were treated by Velcade based regimen (48% together with glucocorticoids and alkylating agens; 30% with anthracycline±dexamethasone; 22% with dexamethasone only). Thalidomide group: A total of 34 (median age 65.1 years; median follow-up 16.2 months; median of previous therapy lines 1 (range 0-4, 64% pts. in first and 20.6% in second relapse) were treated by thalidomide based regimen (94% together with dexamethasone and cyclophosphamide; 6% with dexamethasone only). Both groups were separately examined by cytoplasmic interphase FISH (cIg-FISH) for presence of 1q21 gain/amplification, del13q14, del17p13, t(4;14) and hyperdiploidy/nonhyperdiploidy. *Results.* Chromosomal abnormalities were assessed in 78.4% of all relapsed MM patients. *Results for patients treated by Velcade based regimen.* Amp1q21 was detected in 66% (21/32) patients, del13q14 and del17p13 were detected in 64% (25/39) and 24% (9/37) patients, respectively. Translocation t(4;14) was detected in 34% (11/32) and nonhyperdiploidy in 61% (14/23) patients. A total of 55% (22/40) of patients achieved ORR (CR+PR). Comparison of ORR of positive *vs* negative patients for any aberration was not significant. Comparison of TTP median of positive *vs* negative patients for each aberration was as follows: For amp1q21 reached 8.9 *vs* 7.9 months; p=0.803; for del13q14 reached 7.6 *vs* 8.5 months; p=0.570; for del17p13 reached 5.2 *vs* 9.2 months; p=0.170; and for t(4;14) reached 7.9 *vs* 8.4 months; p=0.525. *Results for patients treated by thalidomide based regimen.* Amp1q21 was detected in 56% (15/27) patients, del13q14 and del17p13 were detected in 45% (14/31) and 6% (2/31) patients, respectively. Translocation t(4;14) was detected in 39% (11/28) and nonhyperdiploidy in 63% (10/16) patients. A total of 67.7% (23/34) of patients achieved ORR. It seems to be a trend towards worst treatment response, whereas 46.7% (7/15) of amp1q21 positive patients achieved ORR *vs* 83.3% (10/12) of patients lacking amp1q21 (p=0.093). ORR was not influenced of any other named chromosomal abnormalities. Comparison of TTP median of positive *vs* negative patients for any aberration was as follows: For amp1q21 reached 8.1 *vs* not yet reached; p=0.015; for del13q14 reached 10.0 *vs* 8.0 months; p=0.906; for del17p13 TTP was not yet reached for positive *vs* 9.2 months; p=0.610; and for t(4;14) reached 9.8 *vs* 9.2 months; p=0.491. *Summary and Conclusions*. Both new drugs can overcome negative prognostic impact of all named chromosomal abnormalities except amp1q21. The difference in TTP median in amp1q21 positive and negative patients treated by thalidomide based regimen suggests that thalidomide is not able to overcome negative prognostic impact of amp1q21 in contrary of treatment based on Velcade regimen, which probably may overcome poor prognosis of amp1q21.

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HOMOZYGOUS DELETION OF P16, P14 AND P15 IS A POOR PROGNOSTIC FACTOR IN ADULT B-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA, NOT IN CHILDHOOD B-ALL: A COMPARISON THROUGH DELETION AND HYPERMETHYLATION STUDY

M. Kim,[1] S.H. Yim,[2] N.S. Cho,[3] S.H. Kang,[4] H.Y. Kim,[5] B. Oh,[5] T.Y. Kim,[5] H.J. Min,[5] C.J. She,[4] H.S. Ahn,[6] S.S. Yoon,[7] H.R. Shin,[2] H.I. Cho,[4] D.S. Lee[4]

[1]Seoul National University Hospital, SEOUL; [2]Research Institute for Cancer Control and Evaluation, National Cancer Center, IL-SAN; [3]Blood Research Institute, Korea Red Cross Center, SEOUL; [4]Department of Laboratory Medicine, Seoul National University Hospital, SEOUL; [5]Cancer Research Institute, Seoul National University College of Medicine, SEOUL; [6]Department of Pediatrics, Seoul National University Hospital, SEOUL; [7]Department of Internal Medicine, Seoul National University Hospital, SEOUL, South-Korea

Backgrounds. The biologic characteristics of childhood acute lymphoblastic leukemia (ALL) is different from those of adult ALL. Tumor suppressor genes, p16, p14, and p15 gene are inactivated either by promoter methylation, deletion or mutation, however, in leukemia, promoter methylation and deletion are the main mechanisms of inactivation. *Aims.* To compare the alteration status of p16, p14, and p15 gene in childhood and adult ALL, we analyzed the incidences and the prognostic significances of deletion and hypermethylation of p16, p14, and p15 in childhood and adult B-ALL. The association between alterations of those genes and known cytogenetic prognostic factors (BCR-ABL, TEL-AML, MLL rearrangement, and numerical changes) were also assessed. *Methods.* A total of 91 newly diagnosed B-ALL patients (61 children, 30 adults) were studied. Interphase fluorescent *in situ* hybridization study (p16, BCR-ABL, TEL-AML, MLL) and methylation specific PCR were performed using bone marrow mononuclear cells. Numerical changes were assessed by FISH and chromosome analysis. Chi-square test, Fisher's exact test, Kaplan and Meier method and Cox proportional hazards regression were applied for statistical analysis. *Results.* The frequencies of homozygous deletion of p16, p14, and p15 were 11.5% in children and 30.0% in adult, showing higher incidence in adults (p=0.029). In overall survival study, homozygous deletion was associated with the worse prognosis in adults (Figure 1, p=0.019), but not in childhood. The incidences of promoter methylation of p16, p14, and p15 were as follows: 34.4%, 14.8%, and 34.4% in children; 26.7%, 10.0%, and 40.0% 26.7% in adults, respectively, with no statistical difference between two groups. No significant association was observed between deletion and hypermethylation. Childhood ALL showed inactivation of p16 (39.3%), p14 (24.6%), and p15 (42.6%), while adult ALL showed inactivation of p16 (46.7%), p14 (33.3%), and p15 (56.7%), with the same order of frequencies, but with higher tendency of methylation in adult ALL. In p14 unmethylated adults, the homozygous deletion had adverse effect on overall survival (OS) (p=0.036). There was no significant association between chromosomal aberrations and promoter methylation in childhood and adult ALL. The children with sole MLL rearrangement showed poorer disease free survival (DFS) than those with sole homozygous deletion with low statistical significance (p=0.059). Homozygous deletion was translated into poor prognosis in OS in adults without MLL rearrangement (p=0.011). Adult with normal karyotype showed shorter OS when accompanied by homozygous deletion, although p value was 0.051. *Summary and Conclusions.* We performed a comprehensive analysis of deletion and hypermethylation of p16, p14, and p15 genes in both childhood and adult B-ALL. Homozygous deletion was more frequent in adults, showing association with shorter OS in adults, but not in children. This difference of distribution and prognostic value between childhood and adult ALL could be one of the explanations for the disparity of clinical outcome. Our results suggest that homozygous deletion is an independent prognostic factor in adult ALL.

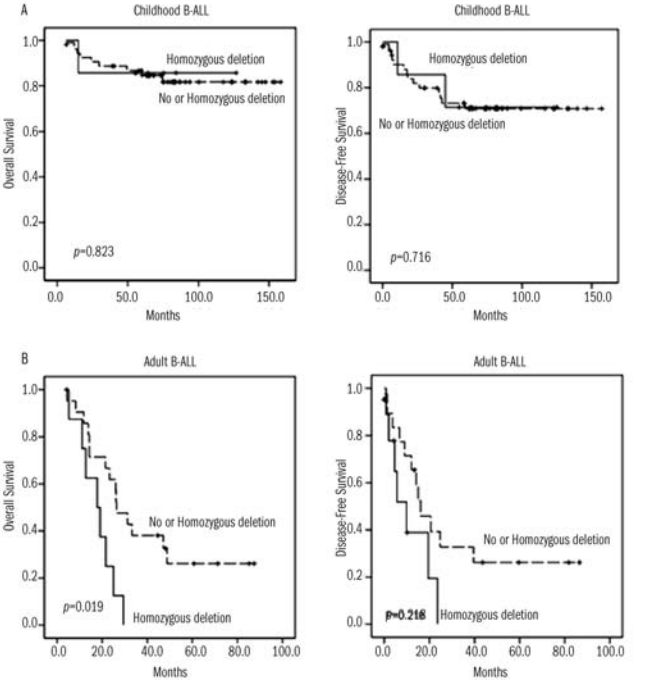

Figure 1. Survival curves for childhood and adult B-ALL.

0563

CHROMOSOMAL INSTABILITY IS CORRELATED WITH PROGNOSIS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

Ch.E. Heilig,[1] H. Löffler,[1] A. Jauch,[2] U, Mahlknecht,[3] A.D. Ho,[3] A. Krämer[1]

[1]Deutsches Krebsforschungszentrum (DKFZ), HEIDELBERG; [2]Institute of Human Genetics, University of Heidelberg, HEIDELBERG; [3]Department of Medicine V, University of Heidelberg, HEIDELBERG, Germany

Background. Chromosomal Instability (CIN) has been proposed to play a pivotal role in both early malignant transformation and tumor progression (Lengauer *et al.* Nature 1998; 396: 643-649). However, data on the presence and extent of CIN in primary malignant cells are lacking. *Aims.* We sought to evaluate the role of CIN in the evolution of myelodysplastic syndromes (MDS), the progression of MDS to acute myeloid leukemia (AML), and the prognosis of patients with these malignancies. *Methods.* We isolated CD34-positive cells from 18 patients with MDS and 27 patients with AML not receiving cytostatic treatment, from 9 healthy controls, and from 9 control patients with malignancies not involving the bone marrow. We performed fluorescence *in situ* hybridisation (FISH) hybridising centromeric probes for chromosomes 1, 6, 7, and 8 to the $CD34^+$ cells and quantitated the cell-to-cell variability of the chromosome content by determining the modal chromosome number of each chromosome and the median percentage of cells whose chromosome number differed thereof. The average of these percentages then was correlated with clinical data. *Results.* Although the CIN values did not differ significantly between subgroups, there was a small number of patients in our cohort with CIN values that were elevated more than 2 SD relative to the mean of healthy control subjects (5.8+3.0%) (Figure 1). Surprisingly, in the group of patients with MDS, all three patients with an elevated CIN value reached the endpoint, defined as progression to AML (2 patients) or death (1 patient) within 4.9, 10.2, and 12.3 months after sample collection, respectively, whereas in the remaining 15 patients with normal CIN values there was only one case of death (3.9 months after sample collection), but no progression to AML after a median follow-up of 10.7 months (range 0-18.3 months). In the group of patients with AML following MDS there were 2 additional samples with clearly elevated CIN values, one of which was from the patient who progressed from MDS. Most remarkably, the CIN value of this patient's CD34-positive cells quickly rose within one month to nearly 50% to remain at this level until 4 months later, when a diagnosis of AML was made. This patient received an allogeneic bone marrow transplant and is alive 13 months after the progression to AML, whereas the other AML patient with an elevated CIN value succumbed to his disease after 4 months. *Conclusions*. The preliminary results of our investigation point out a possible role for CIN in the progression of MDS to AML. Furthermore, our data suggest that the quantification of CIN might be valuable for identifying patients with high-risk MDS more reliably.

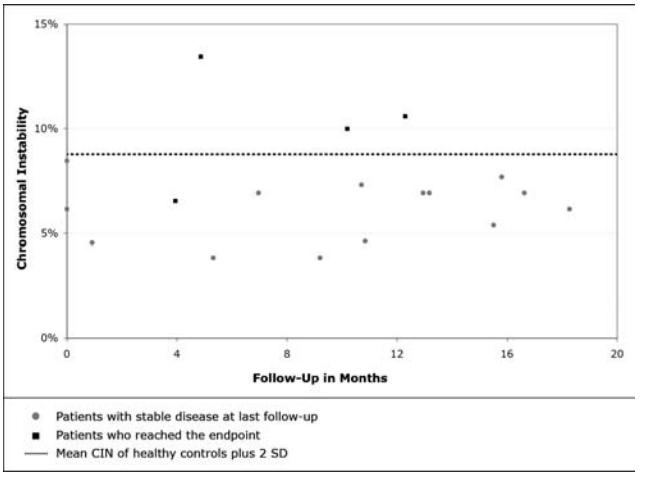

Figure 1.

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AN ANALYSIS OF FMS-LIKE TYROSINE KINASE IN CHILDHOOD ACUTE LEUKAEMIAS

A.S. Leow,[1] H. Ariffin,[2] S.K.Y. Kham,[1] T.C. Quah,[1] A.E.J. Yeoh[1]

[1]National University of Singapore, SINGAPORE, Singapore; [2]University of Malaya, KUALA LUMPUR, Malaysia

Background. FMS-like tyrosine kinase 3 (FLT3) receptor is pivotal in mitogenic pathways of progenitor cells and is expressed in most childhood leukaemias. Certain genetic subgroups demonstrate high-level expression involving a significant percentage harbouring activating mutations, typically FLT3 internal tandem duplication (ITD) and activating loop mutation (ALM). A prevalence of 15-20% of childhood acute myeloid leukemia (cAML) patients harbours either or both mutations contributory to constitutive proliferation and poorer prognosis. Conversely, these mutations may be infrequent or absent in childhood acute lymphoid leukemia (cALL), yet FLT3 over-expression harbours good prognosis in hyperdiploid ALL and infant MLL, showing irrelevance with FLT3 mutation status. *Aims.* We aim to evaluate FLT3 receptor mutations in acute leukaemias in predominantly Asian children; and its associations with any clinical or biological characteristics in these patients. *Methods.*We screened 479 leukemia patients (cAML: n=153; cALL: n=326) for the presence of FLT3 mutations. We screened codon 835 variant using PCR and restriction enzyme digest, and nucleotide change was determined by sequencing. Qualitative and quantitative analysis of FLT3-ITD was performed using Genescan (ABI 377). FLT3 expression was evaluated in 167 patients (cAML: n=95; cALL: n=72) and correlate with age, initial WBC, cytogenetic and FAB classifications, using quantitative real-time PCR (ABI 7900) and normalized with housekeeping gene ABL. The composition in the 95 cAML cohort by FAB classification were: n(M2)=34; n(M3)=14; n(M4)=11; n(M7)=12; whilst cases in each of the remaining subgroup was <7. In 72 cALL cases classified by cytogenetic subtypes, 26 were hyperdiploids, 4 were t(1;19), 12 were t(12;21), 4 were t(9;22), and 26 with no oncogene fusion. *Results.* In cAML, 12.6% (17/153) harbored ITD mutations of varying insertion lengths (15-63bp), whilst 7.2% (11/153) harbored codon 835 variants. Of 17 cAML FLT3-ITD positive, 4 (23.5%) were PML-RARA, 2 (11.8%) were AML1-ETO and 1 (6%) showed 11q23. Low allelic wildtype/ITD ratio (AR: <0.5) constitutes 23.5% (4/17). Two patients harboured both FLT3-ITD and codon 835. High expression levels of FLT-3 in cAML were significantly associated with leukocytosis (TWC>50×10^9/L; p=0.01), and age (3-9yrs > above 9yrs > below 3yrs; p=0.013). FLT3 expression in AML-M7 is significantly down-regulated (mean=0.8; median=0.02; range=0.001-4.4; p=0.022). In cALL, none exhibited FLT3-ITD mutation; only 1.5% (5/326) had codon 835 variant. High expression levels in cALL were significantly associated with age (below 3yrs > 3-9yrs > above 9yrs; p=0.03), and hyperdiploid cytogenetic subtype. *Conclusions*. Our results show a high incidence of 18.3% (28/153) in cAML and a low frequency in cALL, 1.5%. Low AR of FLT3-ITD comprises of 23.5% in our cohort, and this has been shown to have inferior prognosis in adult AML. In cAML, FLT3 expression is significantly down-regulated in AML-M7 subtype, but significantly up-regulated in the 3-9yrs age group and leukocytosis at presentation. In cALL, FLT3 expression is up-regulated in hyperdiploid cALL which is concordant with other published data. We are unable to validate this association in infant ALL due to small sample size.

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GAINS OF CHROMOSOME 1Q IN PATIENTS IN RELAPSE AND PROGRESSION OF MULTIPLE MYELOMA

J. Balcarkova, H. Urbankova, V. Scudla, M. Holzerova, J. Bacovky, M. Zemanova, K. Indrak, M. Jarosova

University Hospital Olomouc, OLOMOUC, Czech Republic

Chromosomal abnormalities have biologic and prognostic significance in multiple myeloma (MM), especially among patients with relapsed and refractory disease. Abnormalities of chromosome 1 are one of the most recurrent chromosomal changes in patients with MM and are found in approximately 45% of patients. Recent publications have shown that 1q gain, consistently involving 1q21, is associated with complex karyotypes and poor-risk genetic features. One of the genes mapping to the band 1q21 is CKS1B that plays a critical role in cell cycle progression. In addition, other oncogenes as BCL9, PDZK1, IRTA1 and IRTA2 were up-regulated by the gain of 1q. It has been suggested that the region 1q21 harbors one or more target genes associated with malignant phenotype of MM, although the critical genetic region and the tar-

get genes for chromosomal gain involving 1q rearrangement in MM remains unknown. In the present study we used FICTION method, arrayCGH or CGH (comparative genomic hybridization) to delineate the minimal region included in 1q gain in 30 bone marrow samples from MM patients, 10 in a progression and 20 with a relapse of MM (14 males and 16 females). Using FICTION method with locus specific probe 1q21/1p36 (Kreatech Biotechnology B.V, The Netherlands) we detected copy number changes of 1q21 in 14 (47 %) out of 30 samples. FICTION method to detect chromosomal changes with known prognostic impact, such as deletion of RB1, IgH translocations and trisomies of chromosomes 7, 9, 11, 15, 17 was done by locus-specific DNA probes (Abbott-Vysis, Des Plaines, Illinois, USA). CGH or 1Mb arrayCGH were performed in 5 selected cases and except other multiple abnormalities revealed gains of whole arm 1q. For rapid mapping of gained region in the other patients we used FICTION method with BAC-derived probes flanking a few selected candidate oncogenes as SF3B4, BCL 9, IRTA 1, ASPM, and ARF1. All patients with proved 1q gains had other cytogenetic abnormalities: deletion of RB1 gene and t(4; 14) were found in 3 (10 %) patients, deletion of RB1 gene and deletion of IgH gene in 2 (7 %), trisomies of examined chromosomes in 5 (17 %), single t(4; 14) in 1(3 %) and complex karyotype in 3 (10 %) patients. We will summarize and discuss our molecular cytogenetic findings with respect to the 1q gains and possible over expression of candidate oncogenes located in this region.

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FLT3-ITD, NPM1 MUTATIONS AND WT1 HYPEREXPRESSION IN CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML)

E. Such,[1] J. Cervera,[1] A. Valencia,[1] O. Fuster,[1] M.L. Senent,[1] I. Luna,[1] E. Marco,[1] M. Mallo,[2] F. Solé,[2] E. Barragán,[1] R. Collado,[3] A. Vicente,[4] V. Amigo,[5] J.C. Hernández-Boluda,[6] E. Luño,[7] S. Oltra,[1] P. Bolufer,[1] M.A. Sanz,[1] G. Sanz[1]

[1]Hospital Universitario La Fe, VALENCIA; [2]Hospital del Mar, BARCELONA; [3]Hospital General Universitario de Valencia, VALENCIA; [4]Hospital de La Ribera, VALENCIA; [5]Hospital Arnau de Vilanova, VALENCIA; [6]Hospital Clinico de Valencia, VALENCIA; [7]Hospital Central de Asturias, OVIEDO, Spain

Background. Chronic myelomonocytic leukemia (CMML) is a heterogeneous disease sharing features of myelodysplastic syndromes (MDS) and chronic myeloproliferative disorders (cMPS). Recent analysis driven to characterize cMPS have shown the important role that tyrosine-kinase cell signalling pathways play in their pathogenesis. However neither comprehensive studies on these pathways nor reports about valid new prognostic factors for MDS and AML applied to CMML are available. FLT3-ITD has been primarily found in patients with AML. However, the fact that approximately 20% of CMML patients develop secondary AML and the high prevalence of FLT3 mutations in myelomonocytic and monocytic variants of *de novo* AML (subtypes M4 and M5 according to the French-American-British [FAB] classification) led us to explore the presence of activating FLT3 mutations in patients with CMML. On the other hand, NPM1 mutations have been detected in patients who had CMML and a short (<1 year) survival, with rapid progression to AML (Caudill, 2006). Additionally, WT1 gene expression in leukemia cells is approximately 105 times higher than in normal peripheral blood (PB) cells and has been inversely correlated with the prognosis of acute leukemia and MDS (Barragán *et al.*, 2004). *Aims.* To study the frequency of FLT3-ITD, NPM1 mutations and WT1 hyperexpression in CMML. *Patients and Methods.* NPM1/FLT3-ITD mutations were studied in PB samples from 66 patients with CMML according to FAB criteria [50M/16F; median age: 72 yr. (range: 37-96); median WBC: 13.5×10^9/L (range: 2.4-170); median Hb level: 10.6 g/dL (range: 6.5-14.7); and median platelet count: 134×10^9/L (range: 4-928)] using a multiplex PCR assay followed by capillary electrophoresis. Genomic DNA was amplified using specific NPM1 and FLT3 primers. PCR products were followed by capillary electrophoresis to simultaneously analyze NPM1 and FLT3 gene alterations on an ABIPRISM 310 DNA Analyzer (Applied Biosystems, Foster City, CA). RNA samples for determining WT1 hyperexpression was available in 31 patients. cDNA was amplified using the kit TM universal Master Mix and specific WT1 primers and probe by real-time quantitative PCR on a LightCycler 1.5 (Roche Diagnostics). *Results.* None FLT3-ITD/NPM1 mutations were found in any of the 66 cases. By contrast, WT1 hyperexpression was identified in 8/31 cases (25%). Due to low numbers no specific clinical correlation or prognostic significance of this finding could be established. *Conclusions.* Our results indicate that FLT3 and NPM1 mutations are uncommon in CMML. By contrat WT1 hyperexpresion is observed in 25% of the cases. Prognostic value of this finding should be explored in large series.

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MINIMAL RESIDUAL DISEASE (MRD) MONITORING IN CML PATIENTS: COMPARISON BETWEEN AUTOMATED FISH ANALYSIS AND RQ-PCR

G. Calabrese,[1] D. Fantasia,[1] F. Pompetti,[2] R. Di Gianfilippo,[3] P. Guanciali-Franchi,[1] D. Romagno,[1] E. Morizio,[1] M. Alfonsi,[1] S. Pulini,[4] A. Spadano,[4] R. Di Lorenzo,[4] P. Di Bartolomeo,[4] A. Iacone,[2] G. Palka[3]

[1]University of Chieti, CHIETI SCALO; [2]Dip. Medicina Trasfusionale, Pescara Hospital, PESCARA; [3]Medical Genetics, Pescara Hospital, PESCARA; [4]Dip. Ematologia, Pescara Hospital, PESCARA, Italy

Background. Real-time quantitative PCR (RQ-PCR) is acknowledged as the gold standard approach for MRD monitoring in CML patients during follow up. *Aims*. To compare RQ-PCR method with a novel FISH analysis approach based on a fully automated FISH slide scanner and image analyzer (Duet BioView, Israel) for picking up rare cell events. *Methods*. Fifty-one CML patients in clinical and cytogenetic remission following imatinib (IM) therapy (44 patients), or bone marrow transplantation (BMT; 7 patients) were investigated. Ninety samples, 75 from bone marrow and 15 from peripheral blood, were tested with both RQ-PCR using TaqMan protocol (Applied BioSystems, USA), and Duet BioView FISH slide scanner by scoring 1600-4500 cells for BCR-ABL rearrangement using a dual-color, dual-fusion FISH probe combination (Kreatech, Denmark). Leukemic cell levels in the samples were arbitrarily grouped in three classes: >1%; 0,99%-0,04%; and <0,04% of scored cells. *Results*. FISH/RQ-PCR concordance was 100% for class >1%, 96% for class 0,99%-0,04%, and 84% for class <0,04% leukemic cells. Samples with FISH/RQ-PCR discordant results showed leukemic cells as evidenced by FISH close to the detection limit of FISH procedure itself (i.e. 0,04%). The discordant cases had <0,01% BCR-ABL transcript level and no recurrence of *molecular* disease in the following 18 months of treatment. In two patients FISH analysis unravelled 3/1900 (0,16%), and 8/3800 (0,21%) leukemic cells carrying two copies of BCR-ABL fusion, i.e. double Ph, which are undistinguishable from those with a single copy of BCR-ABL rearrangement when investigated by RQ-PCR. IM dose escalation (800 mg/day) resulted in disappearance of double BCR-ABL leukemic cells, which are still absent 24 and 30 months from high-dose therapy start, respectively. Peripheral blood samples also showed FISH/RQ-PCR concordant results when >3000 cells were scored by FISH. *Conclusions*. Present data show that automated FISH analysis by scoring >1600 bone marrow or >3000 peripheral blood cells/sample provides useful information for MRD monitoring in CML patients, being FISH results largely overlapping with those obtained with RQ-PCR approach. Furthermore, early occurrence of double Ph-positive cells can also be recognized allowing appropriate therapy protocol modification.

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ANALYSIS OF FLT3-ITD IN 147 PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA

L. Zamora,[1] M. Cabezón,[2] J. Grau,[2] J. Ayats,[3] M. Morgades,[2] S. Marcé,[2] B. Patiño3, D. Dominguez,[2] I. Granada,[2] N. Ruiz-Xivillé,[2] M. Xandri,[2] A. Cisneros,[2] A. Serrano,[2] N. Lloveras,[2] J. Juncà,[2] A. Oriol,[2] M. Arnan,[3] C. Boqué,[3] R.F. Duarte,[3] A. Fernández de Sevilla,[3] J.M. Ribera,[2] F. Millá,[2] E. Feliu[2]

[1]Hospital Germans Trias I Pujol - ICO, BADALONA; [2]Hospital Germans Trias i Pujol - ICO, BADALONA; [3]Hosptial Duran i Reynals - ICO, HOSPITALET, Spain

Background. Cytogentic aberrations are one of the most important independent prognostic factors in AML. However 40-50% of the patients have a normal karyotype conferring an intermediate risk, but their survival is highly variable. A growth factor gene called FMS-like tyrosine kinase3 (FLT3) play an important role in this group of patients. This gene can harbour internal tandem duplication (ITD) or mutations in 25% of adult patients with AML, much of them with normal karyotype. The presence of these FLT3 aberrations is associated with an unfavourable prognosis. Some recent studies have described that increased values of the ITD/wild-type (WT) allele ratio confer a worse outcome. The aim of the present study was to characterise the prognostic significance of the ratio of ITD/WT alleles and the size of the ITD allele. *Patients and Methods.* Bone marrow or peripheral blood samples at diagnosis from 147 patients with AML were retrospectively analysed. They included 86 patients with non promyelocytic AML, 15 with acute promyelocytic leukaemia (APL), 35 AML with multilineage dysplasia and 11 with secondary AML (6 therapy-related and 5 blast crisis of Ph' negative CMPD). Cytogenetic studies were performed from unstimulated bone marrow at diagnosis and karyotypes were described according to ISCN 2005. The presence of FLT3-ITD was performed on genomic DNA using published primer molecules with few modifications (Nakao M *et al.* Leukemia 1996). Polymerase chain reaction (PCR) products were analyzed on standard 3% agarose gels. Patients with ITD were reanalysed by Genescan analysis using PCR primer FLT3 11F labelled with 6-FAM. PCR setup was identical to the standard PCR. This methodology allowed us to establish the mutant to wild-type FLT3-ITD ratio as well as the size of the duplications. *Results.* From the 147 patients, 81 have an abnormal karyotype (55.1%) and 27 harbour an ITD (18.4%). Twenty-one of these 27 patients (78%) had a normal karyotype. FLT3-ITD/WT ratio ranged from 0.041 to 5.431 (median 0.52). FLT3-ITD size ranged from 14.69 to 188.68 (median 43.97). In patients with normal karyotype the presence of FLT3 ITD was associated with inferior OS (p=0.040) but not DFS. Neither the FLT3-ITD/WT ratio nor the FLT3-ITD size had impact on DFS or OS in the present series. *Conclusions.* Although FLT3-ITD had poor prognostic significance in patients with AML and normal karyotype, the FLT3-ITD/WT ratio and the FLT3-ITD size did not have impact on prognosis in patients from the present series.

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MOLECULAR CYTOGENETIC STUDY OF CHILDHOOD T-ALL

L. Babicka,[1] E. Malinova,[1] Z. Zemanova,[1] A. Berkova,[1] J. Tajtova,[1] J. Zuna,[2] E. Mejstrikova,[2] J. Stary,[3] K. Michalova[1]

[1]General Faculty Hospital and 1st Faculty of Medicine, Charles University, PRAGUE; [2]CLIP, 2nd Faculty of Medicine, Charles University, PRAGUE; [3]Uneversity Hospital Motol and 2nd Faculty of Medicine, Charles University, PRAGUE, Czech Republic

Background. T-cell acute lymphoblastic leukemia (T-ALL) represents 10-15% of pediatric ALL cases and differs from B-lineage ALL by clinical, biochemical, immunological and chromosomal features. Acquired cytogenetic aberrations are present in 90% of newly diagnosed B-ALL patients and some of them became an independent prognostic factor. In contrast to leukemia of B-cell origin, chromosomal changes are observed in approximately 30-50% of T-ALL only, and their prognostic implication in patient's outcome still remains unclear. *Aims.* The aim of this study was to - retrospectively and prospectively - analyze by conventional and molecular cytogenetic methods bone marrow cells of children with T-ALL, to evaluate the significance of chromosomal aberrations and to assess prognostic impact of recurrent genomic changes. *Methods.* Karyotypes of all patients were analyzed at the time of diagnosis by G-banding and FISH methods. For detection of the most frequent chromosomal changes, i.e. rearrangements of TCR loci (TCRαδ-14q11, TCRβ-7q34, TCRγ-p15), deletion of p16 (9p21) and amplification of ABL (9q34), we used interphase FISH with locus-specific BAC clones or commercially available probes (DakoTM, Abbott VysisTM). Complex chromosomal rearrangements were proved by multicolor FISH with the *24XCyte* probe kit with combinatorially labeled painting probes specific for each chromosome (MetaSystemsTM). *Results.* During the years 1995-2007 we examined 55 pediatric patients with T-ALL. Archived material was available in 27 of them (9 girls and 18 boys with an average age 8,5 years). Rearrangements of TCR loci were found in 12 children (44%)-TCRαδ in 10 and TCRβ in 2 cases. Deletion of p16 was proved in 11 patients (41%)-homozygous in 8 and heterozygous in 3 cases respectively. Amplification of ABL gene was detected in one patient and in three others a supernumerary copy of this gene was revealed. Twenty one patients are living in the first complete remission. Six children died and none of them showed the rearrangements of TCR loci. The results will be discussed at the poster in detail. *Conclusions.* T-ALL remained for a long time in the shadow of B-ALL because of low incidence and normal karyotype in common. During past decade molecular cytogenetic and genetic methods have been instrumental in finding cryptic chromosomal rearrangements, which have led to the identification of several important oncogenes. In our cohort we detected by molecular cytogenetic methods chromosomal abnormalities in 82% of patients, *vs* 44% by conventional cytogenetic. However, prognostic impact should be evaluated in larger cohorts of patients and longer period of time. Thus, our work attempts to clear up the significance of chromosomal aberrations related to childhood T-ALL in order to facilitate the patients' stratification into prognostic groups similarly like it has been adopted in B-ALL.

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MIRNA PROFILING MAY BE APPLIED TO THE DIAGNOSIS OF ACUTE LEUKEMIAS

I. Sánchez-Ortega, M. Carricondo, A. Lasa, E. Bussaglia, C. Canals, A. Aventín, J. Sierra, J. Nomdedéu

Hospital de la Santa Creu i Sant Pau, BARCELONA, Spain

Background. MicroRNAs (miRNAs) are 18-22 nucleotide non-protein-coding RNAs with regulatory functions. Some miRNA play important roles in development, cell proliferation, differentiation and apoptosis. They can also function as oncogenes and tumor suppressors in multiple cancers. miRNA expression has been shown to be informative in acute leukemias diagnosis based on their differential expression in hematopoietic cell lineages and development stages. *Aims*. To test the potential clinical utility of miRNA profiling, we analyzed the expression of miRNA128a, miRNA128b, miRNAlet7b and miRNA223 in an unselected and consecutive series of acute leukemia cases. *Methods*. Forty-one consecutive acute leukemias diagnosed at the Hospital de la Santa Creu i Sant Pau in Barcelona from 2005 to 2007 were included in the study. For each case morphologic, immunophenotypic, cytogenetic and molecular data were available. The series included: 21 acute myeloid leukemias (FLT3-ITD 2 cases, MLL-ITD 1 case, AML1-ETO 1 patient, PML-RARα 2 cases, CBFβ-MYH11 1 case, complex karyotype 2 cases, other: 12 cases), 18 acute lymphoblastic leukemias (T-cell lineage 2 cases and B cell lineage 16 cases: 2 cases with bcr-abl rearrangements, 1 AF4-MLL+, 1 E2A-PBX+, 12 others) and 2 biphenotypic acute leukemias according to the EGIL criteria (Myeloid-B-cell lineage). Five normal bone marrow samples were used as controls. miRNA128a, miRNA128b, miRNAlet7b and miRNA223 quantification was performed using a stem-loop RT-PCR assay in an ABI PRISM® 7700 genetic analyzer and calculated employing the ΔΔCT method. *Results*. The assessment of the miRNA patterns were useful as cell lineage markers in nearly half of AML cases (10/21) (specificity: 100%) and in fifty percent of ALL patients (9/18) (specificity: 100%) as follows: 1) AML pattern: up-regulation of miRNAlet7b (Ct <22) and miRNA223 (Ct <15) together with the down-regulation of miRNA128a (Ct >31) or miRNA128b (Ct >32). 2) ALL pattern: relative up-regulation of miRNA 128a (Ct <29) and miRNA128b (Ct <31). *Summary*. In the light of our findings, miRNA expression signature of four miRNAs can distinguish AML from a ALL in a subgroup of patients. The use of scoring systems based on the miRNA expression could improve the currently employed immunopenotypic cell-lineage assignment.

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HIGH FREQUENCY OF RUNX1 MUTATIONS IN DE NOVO ACUTE MYELOID LEUKEMIA WITH PARTIAL TANDEM DUPLICATION OF MLL

D.C. Liang,[1] L.Y. Shih,[2] C.F. Huang,[2] T.L. Lin,[2] Y.S. Shih,[1] M.C. Kuo,[2] T.H. Lin,[2] C.L. Lai[2]

[1]Mackay Memorial Hospital, TAIPEI; [2]Chang Gung Memorial Hospital, TAIPEI, Taiwan

Background. Transcription factor AML1/RUNX1 is essential for normal hematopoiesis. RUNX1 mutations have been described in patients with AML-M0 and were rarely found in non-M0 AML. There was no report of RUNX1 mutations in AML with partial tandem duplication of MLL (MLL-PTD). *Aims.* We aimed to investigate the RUNX1 mutations in AML patients with MLL-PTD. Patients and methods. Bone marrow samples from 82 patients with MLL-PTD were examined for RUNX1 mutations. MLL-PTD was screened by Southern-blot analysis followed by RT-PCR or detected by real-time quantitative PCR. Mutation analysis of RUNX1 gene was performed by direct sequencing of all RT-PCR products amplified with 3 overlapping primer pairs which cover the entire coding sequences of RUNX1b gene from exon 3 through exon 8. Samples with abnormal sequencing results were subjected to repeated PCR using genomic DNA with alternative primers. *Results*. RUNX1 mutations were detected in 21 patients (25.6%). Three patients had two RUNX1 mutations. Taken together, 24 mutations were detected; 12 mutations were located in RHD (exons 3-5) and 12 mutations at C-terminal region (exons 6-8). The patterns of 24 mutations consisted of 5 missense mutations, 3 nonsense mutations, 15 frameshift mutations, and 1 silent mutation. Of the 3 patients carrying two RUNX1 mutations, clonal analysis showed that one patient had combined missense and frameshift mutations on the same allele, the other patient had two missense mutations on different alleles, and another patient had combined missense and silent mutations on the same allele. RUNX1 mutations were detected in 2 of 5 patients with AML-M0, 6 of 20 M1, 8 of 36 M2, 3 of 14 M4, 2 of 4 M5 and none of 3 M6 patients with MLL-PTD. Acquired trisomy 21 was found in 2 patients with RUNX1 mutations. Seven of the 23 patients with a duplication of exons 2 to 8(e8e2) had RUNX 1 mutations compared with 14 of 59 patients with exons 2 to 6 duplication (e6e2) (p=0.579). There were no differences in age, sex, blood counts, percentages of blasts in bone marrow or peripheral blood, FAB subtypes, or treatment outcome between patients with and without RUNX1 mutations. Twenty-eight of 44 patients who received induction chemotherapy achieved a complete remission and 15 had leukemia relapse. Twelve patients had relapse samples available for reanalysis, 5 patients harboring RUNX 1 mutations at diagnosis relapsed with the identical mutants, 6 patients had no RUNX 1 mutations at both diagnosis and relapse phases, and the remaining one acquired a C-terminal mutation at relapse. *Conclusions*. Our results showed that patients with *de novo* AML and MLL-PTD had a high frequency of RUNX 1 mutations with frameshift mutations at C-terminal region being the most frequent patterns. RUNX1 mutations play a role in the development and relapse of leukemia in a subset of AML patients with MLL-PTD.

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HIGH EXPRESSION OF ETS-RELATED GENE, ERG, PREDICTS POORER OUTCOME IN AML INTERMEDIATE RISK PATIENTS WITH ABNORMAL KARYOTYPE

V. Kairisto,[1] M. Hämäläinen,[2] V. Juvonen,[1] T. Lakkala,[2] J. Johansson,[1] T.-T. Pelliniemi,[1] T.T. Salmi,[3] K. Remes[4]

[1]Tykslab, TURKU; [2]University of Turku, TURKU; [3]Dept. Paediatrics, Turku University Hospital, TURKU; [4]Dept. Medicine, Turku University Hospital, TURKU, Finland

To date, two studies have suggested that overexpression of the ETS-related gene (ERG) is associated with poor outcome in normal karyotype AML. We investigated the effect of ERG expression on AML survival in a retrospective group of 92 unselected consecutive Finnish AML patients treated at the University Hospital of Turku. The six pediatric patients were treated according to the Nordic NOPHO protocol. Patients, aged 17-65 years, were treated according to the protocols of the Finnish Leukaemia Group. Patients older than 65 yrs were treated with less intensive treatments which typically included low-dose cytarabine for 5-7 d and idarubicine±thioguanine. ERG expression was analysed at presentation in bone marrow mononuclear cells of all patients. Analysis of ERG expression was carried out by quantitative PCR as previously described (Baldus *et al.* PNAS 2004;101:3915) using ABL transcript quantification as the point of reference for each sample. In addition to routine haematological and cytogenetic investigations the mutation analyses for FLT3 length and codon 835 point mutation and nucleophosmin (NPM1) mutation were done for all patients. The cytogenetic classification of the patients was based on the Medical Research Council criteria. 21/92 patients were classified into the favourable cytogenetic group (t(8,21), inv(16) or t(15;17)). Seven patients in this group also had the FLT3 length or point mutation, but none of them had NPM1 mutations. The median ERG/ABL transcript ratio was 6.7 (range 2.6-19.2). 15/92 patients were classified into the adverse prognostic group (-5/5q-, -7, 3q abnormalities or complex karyotypes with >4 abnormalities). One of them had FLT3 codon 835 mutation and one NPM1 mutation. The median ERG/ABL transcript ratio in this group was 6.8 (range 0.05-17.6). The intermediate prognostic group was the largest patient cohort in this study including 56/92 patients. 18/56 patients had abnormal and 38/56 normal karyotypes. 22 patients had mutated NPM1 gene, 15 had the FLT3 length mutation and two the codon 835 point mutation. Of the 22 intermediate risk patients with nucleophosmin mutation all except one had normal karyotype. The median ERG/ABL transcript ratio in this group was 5.2 (range 0.05-15.9). The survival analysis in this retrospective patient group could be done with the endpoint of two years for all patients. To study the effect of ERG expression on survival, each of the above mentioned subgroups were split into two halves using the subgroup specific median ERG/ABL ratio as the cut off -limit. In the intermediate group the high ERG expression tended to associate with poor survival. However, the only statistically significant difference in survival was confined to the subgroup of intermediate risk patients with abnormal karyotype (Figure 1). It is of interest that the previous observations about the clinical significance of ERG expression in AML have been limited to patients with normal karyotype. The only statistically significant effect on survival we could observe was for the patients with abnormal karyotype in the intermediate prognostic group. It remains to be investigated whether this effect is also linked to some additional molecular abnormalities. The Figure 1 below shows overall survival of all intermediate risk group AML patients (left) and of those with abnormal karyotype (right). Solid curves show survival of patients with ERG expression below median and the broken lines of those with ERG expression above median. The difference of the Kaplan-Meier curves on the right is statistically significant (p=0.038).

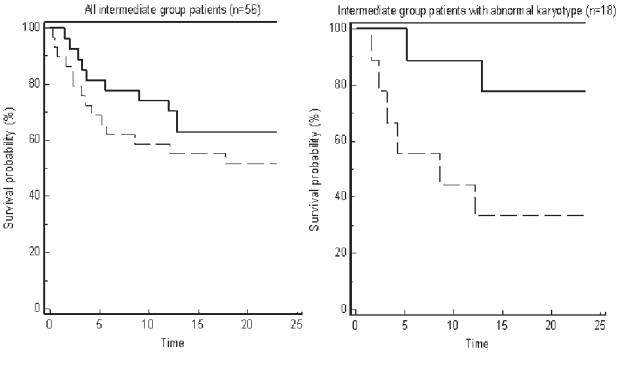

Figure 1. Overall survival (months).

Epigenetics, transcription and signalling

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IN VITRO AND *IN VIVO* STUDY OF TREATMENT WITH THE HDAC INHIBITOR VALPROIC ACID (VPA) IN TWO PATIENTS AFFECTED BY ACUTE MYELOID LEUKAEMIA POST MYELODYSPLASTIC SYNDROME

C. Tatarelli,[1] M.A. Aloe Spiriti,[1] F. Saltarelli,[1] E. Conte,[1] E. Montefusco,[1] G. La Verde,[1] A. Ferrari,[1] B. Monarca,[1] C. Nervi[2]

[1]Azienda Ospedaliera Sant'Andrea, ROME; [2]Departments of Histology and Medical Embryology, University of Rome La Sapienza, ROMA, Italy

Background. Acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) are clonal myeloid diseases that predominantly affect elderly patients, for which therapeutic options are limited. Since both disorders are characterized by impaired maturation of hematopoietic progenitor cells, differentiation induction is an attractive treatment strategy. Histone deacetylase (HDACs) inhibitors are able to induce acetylation of the core histones, allowing recruitment of transcription factors complexes and leading to transcriptional reactivation. Recently it has been demonstrated that treatment with the HDACs drug valproic acid (VPA) potentiates or restores retinoic acid (RA) induced myeloid differentiation in fresh AML blasts from patients. Several works reported a well established efficacy and clinical benefit of VPA or associated with RA in patients with MDS, and in poor risk or chemotherapy- resistant AML patients.Aims.In this work we analyzed the effect of VPA monotherapy in two untreated cases of AML secondary to MDS (sAML/MDS) *in vitro* and *in vivo*. *Patients and Methods*. One 81 year old AML-M2 woman (patient#1) and one 81 year old AML-M6 72 year old men (patient#2), both uneligible for intensive chemotherapeutic regimens, received oral VPA as compassionate treatment. Preclinical *in vitro* studies on the effect of differentiation, proliferation, apoptosis were done five days before starting the *in vivo* treatments. The effect of RA was tested for the eventual *in vivo* administration, in case of resistance to VPA. Serum VPA concentrations were mantained within the therapeutic limits established for the treatment of patients with epylepsy (50-110 ug/mL). Mononuclear cells were isolated for both patients for the *in vitro* study at day -5 and for the *in vivo* study at day 0,7,14,21,28,42,53 for patient #1, and at days 7,14,21,28,43,100 for patient # 2 of treatment from BM and/or PB, and evaluated by: morphology, Flowcytometry, Acetylation of Histones, RNA expression by Real time PCR, Cell Cycle analysis. VPA serum levels were measured at the same above days. Results. VPA alone or with RA (RA+VPA) *in vitro* induced the appearance of cells with meta-myelocte morphology-like morphology in both cases. Moreover the treatment with these agents induced a reduction of the percentage of blasts, that was more evident for the AML-M2 type. Cell cycle analysis revealed a slight increase of cells in G0/G1 phase and G2/M and a decrease of the S-phase of the cell cycle with the RA, VPA and RA+VPA treatments in both patients. The addition of RA, VPA and both agents to the culture of blast cells reduced the percentage of $CD34^+$ cells in AML-M2, a marker present on leukemic blasts. A reduction of the % of the CD235 marker expressed in erythroid AML-M6 blasts, was found after culturing cells with VPA. Moreover, the gene expression analysis showed that myeloid differentiation genes are up-regulated by VPA, in particular MPO and GM-CSF for AML-M2 and G-CSF and GM-CSF for AML-M6.The overall *in vitro* results represented a good rationale for the transcriptional/differential treatment with VPA of these patients *in vivo*. Here we show that achievement of VPA therapeutic serum levels (50ug/mL) correlates with global hyperacetylation of histone H3 and H4 in leukemic blasts and clinical/biological responses in AML patients. Survival from VPA treatment was 85 days for patient #1 and 244 for patient #2. A major neutrophil response (MaR-N) in patient #1 was associated, at the cellular level, with a widespread histone acetylation of the peripheral blood leukemic blasts. In patient#2 the acetylation status correlates with the VPA level that paralleled the increase of the neutrophil count. In this patient a higher proportion of reduction blasts was observed (35%), although he presented a minor neutrophil response (MiR-N). Moreover in this patient a 4 cm diameter lesion at the left upper pulmonary tract visible at a CT chest, that was performed at the time before treatment, disappeared at a subsequent CT thoracic scan after the VPA treatment. While the biospy of the mass was not done because of the low platelet count, and no antibiotics or antifungines were administered between the two CT scan, the lesion was strong suggestive for lung cancer. Valproic has been shown to induce differentiation of bronchial epithelial cells, and regression of lung metastasis of breast carcinoma in animal mice *in vivo* treated with valproic acid has been previously observed. Conclusion.This is the first biological and clinical study that showed that differentiation/transcriptional therapy with VPA was of clinical benefit in these two old patients.

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IDENTIFICATION OF NOVEL EPIGENETICALLY SILENCED TUMOUR SUPPRESSOR GENES IN HODGKIN AND NON-HODGKIN LYMPHOMA

S. Röhrs,[1] J. Romani,[1] A. Rosenwald,[2] W. Dirks,[1] H.G. Drexler,[1] H. Quentmeier[1]

[1]DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen, BRAUNSCHWEIG; [2]University of Würzburg, WÜRZBURG, Germany

Background. Epigenetic inactivation of tumour suppressor genes (TSGs) by promoter CpG island hypermethylation is a common hallmark of human cancer. It is generally agreed that CpG island hypermethylation profiles are specific for different tumour types. Therefore, the methylation patterns of TSGs might prove useful in cancer diagnosis and are potentially valuable for unravelling causes of tumourigenesis. *Aims.* Here, we set out to elucidate whether the methylation profile of TSGs would allow the classification of diverse lymphoma entities, as well as the identification of novel epigenetically silenced genes in these diseases. *Methods.* We analysed the methylation status of 24 different TSGs in combination with copy number changes. Thirty-nine lymphoma cell lines were tested, representing Hodgkin lymphoma plus five distinct subtypes of non-Hodgkin lymphoma (anaplastic large cell lymphoma, Burkitt lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma). For this approach we used a methylation-specific MLPA (Multiplex Ligation-dependent Probe Amplification) assay. The obtained data were validated at the expression level by quantitative real time PCR. *Results.* Using the MLPA assay, we identified a group of TSGs generally methylated or deleted in all analysed lymphoma cell lines (e.g. CDH13, DAPK1, IGSF4, RARbeta). We also found several TSGs that were preferentially methylated in specific lymphoma subtypes. For example, CD44 is epigenetically silenced in all analysed lymphoma entities except for mantle cell lymphoma. Methylation of RASSF1 is characteristic for Hodgkin and Burkitt lymphomas. The inhibitory effect of promoter hypermethylation on transcription could be confirmed at the expression level. Furthermore, expression of methylated genes was reinducible by treatment of cells with the demethylating agent 5-aza-2'-deoxycytidine. *Conclusions.* Our studies on the methylation status of TSGs in lymphoma cell lines support previous methylation analyses (e.g. for RARbeta and RASSF1) performed on primary lymphoma patient material (1, 2), confirming the applicability of cell lines as model systems. In addition, we identified novel methylation patterns and report lymphoma subtype specific methylation of individual TSGs not hitherto described. Future investigations will show, whether our results on lymphoma cell lines can be verified with patient material.

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EPIGENETIC REGULATION OF WNT PATHWAY BY ABERRANT HYPERMETHYLATION OF WNT ANTAGONISTS IN ACUTE MYELOID LEUKEMIA (AML)

A. Valencia,[1] J. Román-Gomez,[2] J. Cervera,[1] E. Such,[1] E. Marco,[1] E. Barragan,[1] P. Bolufer,[1] M.L. Senent,[1] F. Moscardo,[1] M.A. Sanz[1]

[1]Hospital Universitario La Fe, VALENCIA; [2]Hospital Universitario Reina Sofia, CORDOBA, Spain

Background. The canonical Wnt pathway is evolutionary highly conserved and plays a critical role during the cell fate and differentiation decisions during embryogenesis and in adult stem cells such as the self-renewal and proliferation of haematopoietic stem cells. Deregulation of its activity is associated with a variety of human cancers, and several target genes of Wnt act either as tumour suppressor genes or protooncogenes. This signalling pathway is controlled by a number of natural Wnt antagonists that interfere with ligand-receptor interactions that activate the pathway, including members of the Dickkopf (DKK) family and the secreted Frizzled-Related Proteins (sFRP) family. Interestingly, aberrant methylation of Wnt antagonists has been found in some haematological malignancies, leading to activation of the Wnt pathway. *Objectives*.

Given that hypermethylation of CpG islands in the promoter region of several genes is an epigenetic pathway that appears to be common in AML, we studied the role of aberrant gene methylation of a panel of soluble Wnt antagonists including, sFRP1, sFRP2, sFRP4, sFRP5, DKK1 and DKK3 in the activation of the canonical Wnt pathway in AML, as well as the expression of the downstream genes regulated by the Wnt pathway, TCF1 and LEF1, and the Wnt target gene, cyclin D1. *Methods.* We selected 184 AML bone marrow samples at the time of diagnosis (110 male/74 female, median age: 60 yr, range: 16-92). Genomic DNA and RNA were extracted using standard protocols. After bisulphite treatment DNA was PCR amplified with primers specific for the methylated and unmethylated alleles of the genes. LightCycler Fast Start DNA Master SYBR Green I (Roche Diagnostics GmbH Mannheim) was used to detect gene expression by real-time PCR technique. *Results.* Hypermethylation of the gene promoters was observed in all Wnt inhibitors. Among the 184 patients, the methylation frequencies were as follows: 42% sFRP1, 32% DKK1, 31% sFRP2, 22% sFRP5, 16% DKK3 and 4% sFRP4. None methylated gene or just one were found in 56% of patients (Group I) and two or more methylated genes in 44% (Group II). Expression of TCF1, LEF1 and cyclin D1 was significantly higher in patients that had methylation of at least one gene (p=0.015, 0.01 and 0.002, respectively). In order to determine the clinical relevance of the methylation profile we compared patient characteristics between both groups. There were no differences in age, sex, presenting WBC, FAB subtype, cytogenetics risk groups and FLT3 or NPM1 mutations. In univariate analysis patients younger than 60 years with intermediate cytogenetics belonging to the Group II had a significantly reduced disease free survival than Group I at 4 years (49% *vs* 27%, respectively, p=0.001) and relapse free survival (61% *vs* 28%, respectively, p=0.03). In multivariate analysis considering age, sex, WBC, FLT3-ITD and NPM1 mutations and methylation profile of the Wnt antagonist, the combination of FLT3-ITD/NPM1 mutations was the only variable that retained an independent adverse significance. *Conclusions*. We demonstrated that hypermethylation of Wnt antagonists is a frequent event in AML and is associated with activation of the Wnt pathway as demonstrated by the up-regulation of the Wnt target genes. The methylation profile defines a group with a significantly worse prognosis in young patients with intermediate cytogenetics risk.

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EPIGENETIC CONTROL OF MHC2TA TRANSCRIPTION IN HUMAN T CELL ACTIVATION AND IN T LEUKEMIA CELLS

P.J. van den Elsen, M.C.J.A. Van Eggermond, D.R. Boom, T.M. Holling

Leiden University Medical Center, LEIDEN, Netherlands

The co-activator CIITA, encoded by the MHC2TA gene, is essential for transcriptional activation of all MHC-II genes. In humans, activated T cells express CIITA and MHC class II molecules. Expression of CIITA in human activated T cells is associated with chromatin modifications involving the MHC2TA multipromoter region (CIITA-PI, -PII, -PIII and -PIV). By using chromatin immunoprecipitation (ChIP) assays we show that the levels of trimethylated lysine 27 in histone H3 are reduced upon T cell activation. This modification is associated with compact chromatin and transcriptional silent genes and is resulting from the activity of the histone methyltransferase EZH2, a member of the polycomb group protein family. At the same time a strong increase is noted in the levels of histone H3 and H4 acetylation and in triple methylation of lysine 4 in histone H3, modifications which are associated with transcriptional active genes. Bisulfite sequencing showed that DNA methylation of CIITA-PIII and CIITA-PIV is absent in unstimulated T cells lacking CIITA and MHC class II molecule expression. Together these data reveal that epigenetic histone modifications and not DNA methylation modifications are involved in transcriptional activation of MHC2TA during T cell activation. These results are corroborated by the observations made in T leukemia cells lacking CIITA and MHC class II molecule expression. However, in these tumor cells DNA methylation modifications were also noted in addition to the triple methylation modification of lysine 27 in histone H3. To evaluate the contribution of the various epigenetic DNA and histone modifications, we have generated Jurkat T leukemia cells by transfection of a modified histone H3 with a lysine to arginine mutation at position 27. Using these altered Jurkat cells and inhibitors for DNA methylation and histone deacetylase activities we are investigating the contribution of the various epigenetic processes involving DNA and histone methylation in transcriptional silencing of MHC2TA in T leukemia.

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MICRORNA IMPLICATION IN EVI1 OVEREXPRESSION IN ACUTE MYELOID LEUKEMIA

M. Gomez-Benito, I. Vazquez, C. Vicente, A. Conchillo, N. Marcotegui, E. Bandres, M.D. Odero

CIMA, University of Navarra, PAMPLONA, Spain

The EVI1 (ecotropic virus integration site-1) gene codes for a zinc finger transcription factor with important roles both in normal development and in leukemogenesis. High levels of EVI1 expression is a poor prognostic factor in *de novo* acute myeloid leukemia (AML) with and without 3q26 rearrangements. However, its precise role in myeloid disorders is not completely yet understood, and little is known about the pathways by which it promotes leukemic transformation. EVI1 can bind to specific DNA sequences through both of its zinc finger domains independently, and also interacts with transcriptional coactivators and corepressors. In addition, EVI1 has been implicated in signaling through the PI3K/Akt pathway and in blocking JUNK and stress-induced apoptosis. Nonetheless, additional mechanisms may also exist. MicroRNAs are a new class of evolutionary conserved small RNAs affecting gene expression at the post-transcriptional level. miRNAs have been found to participate in regulatory circuits that control development and lineage-differentiation fate of hematopoietic cells. To date, little information is available on transcription factors modulating miRNA transcription and expression at the basal or tissue specific level. Our aim was to study the possible implication of miRNAs in both EVI1 expression levels regulation (mRNA and protein) and EVI1 downstream pathways. miRNA expression, as well as EVI1, is highly regulated and shows restricted expression profiles in adult tissues. We screened the expression of 250 mature miRNAs by real-time RT-PCR in 9 AML cell lines (4 expressing EVI1 and 5 not), and performed a statistical analysis of the two groups. We found 6 miRNAs significantly upregulated in EVI1-expressing cells. Three of these miRNAs have been previously described as having important roles in cell development, proliferation and apoptosis. Besides, the seed region of two of them is entirely complementary to the EVI1 3´UTR, suggesting a possible minicircuitry EVI1-miRNAs and so, a possible implication of these miRNAs in EVI1 regulation. When stably overexpressing EVI1 in cells we have detected an increase in these two miRNA levels, although no change was detected when transient silencing EVI1. We are currently making stably EVI1 silencing to evaluate its effects on miRNA levels. We have also overexpressed these miRNAs and inhibited their activity in AML cells, detecting downregulation and a slight increase of EVI1 protein levels, respectively. Nevertheless, further functional studies must be performed in order to asses their contribution to the leukemic phenotype. In conclusion, even though a lot of interesting data about the functions and ways of action of EVI1 have already been collected, a lot of open questions remain. The miRNA implication in EVI1 overexpression and its functional study could contribute to understand the role EVI1 is exerting in AML development and to identify new EVI1 direct and indirect target genes, which could be of interest for therapeutic approaches.

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THE SECOND SIGNAL IN ANTIGEN-PRESENTING CELLS: COMPLEMENTARY JAK/STAT AND CD40 SIGNALLING MEDIATE PRO-INFLAMMATORY ACTIVATION

T. Luft,[1] M. Conzelmann,[2] E. Rodionova,[1] M. Hess,[1] A. Zota,[2] T. Giese,[3] S. Breit,[4] P. Dreger,[1] T. Luft[1]

[1]University of Heidelberg, HEIDELBERG; [2]DKFZ, HEIDELBERG; [3]Insitute of Immunology, University of Heidelberg, HEIDELBERG; [4]Dept. Paediatrics, University of Heidelberg, HEIDELBERG, Germany

CD40L represents a strong endogenous danger signal associated with inflammation. CD40L induces pro-inflammatory activation of CD40 expressing antigen-presenting cells; however, CD40 activation alone is insufficient to induce IL-12p70 secretion. Cytokines such as IL-4, GM-CSF and IFNg complement the CD40 signal with a JAK/STAT signal, thereby permitting IL-12p70 secretion and at the same time reducing IL-10 secretion. Global inhibition of the JAK/STAT pathway resulted in the loss of IL-12p70 secretion and increased IL-10 secretion. siRNA studies revealed that JAK1, JAK2 and JAK3 enhance whereas Tyk2 inhibits IL-12p70 secretion. In addition, JAK1 directly inhibited IL-10 secretion. The mechanism of JAK/STAT modulation of CD40 signals in DC involved transcriptional regulation of mRNA of IL12p35, p40 and IL-10.

IFNg induces a strong JAK1/JAK2-phosphorylation and a rapid p35mRNA-induction, whereas IL-4 acts with slower kinetics due to a weaker JAK1/JAK2 signal and a stronger Tyk2 signal. Therefore, IL-4 signalling has to persist much longer than IFNg signalling in order to induce similar amounts of IL-12p70. This different dependence on signalling persistence may explain the distinct biological characteristics of Th1- and Th2-cytokines, which both induce IL-12p70 via activation of the same JAKs. Complementary CD40 and JAK/STAT activity is essential for IL-12p70 secretion by all human antigen-presenting cells as it was similarly observed in DC, monocytes and B cells. This strict requirement of both complementary signals opens a new way of interfering with CD40 effects by modulating the *second signal* JAK/STAT.

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JAK KINASE- AND LYSINE-CONTROLLED TRAFFICKING OF G-CSF RECEPTOR (CSF3R) ARE INDEPENDENT MECHANISMS AND SIMULTANEOUS PERTURBATION OF THESE PATHWAYS RESULTS IN G-CSF INDEPENDENT SIGNALING

J.C.M. Meenhuis, M. Irandoust, M. Valkhof, I.P. Touw

ErasmusMC, ROTTERDAM, Netherlands

Background. The G-CSF receptor (CSF3R) plays a major role in granulopoiesis and abnormal signaling from CSF3R has been implicated in severe congenital neutropenia and AML. Previously, we showed that forward routing and ligand-induced lysosomal trafficking of CSF3R are both crucial for appropriate proliferation and differentiation of myeloid progenitors in response to G-CSF. Ubiquitination of cytoplasmic lysine (K) residues, in particular the juxtamembrane K632, plays an imperative role in CSF3R trafficking (Irandoust *et al.*, EMBO J. 2007). In addition to their role in phosphorylation of receptor tyrosines and downstream signaling substrates, JAK kinases have been implicated in controlling cell surface expression of cytokine receptors. Whether JAKs play a role in CSF3R trafficking and how this relates to the ubiquitin-controlled pathway of lysosomal routing has not been established. *Aims.* (1) To determine the role of JAK1, JAK2 and TYK2 in controlling CSF3R expression and intracellular routing. (2) To address to what extent these JAKs interfere with the internalization and lysosomal routing machinery, linked to the conserved lysines within the CSF3R cytoplasmic domain. (3) To determine the consequences of simultaneous perturbation of JAK and receptor lysine-mediated routing for G-CSF signaling. *Methods.* Ba/F3 cells and variants overexpressing JAK1, JAK2 or TYK2 and human fibrosarcoma cells and variants selectively deficient for these JAKs were transduced with CSF3R constructs. CSF3R expression and internalization kinetics were determined by flow cytometry. Stability of the CSF3R protein was determined by Western blotting. CSF3R ubiquitination was assessed by Western blot following ligand affinity purification of CSF3R. Confocal laser scanning microscopy (CLSM) was applied to study intracellular trafficking of CSF3R in conjunction with activated JAKs. *Results.* Elevated levels of JAK1, JAK2 or TYK2 significantly increased CSF3R membrane expression and total CSF3R protein levels. This required the integrity of tryptophan 650 of CSF3R, which we establish to be involved in JAK binding by co-immunoprecipitation. In fibrosarcoma cells, depletion of neither JAK1, JAK2 nor TYK2 modulated CSF3R membrane expression, showing that JAKs are redundant in this respect. JAKs did not enhance CSF3R membrane expression and protein levels by altering receptor internalization kinetics or by reducing ubiquitination of the lysosomal routing determinant K632, suggesting that these mechanisms are independent. This was corroborated by experiments showing that the effects of JAKs and mutation of K632 on CSF3R membrane expression and stability were fully additive. Strikingly, increased JAK2 levels conferred growth factor independent proliferation in conjunction with CSF3R mutant K5R, which lacks all cytoplasmic lysine residues and consequently fails to route to lysosomes. *Summary and Conclusions.* JAK1, JAK2 or TYK2 elevate steady state CSF3R cell surface expression and enhance total CSF3R protein levels in hematopoietic cells. These effects are not due to masking critical motifs involved in lysosomal targeting. The finding that increased JAK2 levels confer spontaneous signaling from K5R CSF3R provides a potential novel mechanism for transformation, worth studying in further detail in myeloid disorders.

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TOLL-LIKE RECEPTOR 4 ACTIVATION IN THE BONE MARROW OF PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA MAY CONTRIBUTE TO THE INFLAMMATORY MARROW ENVIRONMENT

M. Velegraki, N. Kanellias, I. Mavroudi, G. Eliopoulos, H. Papadaki

University of Crete School of Medicine, HERAKLION, CRETE, Greece

Background. Chronic idiopathic neutropenia (CIN) is an acquired disorder of granulopoiesis characterized by impaired neutrophil production in the bone marrow (BM) mainly due to the local over-production of inflammatory mediators that induced the apoptotic death of the granulocytic progenitor cells. We have previously reported increased expression of Toll-like Receptor (TLR)-4 in patient BM $CD14^+$ cells. The ligand(s) and pathophysiologic significance of TLR-4 up-modulation in CIN have not been investigated. *Aims*. To probe the potential role of TLR-4 in the generation of the inflammatory BM milieu in patients with CIN and to evaluate the local levels of the High Mobility Group Box 1 (HMGB1) protein as potential TLR-4 endogenous ligand. *Methods.* BM aspirates were obtained from 20 CIN patients and 11 healthy individuals after informed consent. To determine whether TLR-4 upmodulation in CIN BM is associated with activated TLR-mediated signal transduction pathway that might result in increased local production of pro-inflammatory cytokines, we examined the expression of 84 genes associated with TLR-mediated signal transduction. For this purpose mRNA was extracted from immunomagnetically sorted BM $CD14^+$ cells from patients and controls and a quantitative RT-PCR was performed using the PCR array technology. To examine the involvement of TLR signaling in BM cytokine production, plastic adherent BM monocytes from CIN patients were treated for 24-hours with autologous BM plasma in the presence or absence of a specific TLR-4 inhibitor or a placebo and the levels of interleukin (IL)-6, IL-8, IL-1beta and tumor necrosis factor (TNF)a were evaluated to determine the percentage of inhibition of cytokine production. Finally, HMGB1 levels were evaluated in long-term BM culture (LTBMC) supernatants by means of ELISA. *Results.* Quantitative RT-PCR analysis of 84 genes involved in TLR-4 signaling, demonstrated increased expression of 43 genes in BM $CD14^+$ cells of CIN patients compared to healthy controls. The most prominent expression were obtained for genes considered as key-mediators of the TLR signaling such as TRAF6 (3.44 fold up-regulation), MyD88 (2.23 fold up-regulation), TICAM2 (4.49 fold up-regulation), IRAK1 (3.16 fold up-regulation), and TIRAP (4.25 fold up-regulation). These data suggest that the TLR downstream signaling pathway is activated in the BM CD14+ cells of CIN patients. Furthermore, BM plasma from CIN patients induced the production of IL-6, IL-8, IL-1beta and TNFa by autologous BM monocytes in a TLR-4 dependent manner, since percentage of inhibition of cytokine production was significantly higher in the presence of TLR-4 inhibitor (72.5%±32.31%, 49.41%±18.41%, 83.86%±4.53% and 67.87%±1.79%, respectively) compared to placebo (4.18%±4.84%, 0.341%±0.30%, 5.61%±4.57% and 11.47%±8.37%, respectively) (p=0.022, p=0.009, p=0.0003, p=0.00003, respectively). Finally, CIN patients displayed significantly increased levels of HMGB1 in LTBMC supernatants (4.63±4.45 ng/mL) compared to controls (1.73±1.35 ng/mL, p= 0.045). *Conclusions*. TLR4 up-modulation in the BM $CD14^+$ cell compartment of CIN patients has a significant role in the pathophysiology of CIN contributing, at least in part, in the pro-inflammatory cytokine over-production in CIN BM. The increased levels of HMGB1, possibly derived from the late apoptotic/dead granulocytic progenitor cells, may represent the TLR-4 activating ligand.

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THE ONCOGENIC C-KIT/D816V MUTANT CIRCUMVENTS THE REQUIREMENT OF SRC FAMILY KINASES FOR C-KIT SIGNAL TRANSDUCTION

J. Sun, E. Pedersen, L. Ronnstrand

Laboratory Medicine, MALMÖ, Sweden

Background. The receptor tyrosine kinase c-Kit plays a critical role in hematopoiesis and gain-of-function mutations of the receptor are frequently seen in acute myeloid leukemia (AML) and mastocytosis. The most common mutation of c-Kit in these disorders is a substitution of the aspartic acid residue in position 816 to a valine (D816V), leading to constitutive activation of the receptor. *Aims.* In this study we aimed to investigate the role of Src family kinases in c-Kit/D816V signaling. Src family kinases are necessary for the phosphorylation of wild-type c-Kit as well as of activation of downstream signaling pathways including

receptor ubiquitination and the Ras/Mek/Erk pathway. *Methods.* Tyrosine 568 of c-Kit, that is responsible for Src activation in wild-type c-Kit signaling, was mutated to phenylalanine on both wild-type c-Kit and c-Kit/D816V and stably transfected into the hematopoietic cell line Ba/F3. *Results.* Our data demonstrate that, unlike wild-type c-Kit, the phosphorylation of c-Kit/D816V is not dependent on Src family kinases. In addition we found that neither receptor ubiquitination nor Ras/Mek/Erk activation of c-Kit/D816V required Src family kinases. Using a kinase activity assay we revealed that c-Kit/D816V gains Src activity of its own. The serine/threonine kinases Akt and Erk play important roles in receptor tyrosine kinases mediated cell survival and proliferation. We could show constitutive activation of both PI3-kinase pathway and Ras/Mek/Erk pathway in Ba/F3 cells expressing c-Kit/D816V, although ligand stimulation induced even stronger activation. We further present evidence of a critical role of Src family kinases in cell survival of SCF stimulated cells expressing wild-type c-Kit but not in cells expressing c-Kit/D816V. *Conclusions.* Taken together, this is the first demonstration that c-Kit/D816V circumvents the requirement of Src family kinases in its signal transduction, contributing to its oncogenic potential.

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G-CSF INDUCES A SPECIFIC TOLEROGENEIC T CELL SUBSET IN THE HUMAN SYSTEM

A. Franzke,[1] S.N. Ukena,[1] S. Lauszus,[1] J. Grosse,[1] R. Geffers,[2] A. Ganser[1]

[1]Hannover Medical School, HANNOVER; [2]Helmholtz Center of Infectious Diseases, BRAUNSCHWEIG, Germany

Background. Granulocyte colony-stimulating factor (G-CSF) is a pleiotropic cytokine playing a major role in the complex regulation of hematopoiesis and innate immune responses. Recent data indicate that G-CSF also exerts regulatory function in the adaptive immune system and promotes tolerogeneic cells at both poles of APC/ T cell interaction. In an earlier study we have demonstrated that T lymphocytes may express a functionally active G-CSF receptor. *Aims.* Here, we characterize a regulatory T cell subpopulation directly generated after *in vitro* stimulation with G-CSF. *Methods.* Human peripheral $CD4^+CD25^-$ T lymphocytes were isolated by MACS separation from healthy donors and kinetic assays were subsequently performed by stimulation with G-CSF (± anti-CD3 monoclonal antibody). Marker molecules of regulatory T cells were analyzed by realtime RT-PCR in the stimulated T cell populations. Proliferation and inhibition assays were performed in order to determine the suppressive capacity of G-CSF stimulated T cells. *Results. in vitro* stimulation of $CD4^+CD25^-$ T lymphocytes with G-CSF revealed a time dependent up-regulation of FOXP3 gene expression which was significant after 4h of stimulation ($p<0.05$ *vs* non stimulated control cells). Gene expression analysis of further regulatory T cell marker resulted in increased mRNA levels for CD73 and CD83. In contrast, mRNA expression of GATA3, GITR, CD127, GRP83 and CLTA4 was not affected by G-CSF. Furthermore, functional assays demonstrated that G-CSF may slightly increase the proliferation of stimulated $CD4^+CD25^-$ T lymphocytes. However, $CD4^+CD25^-$ T cells preincubated with G-CSF suppress anti-CD3 induced proliferation of autologous T cells in independent inhibition assays. *Conclusions.* Our results demonstrate that G-CSF modulates $CD4^+CD25^-$ T lymphocytes in terms of gene expression and functional capacities exhibiting an immunoregulatory phenotype. Most importantly, G-CSF stimulated T lymphocytes seem to have a suppressive effect on the proliferation of autologous T lymphocytes and up-regulate important marker molecules of regulatory T cells. Thus, G-CSF represents an interesting candidate for specific immune modulation in transplantation medicine and autoimmune diseases.

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THE GLYCOGENSYNTHASE KINASE 3β IS CRITICALLY INVOLVED IN THE APOPTOTIC RESPONSE IN HEMATOPOIETIC CELLS

J. Basecke,[1] S. Horn,[2] A. Lehmann,[3] U. Bergholz,[3] J.A. McCubrey,[4] L. Trümper,[5] C. Stocking,[3] J. Basecke[5]

[1]University of Goettingen, GOETTINGEN, Germany; [2]Department of Hematology/Oncology, University of Goettingen, GOETTINGEN, Germany; [3]Molecular Pathology Group at the Heinrich-Pette-Institute, HAMBURG, Germany; [4]Department of Microbiology and Immunology, Brody School of Medicine, GREENVILLE, USA; [5]Department of Hematology/Oncology, University of Göttingen, GOETTINGEN, Germany

Class III receptor tyrosine kinases (RTK) are frequently mutated in acute myeloid leukemia and are used for diagnosis and therapeutical stratification. Activated RTK's stimulate the proliferation and survival of hematopoietic cells through multiple signaling pathways including the PI3K/AKT/GSK-3β cascade. GSK-3β is inactivated by AKT-mediated phosphorylation which implicates GSK-3β inhibition in the deregulated signal transduction of mutant RTK. We tested the relevance of GSK-3β inactivation in the transformation process of hematopoietic cells by using an *in vitro* factor-independent growth assay and GSK-3b inhibitors. We demonstrate that inactivation of GSK-3b alone was not sufficient to induce factor-independent growth in early hematopoietic cells (Ba/F3). Induction of apoptosis upon growth factor withdrawal was suppressed, but not prevented, in the presence of GSK-3b inhibitors, leading to a delayed Caspase 3 activation and DNA fragmentation. In synergy with Bcl-XL overexpression, GSK-3b inhibition resulted in the establishement of several growth factor-independent cell lines, which were characterized by the activation of multiple signaling cascades including AKT, MAPK, STAT5, but not STAT3. Our data show that GSK-3β is part of the apoptotic response to growth factor withdrawal and suggest that GSK-3β inactivation may be causaly involved in the transformation process of hematopoietic cells.

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DISTRIBUTION AND FUNCTION OF CD40/CD40L IN THE BONE MARROW GRANULOCYTIC PROGENITOR AND PRECURSOR CELLS. POSSIBLE PATHOPHYSIOLOGIC SIGNIFICANCE

I. Mavroudi, V. Papadaki, M. Velegraki, A. Eliopoulos, H. Papadaki

University of Crete School of Medicine, HERAKLION, CRETE, Greece

Background. Chronic Idiopathic Neutropenia (CIN) is a bone marrow (BM) failure syndrome characterized by inefficient granulopoiesis, low frequency of BM granulocytic progenitor cells, increased Fas antigen expression on the $CD34^+/CD33^+$ cell subpopulation and increased apoptosis of Fas-positive granulocytic progenitor cells. BM stromal cells of CIN patients have also been shown to express high levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-a and FasL. CD40 and CD40L are two molecules belonging to the TNF-Receptor/TNF family and have been implicated in neutropenia associated with the hyper-IgM Syndrome. The role of these molecules in the pathogenesis of CIN is currently unknown. *Aims.* To study the distribution of CD40-CD40L in normal BM granulocytic progenitor/precursor cells and probe the possible involvement of these molecules in the pathophysiology of CIN. *Methods.* We evaluated the surface expression of CD40 on immunomagnetically sorted $CD34^+$, $CD34^-/CD33^+$ and $CD34^-/CD33^-/CD15^+$ BM cells, representing sequential stages of the granulocytic development, in five haematologically normal subjects, after informed consent, using a sequential immunomagnetic separation system and 2-colour flow-cytometry. The role of CD40 in the survival characteristics of BM $CD34^+$, $CD34^-/CD33^+$ and $CD34^-/CD33^-/CD15^+$ cells was studied (a) by evaluating the proportion of apoptotic cells within the $CD34^+$, $CD34^-/CD33^+$ and $CD34^-/CD33^-/CD15^+$ cell compartment by flow-cytometry and 7-aminoactinomycin-D, (b) by enumerating the colony-forming cells (CFCs) in the BM mononuclear cell (BMMC) fraction in the presence of recombinant human (rh)CD40L using clonogenic assays. We also evaluated serum levels of CD40L in 38 CIN patients and 11 healthy controls by ELISA. *Results.* Serum CD40L was significantly increased in CIN patients (6.40±4.96 ng/mL) compared to controls (3.75±1.65 ng/mL; $p=0.0195$). CD40 was minimally expressed on $CD34^+$, $CD34^-/CD33^+$ and $CD34^-/CD33^-/CD15^+$ cells under steady state conditions (4.54±6.73%, 12.46±9.22% and 6.1±5.03%, respectively). However, in the presence of rhTNFa (25 ng/mL) the proportion of $CD40^+$ cells within the above populations was significantly increased (38.08±12.72%, 27.06±10.71% and 16.83±7.26% respectively; $p=0.0076$, $p=0.0187$,

p=0.0487, respectively) compared to baseline. In the presence of both rhTNFa and rhCD40L (200ng/mL and 5μg/mL, respectively) a significant increase was obtained in the proportion of apoptotic cells within the $CD34^+$, $CD34^-/CD33^+$ and $CD34^-/CD33^-/CD15^+$ cell compartments (32.18±40.44, 42.60±26.62, 37.25±21.92 respectively) compared to baseline (11.40±20.53, 3.86±1.72, 9.15±4.25, respectively; p=0.0270, p=0.0011, p=0.0015, respectively). In keeping with these findings was the decreased colony recovery following incubation of normal BMMCs with rhCD40L (33±28 CFC per 10×10^5 BMMCs) compared to baseline (58±41 CFC per 10×10^5 BMMCs, p=0.005). *Conclusions*. CD40 is minimally expressed under normal conditions in BM granulocytic progenitor/precursor cells. Its expression, however, is upregulated in the presence of TNFa. CD40L may induce apoptosis in the granulocytic progenitor cells upon CD40 induction. Since TNFa is highly produced in the BM microenvironment of CIN patients and CD40L is increased in patient sera, our findings suggest a mechanism contributing to apoptosis of BM progenitor cells in CIN patients.

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HEMATOPOIETIC STEM CELLS MOBILIZATION BY G-CSF OR LTB4 WAS SIGNIFICANTLY SUPPRESSED BY AN OXYGEN RADICAL SCAVENGER OR AN INHIBITOR OF NADPH OXIDASE

Y.C. Mun,[1] S.A. Oh,[1] K.E. Lee,[1] E.S. Yoo,[1] M.Y. Choi,[1] J.Y. Ahn,[1] J.H. Kim,[2] C.M. Seong[1]

[1]Ewha Womans University Hospital, SEOUL; [2]School of Life Sciences and Biotechnology, Korea University, SEOUL, South-Korea

Backgrounds. We previously had reported that LTB4 was able to mobilize HSC within 4 hours without significant side effects and LTB4 receptor was involved in the both pathway of mobilization induced by G-CSF and LTB4 in the murine model. Though the roles of protease (ie. NE, MMPs) seem to be important, the precise mechanisms are still unknown. ROS (reactive oxygen species) have a cell signaling roles that are involved in signal transduction cascades of numerous growth factor-, cytokine-, and hormone-mediated pathways, and regulate many biological systems. Because a variety of inflammatory cytokines and chemokines, including G-CSF and LTB4, induce a rapid increase of intracellular ROS, we hypothesized the role of ROS on HSC mobilization induced by G-CSF and LTB4 might be important. *Aims*. We investigated the role of ROS on HSC mobilization induced by G-CSF and LTB4. *Methods*. MS5, murine stromal cell line cells, or bEnd3, murine microvascular cell line cells, were grown to confluence on microporous transwell membrane. Murine marrow cells were placed on top of the prepared transwell membrane. The transwells were then seated in wells containing media and G-CSF or LTB4 with or without pretreatment of NAC, an oxygen free radical scavenger, or DPI, an inhibitor of NADPH oxidase-like flavoproteins. Cells that migrated through the stromal or endothelial layer into the wells were assayed for mobilization. NAC and DPI were given to C57BL/6 mice followed by rhG-CSF (5μg, IV) or LTB4 (1μg, IV) 2 hours later. 24 hours after the rhG-CSF injection or 4 hours after the LTB4 injection, peripheral blood samples were obtained via cardiac puncture. The samples were analyzed for TNC using a trypan blue stain and FACS analysis were performed using Sca-1 and lineage markers, including CD45R (B220), CD116, Gr-1 and TER119. *Results*. The numbers of migrated cell through the MS5 or bEnd3 were increased by treatment of G-CSF or LTB4. However, increasing effects of G-CSF or LTB4 to the transmigration through the MS5 or bEnd3 were inhibited by pretreatment of NAC or DPI. Comparing the control arm, the numbers of WBC and HSC (Sca-1+Lin-) in blood were decreased in the G-CSF or LTB4 mobilized mice (N=4), in which NAC or DPI were pretreated (Sca-1+Lin- fraction in peripheral blood: 1.91±0.80% in G-CSF alone, 0.55±0.37% in G-CSF with NAC, 0.36±0.09% in G-CSF with DPI, p<0.05; 1.92±0.49% in LTB4 alone, 0.55±0.29% in LTB4 with NAC, 0.67±0.52% in LTB4 with DPI, p<0.05). *Summary and Conclusions*. Through our data, it is suggested that ROS are involved on the HSC mobilization induced by G-CSF and LTB4 in murine model. It would be very interesting to test the effect of G-CSF or LTB4 on ROS knock-out mice in the future. Now, we try to find the critical signal transduction pathway, through which we may find more effective and efficient molecules for HSC mobilization.

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BENDAMUSTINE (TREANDA) CYTOTOXICITY IN B-CELL NEOPLASMS REQUIRES PRODUCTION OF REACTIVE OXYGEN SPECIES AND CASPASE-UNRELATED PROCESS IRRESPECTIVE OF P53 STATUS

G. Roue,[1] M. López-Guerra,[1] P. Milpied,[1] P. Pérez-Galán,[1] N. Villamor,[1] E. Montserrat,[2] E. Campo,[1] D. Colomer[1]

[1]Hospital Clinic - Hematopathology Unit, BARCELONA; [2]Hospital Clinic - Department of Haematology, BARCELONA, Spain

Background. Chronic lymphocytic leukaemia (CLL) and mantle cell lymphoma (MCL) are two types of B-cell lymphoid neoplasms characterized respectively by a relatively indolent natural history and an aggressive course. These two diseases remain incurable. Bendamustine hydrochloride (TREANDA) is a multifunctional, alkylating agent with a purine-like ring system that exhibits activity in multiple cancer models, but whose mechanism of action is partially unknown. *Aims.* To analyse the apoptotic pathways activated by bendamustine in CLL and MCL, together with the relevance of p53 mutation in determining the response of malignant B cells to this drug alone or combined with nucleoside analogues. *Methods.* 13 CLL/MCL cell lines and primary tumor cells from 8 MCL and 25 CLL patients were cultured for up to 24 hours with bendamustine (Treanda, provided by Cephalon Inc.). Cytotoxic assays, flow cytometry, immunofluorescence and western blot analysis of DNA-damage response pathway and apoptosis-related factors. *Results.* We show that bendamustine exerts a cytotoxic effect on most CLL and MCL primary cells and cell lines, irrespective of ZAP-70 expression and p53 status. Bendamustine cytotoxicity was mediated by the generation of reactive oxygen species, and p53-dependent and p53-independent triggering of the intrinsic apoptotic pathway involving up-regulation of PUMA and NOXA, conformational activation of BAX and BAK, and cytosolic release of caspase-related and caspase-unrelated mitochondrial apoptogenic proteins. More importantly, bendamustine was found to act synergistically with nucleoside analogues, this combination being effective in non-functional p53 CLL and MCL cases resistant to standard chemotherapy. *Conclusions.* our findings support the use of bendamustine as a therapeutic agent for CLL and MCL and establish the basis for its combination with conventional genotoxic agents.

0587

AURORA KINASE-A AND -B INHIBITION ENHANCE THE CHEMOTHERAPY-INDUCED CELL DEATH IN MYELOID LEUKEMIA CELLS THROUGH CASPASE-DEPENDENT PATHWAY OR MITOTIC CATASTROPHE ACCORDING TO CHEMOTHERAPEUTIC AGENTS

J.-W. Cheong,[1] J.I. Eom,[1] H.I. Jung,[1] H.K. Jeung,[1] J.S. Kim,[1] Y.H. Min[2]

[1]Yonsei University College of Medicine, SEOUL; [2]BrainKorea21 Research Team of Nanobiomaterials for the Cell-Based Implants, YUMC, SEOUL, South-Korea

Despite the development of multiple new agents that are effective at reducing the tumor burden in patients with acute myeloid leukemia (AML), relapse continues to be the most common cause of death. Novel therapeutic strategies should be developed to overcome drug resistance in this fatal disorder. Aurora family of serine/threonine kinases plays a critical role in chromosome alignment, segregation, and cytokinesis during mitosis. It has been shown that Aurora kinase A (AurA) and AurB were aberrantly expressed in a variety of human cancers including leukemia disorders, and associated with advanced clinical stage in several cancers. Evidences are accumulating that Aurora kinases may be a promising molecular target in human cancers. We evaluated whether inhibition of Aurora kinases with specific RNA interference (siRNA) or inhibitory molecule might lead to increase the extent of chemotherapy-induced cell death in myeloid leukemia cells. Western blot analyses demonstrated that phospho(p)-AurA and p-AurB were observed in 40 (80%) and 45 (90%) of fifty AML specimens obtained from AML patients. Correlation between levels of p-AurA and p-AurB were not observed. Levels of p-AurA or p-AurB expression were significantly higher in AML blasts compared with normal bone marrow (BM) mononuclear cells (p<0.001 for p-AurA and p<0.001 for p-AurB, respectively). With silencing of AurA or AurB in U937 leukemia cell line by specific siRNA transfection, the cell death was observed in 16.7±4.3% and 19.2±3.5%, respectively. A significant increase in the G2-M population was observed in AurA siRNA (39.8±2.5%) and AurB siRNA (64.2±9.3%) treatment with a concomitant decrease in the G0-G1 population. *in vitro* cell death induced by 48-hour treatment of cytosine arabinoside (AraC, 20μM) was minimal (11.0±2.3%) in U937 cells. However, when AraC treatment was combined with AurA siRNA (AraC/AurA)or AurB siRNA (AraC/AurB) knock-down, cell death was significantly increased to the

level of 33.4.0±2.9% (p<0.05) and 52.2.0±9.2% (p<0.001), respectively. Synergistic increase in the cell death was also observed in primary leukemic blasts obtained from untreated AML patients. Similar results were obtained using chemical aurora kinase inhibitor instead of siRNA transfection. We also observed a remarkable increase in cell death rate when etoposide (50 μg/mL) was combined with AurA siRNA or AurB siRNA treatment (p<0.01 for Eto/AurA; p<0.001 for Eto/AurB). Cell death was accompanied by disruption of mitochondrial membrane potential when AraC or Eto was combined with Aurora kinase inhibition. Cleavage of caspase-3, -8, -9, and PARP was not observed in the AraC/AurA or AraC/AurB treatment, whereas the activation of caspase cascade was observed in the Eto/AurA or Eto/AurB treatment. Both in AraC/AurA and AraC/AurB treatment, synergistic increase in cell death was associated with multi-nucleation and shrink of mitochondria membrane, an increase in cyclin B1 level, and reduced level of Plk1 and cdc20, indicating this cell death occurred through caspase-independent, mitotic catastrophe. In contrast, Eto/AurA or Eto/AurB-induced cell death occurred in a caspase-dependent manner. Taken together, an inhibition of aurora kinases can be combined with conventional chemotherapy to increase the response rate in AML. Novel therapeutic strategies can be designed by considering the mechanisms involved in the synergistic interaction between chemotherapy and Aurora kinase inhibition.

0588

CYCLIN D1 DE NOVO EXPRESSION IN B LYMPHOCYTES MEDIATES RESISTANCE TOWARDS APOPTOSIS THROUGH UP-REGULATION OF MOLECULAR CHAPERONES AND REDISTRIBUTION OF CELL DEATH REGULATORS

G. Roue,[1] V. Pichereau,[2] H. Lincet,[3] D. Colomer,[1] B. Sola[4]

[1]Hospital Clinic - Hematopathology Unit, BARCELONA, Spain; [2]Laboratoire de Microbiologie de l'Environnement-USC INRA 2017-EA 956, IFR 146, CAEN, France; [3]Grecan - EA1773, IFR 146, Centre de Lutte Contre le Cancer François Baclesse, CAEN, France; 4Biologie moléculaire et cellulaire de la signalisation - EA 3919, IFR 146, CAEN, France

Background. cyclin D1 is a key regulator of cell proliferation. It controls also other aspects of the cellular fate as cellular senescence, apoptosis or tumourigenesis. *Aims.* to understand the role of cyclin D1 expression associated with some B-cell lymphomas and leukaemias. *Methods.* we have established B-lymphoid cell lines expressing cyclin D1 and analysed the molecular events associated with variability in cell response to apoptotic stimuli. *Results.* we show that constitutive low levels of cyclin D1 having no effect *per se* on cell proliferation, confer to B cells resistance towards various apoptotic stimuli. Following cytokine withdrawal, cyclin D1-expressing cells presented a reduced activation of the pro-apoptotic protein Bax and a delay in mitochondrial permeabilisation and phosphatidylserine exposure. Proteomic analysis further pointed out that cyclin D1 expression led to intracellular accumulation of various molecular chaperones. Focusing on Hsp70, we observed that this chaperone associated with both Bax and the mitochondrial apoptosis inducing factor after cytokine starvation, and impeded IkappaB-mediated inhibition of NF-kappaB anti-apoptotic signalling. Impairment of Hsp70 activity by using a pharmacological Hsp inhibitor or by transfecting cells with an Hsp70 blocking antibody restored cell response to mitochondrial apoptosis triggering. *Conclusions.* the constitutive *de novo* expression of cyclin D1 in B cells may delay apoptosis commitment by induction of Hsp70 chaperoning activity on pre- and post-mitochondrial pro-apoptotic factors.

0589

INHIBITION OF BOTH PROTEIN KINASE CK2? AND PI3K/AKT SYNERGISTICALLY INDUCES APOPTOSIS OF CD34+CD38- LEUKEMIA STEM CELLS WHILE SPARING NORMAL HEMATOPOIETIC STEM CELLS THROUGH INHIBITION OF MULTIPLE DEREGULATED SIGNALING PATHWAYS

J.-W. Cheong,[1] Y.H. Min,[2] J.I. Eom,[1] H.Y. Choi,[1] S.H. Yoon,[1] J.S. Kim[1]

[1]Yonsei University College of Medicine, SEOUL; [2]BrainKorea21 Research Team of Nanobiomaterials for the Cell-Based Implants,YUMC, SEOUL, South-Korea

Given the critical role of leukemia stem cells (LSC) in drug resistance, an identification of novel therapeutic approach to eradicate LSC without harming normal hematopoietic stem cells (HSC) is urgently required. PI3K/Akt pathway deregulation has been shown to play an important role in acute myeloid leukemia (AML) development and response to anti-AML treatment. Similarly, we previously demonstrated that levels of protein kinase CK2α are frequently elevated in AML and correlated with deregulated apoptosis and poor survival in AML. Previous reports raise the possibility that CK2 and PI3K/Akt might be implicated in common pathway that control cell proliferation and apoptosis. In this study, we therefore examined whether apigenin, one of CK2 inhibitors, potentiates the LY294002-induced apoptosis of $CD34^+CD38^-$ leukemia stem cells. In representative myeloid leukemia cell lines, combination of subtoxic concentrations of LY294002 and apigenin (LY/Api) for 24-48 hours produced a synergistic increase in the level of caspase-dependent cell death in a dose- and time-dependent manner (2.2±0.3% with 25μM LY294002 *vs* 4.1±1.4% with 25μM apigenin *vs* 78±4.7% with LY/Api, p<0.001). These findings were obviously documented in the $CD34^+CD38^-$ leukemia stem cell candidate isolated from primary AML (p<0.001). In contrast, LY/Api-induced cell death was very negligible in normal bone marrow $CD34^+CD38^-$ cells. When CK2α was knockdowned with specific CK2α siRNA treatment instead of using apigenin, the synergistic effect of LY294002 was also observed. The synergistic effect of apigenin treatment and transfection with dominant negative Akt cDNA, instead of using LY294002, on inducing apoptosis was obviously observed in these leukemia cells. LY/Api combination treatment was associated with notable decrease in the level of p85-PI3K, p-Akt(Ser473), p-PDK1, p-FKHR, p-GSK-3β, and p-Bad. Levels of p-mTOR, p-p70 S6K, p-4E-BP1 were also markedly decreased with LY/Api treatment. Although subtoxic concentration of apigenin or LY294002 alone did not affect the levels of anti-apoptotic proteins including Bcl-2, Bcl-xL, Mcl-1, XIAP, survivin, LY/Api co-treatment for 48 hours resulted in a near complete downregulation of these antiapoptotic proteins in leukemia cell lines, primary AML cells, and $CD34^+CD38^-$ leukemia stem cells. However, these events did not occur in the $CD34^+CD38^-$ normal BM mononuclear cells. Taken together, these findings suggest that Api/LY combination may be a promising LSC-targeted therapeutic treatment in AML.

Gene therapy and cellular immunotherapy and vaccination

0590

INDUCTION OF GAMMA-GLOBIN EXPRESSION AND FETAL HEMOGLOBIN PRODUCTION AFTER EPISOMAL GENE TRANSFER OF A SYNTHETIC ZINC-FINGER ACTIVATOR, IN K562 AND MURINE PROGENITOR CELLS

F.E. Stavrou,[1] E. Lagadinou,[2] E. Papapetrou,[3] N. Zoumbos,[2] C. Barbas,[4] K. Peterson,[5] A. Athanassiadou[3]

[1]University of Patras, PATRAS, Greece; [2]Department of Hematology, University of Patras, PATRAS, Greece; [3]Department of General Biology, University of Patras, PATRAS, Greece; [4]Scripps Research Institute, LA JOLLA, CALIFORNIA, USA; [5]University of Kansas Medical Center, KANSAS CITY, KANSAS, USA

Background. The increase of HbF through the activation of the gamma-globin gene for the treatment of sickle-cell anemia and beta-thalassaemia has been pursued for several years, through the use of pharmacological compounds and in recent years by attempts of gamma-globin gene transfer. The development of a selective, synthetic activator of gamma-globin gene, Zif-VP64, based on a zinc-finger DNA binding protein specially designed to bind at 18 bases at the gamma-globin promoter -117HPFH area, has been presented (Graslund *et al.*, 2005) showing significant increase of gamma-globin, potentially therapeutic levels as documented after viral gene transfer in K562 cells. *Aims.* Construction of a non-viral episomal vector containing the synthetic activator of gamma-globin gene, Zif-VP64 and investigation of its ability to i) transfect K562 cells and maintain in episomal status with trangenes constantly expressed ii) to increase the gamma-globin levels for a long period of time, iii) to transfect murine progenitor beta-cells carrying a YAC with the whole of the human beta-globin locus, in which human beta-globin but not human gamma-globin is expressed and vi) to activate the human gamma-globin gene in the beta-YAC in the murine progenitor cells. *Methods.* The constructed episomal vector contained the activator Zif-VP64, the reporter gene eGFP driven by the CMV promoter and the S/MAR element that is necessary for the retention of the transferred plasmids in the nucleus of the host cell. Gene transfer was done in all cases by electroporation. The fate of Zif-VP64-Ep1 in the cells was studied by Southern Blot and plasmid rescue experiments and eGFP expression was documented by Flow Cytometry and Florescent Microscopy. Real time PCR, Western blotting and Intracellular Flow Cytometry were employed to investigate gamma-globin mRNA, gamma-globin protein and HbF protein levels respectively. Chromatin Immunoprecipitation (ChIP) was employed to check the binding specificity of the synthetic activator. *Results.* Transfection efficiencies were around 65% in K562 cells and 25% in the murine beta-Yac cells. This episomal vector specifically binds to the designed promoter position of the human gamma-globin gene in K562 cells. Gamma-globin mRNA levels showed an increase of 250%, gamma-globin protein of 350% and HbF protein of 165%, as compared to the corresponding levels in the untransfected K562 cells, at least 200 generations post-transfection. Most significantly, Zif-VP64-Ep1 was able to stably transfect murine beta-YAC Bone Marrow cells. Furthermore, it was able to activate the expression of the gamma-globin gene of the human beta-YAC in these cells, as compared to the control, untransfected with the episomal DNA cells, in which normally gamma-globin gene is not expressed. *Summary*. Activation of human gamma-globin, by gene transfer of a synthetic activator, is documented. This is the first time that an episomal vector for gene transfer is applied for gene transactivation in a cell line and progenitor cells, aiming at specific gene therapy.

0591

BCR-ABL PEPTIDE VACCINATION DOES NOT INDUCE TOLERANCE IN CML PATIENTS: EVIDENCE FROM THE EPIC TRIAL

J.M. Rojas, K. Knight, L.H. Wang, R.E. Clark

University of Liverpool, LIVERPOOL, UK

Chronic Myeloid Leukaemia (CML) is characterised by the BCR-ABL oncoprotein. We have previously reported that vaccination of CML patients over 9 weeks with BCR-ABL junctional peptides can elicit anti-BCR-ABL T cell responses, and that these immune responses correlate with a decrease in BCR-ABL transcript levels (Rojas *et al.* Leukemia 2007; 2007; 21: 2287-2295). However, these are only sustained for up to 3 months following cessation of vaccination, with no detectable responses thereafter, despite evidence of memory cell phenotype in some patients. It is not known whether these responses disappear because of the induction of tolerance by the vaccine. In the present study, we investigated whether booster vaccinations can restore these anti BCR-ABL immune responses, in all 12 available CML patients who had previously showed BCR-ABL immune responses to an initial 9-week vaccination course. All cases were in first chronic phase and remained on imatinib throughout. Booster vaccinations were identical to the original vaccine, consisting of a cocktail of 3 BCR-ABL peptides: (1) a 9-mer spanning the e14a2 region, (2) this same 9-mer linked to a PADRE (a 15-mer non-natural peptide shown to activate $CD4^+$ T cells, to which all patients are immunologically naive), and (3) a 13-mer consensus e14a2 junctional peptide linked to PADRE. These were administered intradermally at a dose of 600mg each with sargramostim, and at 3 monthly intervals. Immune responses were monitored by IFN-γ, Granzyme-B and IL-5 ELISPOT, proliferation assays to the vaccination peptides and IFN-γ capture assay by flow cytometry on peripheral blood mononuclear cells. At entry, no patients showed detectable anti BCR-ABL immune responses. After the first booster vaccination, IFN-γ producing cells specifically to BCR-ABL peptides were detected in 10/12 patients and Granzyme-B producing cells in 6/12 patients, indicating that BCR-ABL vaccination can potentially induce cytotoxic T cells. No IL-5 production was observed, suggesting that the vaccination regimen favours type 1 immune responses. Although these had all disappeared in all cases over the next 2 months, a second booster vaccination produced a detectable IFN-γ producing cell population to the BCR-ABL peptides in 6/7 patients tested thus far; again no IL-5 production was detected. CFSE-based proliferation assays were also used to confirm the immunogenicity of the vaccine peptides. Proliferative immune responses to the vaccine peptides were detected in 3/6 patients following the first booster vaccination and in 4/6 patients after the second booster vaccination, indicating that cytokine production correlated with a proliferative response. Although all patients had local cutaneous reactions to the vaccine that were typically asymptomatic and transient, no patient developed any other adverse events. These data suggest that the booster vaccination procedure does not induce tolerance to BCR-ABL peptides. Booster vaccinations appear safe, and may prove a useful tool in order to sustain anti-leukaemia responses in CML.

0592

VIGOROUS PRIMING OF MINOR HISTOCOMPATIBILITY ANTIGEN SPECIFIC T CELLS BY FREQUENT HIGH DOSE PEPTIDE VACCINATION MAY LEAD TO PROLONGED T CELL RECEPTOR DOWNREGULATION, IMPAIRED T CELL FUNCTIONALITY, AND PROMOTION OF TUMOR ESCAPE

I. Jedema, L. van Dreunen, M.M. van Loenen, M.H.M. Heemskerk, R. Willemze, J.H.F. Falkenburg

Leiden University Medical Center, LEIDEN, Netherlands

Allogeneic stem cell transplantation can be successfully applied in the treatment of hematological malignancies and relies on the graft versus leukemia (GVL) effect mediated by donor T cells directed against minor histocompatibility antigens (mHag) selectively expressed on malignant hematopoietic cells of the patient. However, due to insufficient in-vivo priming of donor T cells the GVL response may not be adequately initiated or amplified. Vaccination strategies using immunogenic peptides derived from hematopoiesis specific mHag like HA-1 may form a strategy to initiate or boost the in-vivo GVL response. However, it has been reported that repetitive vaccination with HLA-class I binding 9-mer peptides can lead to the induction of T cell anergy. We hypothesized that repetitive strong stimulation of the mHag specific T cells may also lead to prolonged downregulation of the T cell receptor (TCR) resulting in temporary inability of the T cells to subsequently attack tumor cells expressing the mHag, allowing tumor escape. We tested this hypothesis in an in-vitro model using clonal HA-1 specific $CD8^+$ T cells as responder/effector cells. To mimick a peptide vaccination strategy, we used HLA-A2+ monocytes loaded with E-12-E-6M of the 9-mer HA-1 peptide as stimulator cells, and investigated the direct and residual functional cytotoxic capacity of the HA-1 specific T cells against HA-1+ CML cells, as well as their specific TCR expression. After a single stimulation of the T cells we observed strong dose-dependent TCR downregulation as measured by specific tetramer staining (20%-78% decrease in fluorescence intensity after 24 hours of exposure to targets loaded with E-12-E-6M HA-1 peptide). This drop in TCR expression was coincided by an equal loss of functional cytotoxic capacity of the T cells against HA-1+ $CD34^+$ CML cells, and even allowed the outgrowth of malignant progeny from these precursor cells. At high peptide concentrations it took 6-

9 days before proper functional TCR expression could be demonstrated again. Since peptide vaccination strategies normally consist of repetitive vaccination cycles in a small time-frame, we investigated whether repetitive stimulation of Ag-specific T cells could result in a prolonged period of TCR downregulation and impaired functionality of the T cells. Therefore, we stimulated HA-1 specific T cells at 2-3 day intervals with HA-1 peptide loaded monocytes and monitored their TCR expression and residual cytotoxic capacity for 3 weeks. Peptide-stimulated T cells showed a continuous decrease in TCR expression to 20-30% of non-stimulated control T cells and the T cells stimulated with non-peptide loaded monocytes. This correlated with impaired cytotoxic capacity. In conclusion, we here demonstrate that high affinity HA-1 specific T cells show prolonged TCR downregulation after continuous vigorous stimulation by peptide loaded target cells. In this period the T cells showed a dramatic loss of function and allowed the outgrowth of a leukemic subpopulation expressing the HA-1 antigen. Milder vaccination strategies using longer peptides, requiring uptake and processing by the target cells, may lead to expression of more physiological levels of the mHag, and less vigorous priming of the mHag specific T cells, thereby improving their residual functional capacity and responsiveness.

0593

EX VIVO GENERATION OF CLINICALLY RELEVANT AMOUNTS OF HUMAN NATURAL KILLER CELLS FOR IMMUNOTHERAPY AGAINST ACUTE MYELOID LEUKEMIA

J. Spanholtz,[1] M. Tordoir,[2] T. De Witte,[3] H. Dolstra[4]

[1]UMCN, NIJMEGEN; [2]Central Hematology Laboratoy, UMC St. Radboud, NIJMEGEN; [3]Central Hematology Laboratory and Department of Hematology, UMC St. Radboud, NIJMEGEN; [4]Central Hematology Laboratory, UMC St. Radboud, NIJMEGEN, Netherlands

Background. Adoptive transfer of Natural Killer (*NK*) cells is an attractive option for immunotherapy. NK-cells can produce a strong Graft Versus Leukemia response after haploidentical stem cell transplantation without significant Graft Versus Host Disease due a KIR-ligand mismatch between donor and recipient cells. So far, the inability of isolating sufficient numbers of tumor-reactive NK cells limits the collection of data about their safety and clinical effects. Several studies suggest that the generation of NK cells from CD34$^+$ cells could be an alternative strategy to provide NK cells for immunotherapy. *Aims*. We wanted to develop a clinical grade cell culture system that allows the generation of clinically relevant amounts of functional NK cells for immunotherapy. We tested different serum free media, regarding their potential for supporting the outgrowth of NK cells under standardized culture conditions. *Methods*. We used a cell culture system without feeder cells for generating NK cells from CD34$^+$ hematopoietic progenitor cells, isolated from cord blood (*CB*), bone marrow (*BM*) or G-CSF mobilized peripheral blood (*PB*). This system is based on a two-step procedure, comprising an expansion and a differentiation step. This NK cell generation system uses mainly cytokines such as SCF, Flt3-L, IL-2 and IL-15 to direct and control the two steps. The development and the final NK cell product are characterized using multi-color flow cytometry and CFSE-based cytotoxicity assays against various tumor cells.

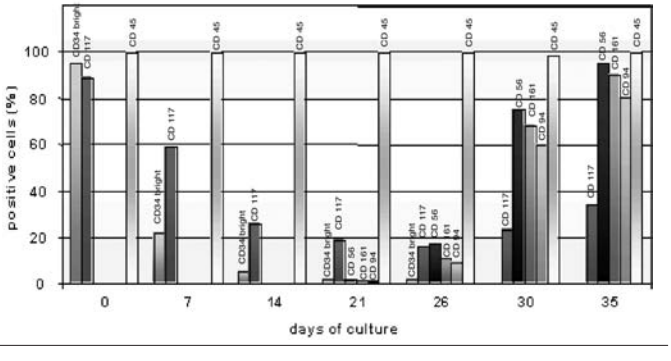

Figure 1.

Results. The described system generates a homogeneous final cell product of CD56$^+$/CD3$^-$ cells with a purity >95% of total cells. The use of Glycostem® Basal Growth Medium (GBGM) enables a total cell expansion of more than 50,000 fold, which facilitates the generation of 5×10^{10} NK cells from 10^6 CB CD34$^+$ stem and progenitor cells from within 4-5 weeks of culture. For BM cells an expansion rate of more than 10.000 fold was detected after a 5-6 week cell culture period, whereas with PB cells an expansion of more than 1,000 fold was achieved. The phases of development show a decrease of stem cell-specific antigens (i.e. CD34, CD117) during the first three weeks, whereas antigens specific for NK cells and NK cell progenitors (i.e. CD56, CD94, CD161) are up-regulated after initiating differentiation at day 14 (Figure 1). The effective differentiation of the expanded progenitor cells into NK cells is characterised by the expression of NK cell-specific antigens including CD56, CD94, KIR, NKG2A, NKG2D and NCRs as well as homing receptors such as CD62L, CXCR4 and CCR7. The NK cell product shows high expression levels of inhibitory and activating receptors such as KIRs, indicating that the final product is highly activated. Cytotoxicity assays demonstrated robust lysis of more than 90% against AML as well as melanoma tumor cell lines. *Conclusions*. This system used with Glycostem® Basal Growth Medium (GBGM) enables the generation of functional NK cells from CD34$^+$ cells from different sources. The generation of highly activated NK cells with homing capability is the basis for a first clinical trial in 2008, in which haploidentical NK cells generated from CD34$^+$ cells will be infused in poor-prognosis AML patients.

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DENDRITIC CELLS (DC) AS PROGNOSTIC INDICATORS OR IMMUNOTHERAPEUTIC TOOLS TO TREAT ACUTE MYELOID LEUKEMIA: ROLE OF THE QUALITY OF LEUKEMIA-DERIVED DC TO PREDICT THE SPECIFIC ANTI-LEUKEMIC POTENTIAL OF (DC-TRAINED) T-CELLS

M. Schmetzer,[1] C. Grabrucker,[1] A. Liepert,[1] A. Kremser,[1] J. Loibl,[1] M. Freudenreich,[1] R. Reibke,[1] R. Buhmann,[1] T. Yang,[2] C. Schmid,[3] T. Kroell,[1] H.-J. Kolb[1]

[1]University of Munich, MUNICH; [2]Helmholtz Center, MUNICH; [3]Municipal Hospital, AUGSBURG, Germany

Background. The presentation of leukemic antigens can be improved by conversion of leukemic cells to leukemia-derived DC (DCleu), thereby forming a platform for the generation of leukemia-specific cytotoxic lymphocytes. We could already show:1) the generation of DC/ DCleu is possible from every AML/MDS-case, independent of karyotype and FAB-type with at least one of 3 different DC-generating methods 2)DC/DCleu can be quantified by combination of suitable blast and DC-antigens and 3)The antileukemic reactivity of DC-trained T-cells can be specified in a fluorolysis assay (Schmetzer 2007). *Aims.* We want 1)to enlight the role of the composition and quality of DC/ DCleu and (DC-trained) T-cells to mediate leukemia-cytotoxic reactions *ex vivo*, 2)to predict or correlate the clinical response to a DC/DLI-based immunotherapy *in vivo* and to develop a targeted, DC-based adoptive immunotherapy to treat AML. *Methods.* DC were generated from 27 AML-cases in blast-rich phases with the best of 3 DC-generating methods and used to train T-cells (autologous patients'- (n=10), allogeneic donor- (n=11) or T-cells at relapse after allogeneic SCT (n=6)) in a 'Mixed lymphocyte culture' (MLC). The leukemia-lytic activity of DC- (or blast- or untrained) T-cells against naïve blasts was quantified after 3/24 hours. Results were correlated with clinical data. *Results.* 1) DC can be generated in all 27 AML-cases. 2) In Ø 44% (n=12) of the cases T-cells gained a CTL-activity after DC-training. 3)The leukemia-cytotoxic T-cell training efficacy with DC was superior to a blast-training (Ø 42% *vs* 34% lysed blasts). 4) A comparison of cases with or without a gain of lytic T-cell activity showed 68 *vs* 60% DCleu, 52 *vs* 30 % mature and 27 *vs* 17% migratory DC and 50 *vs* 40% proliferating T-cells, 53 *vs* 46% memory T-cells, 68 *vs* 56% CD4$^+$ T-cells. 5) In cases with a training with > 65% DCleu/ >40% mature DC/>65% CD4$^+$ T-cells 70%/80%/80% of trained T-cells gained a lytic activity. 6) In AML-patients with(n=7)/ without (n=9) successful response to a GM-CSF-DLI-based therapy of relapse after SCT we could demonstrate 43 *vs* 29% DC; 78 *vs* 56% mature DC; 61 *vs* 52% blasts convertible to DCleu. Moreover we found, that cases with more *vs* less than 60% blasts convertible to DCleu the overall survival from transplantation to endpoint was 612 *vs* 168 days. *Summary and Conclusions.* That means, that the generability and composition of DC/ DCleu and of DC/MNC-trained T-cells could contribute to predict the clinical course of the disease after SCT in individual cases and could help to create specific anti-leukemic T-cells for immunotherapy of AML.

0595

DONOR LYMPHOCYTE INFUSION FOR MIXED CHIMERISM AT 7 MONTHS AFTER T CELL DEPLETED ALLOGENEIC STEM CELL TRANSPLANTATION REDUCES RELAPSE RISK

W.A. Marijt, A., von dem Borne, M.Y. Barge, P. Deutz-Terlouw, M. Beaumont, C.W.J. Starrenburg, E. Fibbe, R. Willemze, J.H.F. Falkenburg

Leiden University Medical Center, LEIDEN, Netherlands

Background. T cell depleted allogeneic stem cell transplantation (TCD alloSCT) is associated with both an increased relapse rate (RR) and mixed chimerism (MC) compared to non-TCD alloSCT. MC is in turn associated with an increased RR compared to complete chimerism (CC). Since donor lymphocyte infusion (DLI) for hematological relapse of acute leukemia cures only 20-30% of patients (pts), pre-emptive DLI might restore CC and decrease the RR. *Aims.* We evaluated whether low dose DLI administered 6-9 months (mo) after Tx for acute leukemia in pts with MC without GVHD decreased the RR without inducing severe GVHD. The effect on RR in this group of patients was compared with that of a historical control group. *Patients and Methods.* In group 1 (DLI group) 42 pts (AML 25, ALL 12, high risk MDS 5) and in group 2 45 pts (AML 29, ALL 13, HR-MDS 3) were transplanted in CR after conditioning with TBI and cyclophosfamide. Pts in group 1 received stem cells from HLA-identical sibling donors (27), HLA-matched unrelated donors (MUD)(13), or 1/2 antigen mismatched family donors (2). Pts in group 2 were transplanted with an HLA-sib (41) or a MUD (4). Grafts were T cell depleted with 20 mg Campath. Only MUD pts received ciclosporin for 6-12 weeks. Bone marrow morphology and chimerism analysis (STR-PCR or XY-FISH) was performed at 3 and 6 months (mo) after Tx and at regular intervals after DLI. *Results*. 6 Mo after Tx 9/42 pts in group 1 had relapsed, and 6 pts had died due to infections. 7/26 MC pts had GVHD, which resolved in 3 pts. 3 MC pts did not receive DLI due to logistical problems. In total, 20 MC pts received a 1st DLI (3×10^6 $CD3^+$ T cells/kg body weight) at a median of 7 (range 5-22) mo after Tx. When no CC and no GVHD was present subsequent DLI's were administered in escalating doses of $1x10^7$ (4x), 3×10^7 (3x), and 1×10^8 T cells/kg (1x). The median follow-up after the 1st DLI was 16 (range 2-41) mo. In 14 pts the percentage patient cells decreased to <1% after DLI ($p<0.05$). In 6 pts MC did not change. In 6 pts GVHD grade I-III developed after DLI of whom 1pat died. 4 Pts in group 1 not receiving DLI relapsed within 9 mo after Tx. None of the 20 MC pts treated with DLI developed a relapse (RR in group 1 13/42). In group 2 13 pts relapsed in the first 9 mo post Tx. Thereafter, 6 additional patients developed a relapse, a statistically significant difference ($p<0.05$). Non-relapse mortality in both groups was identical (19% group 1, 22% group 2). *Conclusions*. Our results indicate that low dose DLI administered at a median of 7 mo after Tx decreased MC in the majority of pts and significantly reduced the RR without severe GVHD. Since the majority of relapses occurred from 3-6 mo after Tx, we aim to administer low dose DLI 3 mo after Tx in our next group of pts.

0596

DEVELOPMENT OF A LARGE SCALE, NON-VIRAL PLATFORM FOR GENERATION OF REDIRECTED T CELLS FOR ADOPTIVE TRANSFER

E. Rian,[1] H. Almåsbak,[1] A.M. Rasmussen,[1] G. Borelli,[2] M. Lundby,[2] G. Kvalheim,[2] J. Olweus,[1] M. Pule,[3] C. Rossig,[4] G. Gaudernack[1]

[1]Institute of Cancer Research, Rikshospitalet University Hospital, OSLO, Norway; [2]Department of Cellular Therapy, Rikshospitalet University Hospital, OSLO, Norway; [3]UCL Cancer Institute, University College London, LONDON, UK; [4]Westfälishe Wilhelms-Universität, MUENSTER, Germany

Background. Adoptive immunotherapy using redirected T cells serves as a promising tool for cancer treatment, and a number of clinical protocols targeting a handful of tumor associated antigens are currently being implemented in Europe and the US. As of today, integrating vectors represent the major tool for genetic modification of T cells. However, there are several advantages associated with non-viral approaches resulting in transient, rather that permanent genetic modification; 1) A less demanding regulatory regime provides a faster track into clinical trials for the continuous testing of novel and improved protocols for genetic modification. 2) The potential dangers of insertional mutagenesis, and 3) the long-term persistence of antigen-targeting T cells that may cause damage to vital cells expressing the target antigen are avoided 4) Lower costs than those associated with virally transduced T cells for clinical studies. Thus, the development of a clinical grade, non-viral platform for transient redirection of T cell is warranted. *Aims.* The CHILDHOPE project (EU contract #037381) is aiming at further developing the technology for redirecting T cells for adoptive immunotherapy, and to offer this treatment to children with hematological malignancies. As a part of this project, we are developing the alternative non-viral approach using mRNA electroporation of expanded T cells and transferring this method to a large scale platform. The current work describes the optimization of *ex vivo* T cell expansion, electroporation of mRNA for chimaeric antigen receptors (CAR, scFv for CD19 fused to CD3) and analyses of transient CAR expression and T cell efficacy as a function of a set of variables. *Methods.* T cells from patients undergoing leukapheresis were expanded for 10 days using the Dynabeads ClinExVivo CD3/CD28 and the Wave Bioreactor. mRNA was synthesized from CAR expression cassettes subcloned into the pCIpA102 *in vitro* transcription vector. This mRNA was electroporated into expanded T cells using the BTX ECM830 square wave electroporator (small and large scale). CAR expression and the T cell phenotype were analyzed by flow cytometry and T cell function was measured in cytotox assays. *Results.* The T cell expanded up to 400 fold during 10 days culture and the CD4/CD8 ratio and a set of differentiation markers were monitored regularly. Square wave electroporation of these cells resulted in 95% expression of different CARs and high T cell recovery and viability. CAR expression kinetics on proliferating T cells in culture was determined for a 7-day period. The T cells were still functional in 51Cr cytotoxic assay at time points where the CAR was beyond the detection limit. Work is in progress to optimize parameters influencing the longevity of CAR and the T cell function. Moreover, we are currently scaling up the electroporation to allow gene modification of high numbers of T cells suitable for clinical studies. *Summary*. We have combined a large scale clinical grade T cell expansion platform with mRNA electroporation technology to generate T cells for the transient expression of chimeric antigen receptors targeting leukemia and lymphoma antigens. The platform is currently being set up for clinical studies.

0597

AUTOLOGOUS DENDRITIC CELL VACCINATION IN MULTIPLE MYELOMA PATIENTS - A FIRST CLINICAL RESULTS AND SAFETY EVALUATION IN A PHASE II CLINICAL TRIAL

L.Z. Zahradova,[1] D. Ocadlikova,[2] L. Kovarova,[2] J. Smejkalova,[2] L. Pour,[3] M. Penka,[4] J. Michalek,[2] R. Hajek[3]

[1]University Hospital, BRNO; [2]University Cell Immunotherapy Center, BRNO; [3]Department of Hematooncology, University Hospital, BRNO; [4]Department of Hematology, University Hospital, BRNO, Czech Republic

Background. Dendritic cells (DCs) are antigen-presenting cells that play a key role in the induction of cytotoxic T-lymphocytes. Adjuvant immunotherapy with antigen-loaded DCs represents a relatively non-toxic anticancer strategy for multiple myeloma (MM). Malignant plasma cells in MM produce a monoclonal immunoglobulin (idiotypic protein) that can be used for the induction of myeloma-reactive T lymphocytes. The vaccination using DCs loaded with the idiotypic protein (Id-protein) is supposed to induce humoral and cellular anti-myeloma immune responses. *Aims.* The aim of this phase II clinical study was a clinical evaluation of efficacy, safety and toxicity of DC-based vaccine as well as the immune response in patients with MM. *Patients and Methods.* 6 patients with MM with stable disease or with slow progression according EBMT criteria not requiring systemic therapy was enrolled after signing the informed consent. Patients received 6 doses of DCs loaded with autologous Id-protein every 4 weeks. The vaccine was administred subcutaneously. Autologous mature DCs derived from peripherial blood monocytes were prepared *in vitro* and loaded with Id-protein under the GMP conditions. A clinical response was evaluated by measuring the monoclonal immunoglobulin levels in peripheral blood. An immune response was monitored by flow cytometry every 4 weeks as well as the production of interferon gamma (evaluated by Elispot) by myeloma-reactive T lymphocytes. A delayed type hypersensitivity test was performed every 3 months. *Results.* Vaccination was well tolerated with only mild fever up to 37,5°C in 1 patient, no other grade II-IV toxicities were observed. A local reaction (erythema or induration) was observed in all patients and disappeared in 2-6 days. The immune response to the vaccine was noticed in 3 of 4 patients who completed the vaccination and these 3 patients remained in stable disease while one evaluable patient with no immune response revealed progressive disease (the follow-up of these patients is 19-20 months, median 20 months). The trial is ongoing and a total number of 12 patients is planned to be enrolled. *Conclusions.* Vaccination with Id-protein loaded autologous DCs is a safe therapeutic strategy with no significant side effects. The final evaluation of clinical response needs to be performed in a longer follow-up.

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0598

OXIDIZED MANNAN POTENTIATES DNA VACCINE FOR USE IN CANCER IMMUNOTHERAPY

C.K. Tang, G. Pietersz, V. Apostolopoulos

Burnet Institute, MELBOURNE, Australia

There has been an increasing awareness of the importance of the Toll-like receptors and its ligands in preparations of adjuvants for use in vaccines. Our laboratory have demonstrated that mannan in its oxidized form is suitable for use as carrier in MUC1 peptide vaccine for cancer immunotherapy. This has been proven in pre-clinical and clinical studies. More recently, we have shown that the immune augmenting property of oxidised mannan could also be applied in DNA vaccines for cancer immunotherapy (Apostolopoulous *et al.*; Immunology 2007; 120(3):325-35). Using MUC1 antigen as tumor model, we have demonstrated that oxidized mannan complexed DNA vaccine induced better immunogenicity against the target antigen and more importantly offer mice better prophylactic and therapeutic tumor protection. Mechanism studies revealed that among its many properties that enable better antigen processing and immune responses, the stimulation of toll-like receptor is one of them. With the use of knock-out mice, we show that oxidized mannan stimulates via Toll-like receptor 4 and the addition of a polypeptide to oxidized mannan was able to switch this interaction to Toll-like receptor 2. In conclusion, results presented here indicate the potential of using oxidized mannan as a carrier for DNA vaccine for use in cancer immunotherapy and the importance of adjuvants that targets the innate immune system and its role in increasing the immunogenicity of vaccine preparations.

0599

IMMUNE AUGMENTATION OF DENDRITIC CELL VACCINES FOR ACUTE AND CHRONIC LEUKAEMIAS - USE OF MONOCLONAL ANTIBODIES AND LENALIDOMIDE

J. Duncan,[1] H. Roddie[2]

[1]Centre for Regenerative Medicine, EDINBURGH; [2]Western General Hospital, EDINBURGH, UK

Background. Dendritic cell (DC) vaccines in leukaemia show promise as a novel treatment modality. Despite demonstration of immunological responses to vaccination clinical responses have proven more elusive. Responses may be improved by vaccination therapy taking place at a time of low disease burden and by the use of immune adjuncts. Monoclonal antibodies (MoAbs) have been used to treat malignant cells prior to co-culture with DCs to enhance cross presentation and generation of cytotoxic T cell responses.[1] Lenalidomide, a thalidomide analogue and immunomodulatory agent, is a powerful potentiator of CTLs and NK cells.[2,3] It may be useful in DC vaccine therapy as an immune adjunct and has the advantage of clinical tolerability. *Aims*. The aim of this study is to generate an effective autologous vaccination approach for patients with acute and chronic leukaemia who are in a state of low disease burden following conventional chemotherapy. In order to improve vaccine therapy two different approaches to immune augmentation were tested. Firstly leukaemia cells were treated with leukaemia specific monoclonal antibodies an an effort to improve DC uptake and presentation of leukaemia antigens. Secondly the immunomodulatory agent Lenalidomide was used to augment leukaemia specific CTL responses. *Methods*. In this *in vitro* study DCs were generated from monocytes of patients in remission following chemotherapy for acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML) and chronic lymphocytic leukaemia (CLL) with standard cytokine protocols. The immature DCs were loaded with autologous leukaemia cells from the patients' presentation samples. The presentation leukaemia cells were treated with either UVB irradiation or appropriate MoAbs (the anti-CD33 MoAb Mylotarg in AML and CML; the anti CD20 MoAb Rituximab or the anti-CD 52 MoAb Alemtuzumab in CLL). DCs were matured with TNF alpha for two days then co-cultured with autologous T cells for one week with or without the addition of Lenalidomide at ten micromolar. The T cells were harvested and their cytoxicity assessed in an Interferon Gamma (IFNγ) ELISPOT assay where the stimulators used were the unmodified blasts. *Results*. Results in six patients show the leukaemia specific CTL responses were markedly improved in the groups treated with MoAbs or irradiation compared with CTL responses against unmodified leukaemia cells although to date no clear advantage has been seen with any one MoAb. In two patients we have demonstrated further improvement in the leukaemia specific CTL responses when Lenalidomide was added to MoAb or irradiated treated groups. *Conclusions*. These early results show promise for this approach to immune augmentation of DC vaccination in leukaemia and further investigation is in progress to carry out this protocol in more patients.

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0600

CELL-FREE VACCINATION WITH ACUTE MYELOID LEUKEMIA-CELL DERIVED EXOSOMES PROLONGS SURVIVAL IN MURINE LEUKEMIA MODEL THROUGH EFFECTIVE INDUCING CYTOTOXIC T-CELL GENERATION

J.-W. Cheong,[1] Y.H. Min,[2] A.J. Choi,[1] H.K. Jeung,[1] H.Y. Choi,[1] S.J. Kim,[1] J.S. Kim[1]

[1]Yonsei University College of Medicine, SEOUL; [2]BrainKorea21 Research Team of Nanobiomaterials for the Cell-Based Implants, YUMC, SEOUL, South-Korea

Exosomes are a population of nanometer-sized vesicles, actively secreted by various kinds of hematopoietic cells and tumor cells, with physiologic functions that include immune modulation. Both dendritic cell (DC)-derived exosomes and tumor-derived exosomes have potential therapeutic efficacy as cancer vaccine. Since DC preparation *in vitro* is time-consuming, expensive, and difficult to handle for scale-up, tumor-derived exosomes can be a preferable source of exosomes including MHC class I molecules, shared tumor antigen, and heat shock proteins (Hsp). In this study, we aimed to isolate and characterize leukemia cell-derived exosomes (LEX) and to evaluate its efficacy as leukemia vaccination therapy in murine leukemia model. C1498 (H-2K) acute myeloid leukemia cells were grown in RPMI 1640 medium supplemented with GM-CSF. Exosomes were harvested from the culture media using multi-step centrifugation, and then characterized by electronmicroscopy, flow cytometry, and Western blot analysis analysis. Western blot analysis demonstrated the presence of Hsp70 and survivin. The efficacy of exosomes as a prophylactic vaccine against C1498 leukemia was examined in a syngeneic C57BL/6J female mice. Mice were subcutaneously immunized three times at 1-week interval on the flanks with 10μg of exosomes. One week after final immunization, 0.2mL of 5×10^6 C1498 leukemia cells were injected subcutaneously in the side opposite to the exosome injection. No leukemia was developed in LEX-immunized mice until the 25th day, whereas over 90% of control mice succumbed to leukemia ($p<0.001$). LEX vaccines significantly improved survival compared to the non-vaccinated controls ($p<0.001$). In the control mice developed leukemia, the proportions of splenic $CD3^+CD4^+$ and $CD3^+CD8\alpha$ T-cells were markedly decreased compared to the LEX-vaccinated group (6.1±2.3% *vs* 15.2±3.5% for CD3+CD4+, $p<0.01$; 4.2±1.2 *vs* 12.5±1.9% for CD3+CD8α T-cells, $p<0.01$). C1498 LEX could stimulate cytotoxic T-lymphocytes (CTL) *in vitro*, and these CTL effectively lysed C1498 leukemia cells. Significantly higher frequency of IFNÁ-producing splenocytes was observed in the LEX-vaccinated group ($p<0.01$). These effects were only observed in spleens from LEX-vaccinated mice. We demonstrated that LEX can induce leukemia-specific immunity and be considered as a potential strategy for novel immunotherapy in acute myeloid leukemia.

0601

ATTENUATED DOSES OF RITUXIMAB FOR THE TREATMENT OF ADULTS WITH AUTOIMMUNE CYTOPENIAS

G. D'Arena, P.R. Scalzulli, M. Nobile, A. La Sala, C. Bodenizza, M. Dell'Olio, S. Mantuano, G. Rossi, N. Cascavilla

Casa Sollievo della Sofferenza Hospital, SAN GIOVANNI ROTONDO, Italy

Background. A new paradigm of treatment for autoimmune cytopenias has recently emerged based on the use of monoclonal antibodies such as rituximab. This drug was generally infused at 375 mg/sqm weekly for 4 consecutive weeks according to the schedules currently used for the treatment of non-Hodgkin's lymphoma (NHL), in which a high tumor burden is usually seen at diagnosis. However, the number of B lymphocytes in autoimmune disorders is considered normal. For this reason, some attempts have been made using low dose rituximab to treat autoimmune cytopenias in adults (Provan *et al.*, Haematologica 2007). Aims. On this basis, 5 patients (4 M; 1 F; mean age 52 years; range 41 - 66 years) with autoimmune cytopenias were treated at our Institution with attenuated doses of rituximab (100 mg) for 4 times weekly, after informed written consent was given. *Methods*. Three patients had idiopathic thrombocytopenic purpura (ITP) and 2 patients had autoimmune hemolytic anemia (AIHA) The median disease duration before giving rituximab was 52 months (range 1 - 144 months), while the median number of previous therapies was 1 (range 1 - 3). No patient was found positive for hepatitis B and C and/or HIV serology. *Results*. Four patients completed the 4 scheduled cycles. One non-responding patient with ITP did not respond and rituximab was stopped after the third infusion and patient successfully underwent to splenectomy. For all patients infusion-related side effects were minimal. Complete responses (CR) were seen in 1 (33%) of the 3 patients with ITP and in both (100%) patients with AIHA. Two patients with ITP showed an early increase of platelets after the first rituximab infusion followed by a progressive decrease and were considered non responders. The time to maximum response ranged from 2 to 3 weeks. In all responding patients the remission status is maintained at last follow-up (median 6,2 months; range 2-11 months). *Conclusions*. Despite the number of patients treated so far with attenuated doses of rituximab is too small to draw any firm conclusion, these encouraging results support this therapeutic approach that appears less expensive but with the same efficacy as the conventional dosage to treat autoimmune disorders. In addition, efforts should be directed towards the identification of patients with potentially responsive chronic ITP.

0602

SELECTIVE DEPLETION OF ALLOREACTIVE DONOR T LYMPHOCYTES AND STUDY OF ANTI-TUMOR ACTIVITY OF SPECIFIC T CELL CLONES IN PATIENS WITH LEUKEMIA AND MULTIPLE MYELOMA

E. Matejkova,[1] D. Ocadlikova,[1] J. Muzikova,[1] P. Vidlakova,[1] D. Kyjovska,[1] E.S. Vitetta,[2] R. Hajek,[3] J. Michalek[1]

[1]*Masaryk University, BRNO, Czech Republic;* [2]*Cancer Immunobiology Center, University of Texas Southwestern Medical Center, DALLAS, TEXAS, USA;* [3]*University Research Center - Czech Myeloma Group, BRNO, Czech Republic*

Background. Graft-versus-host disease (GVHD) is a severe complication of allogeneic transplantation of hematopoietic stem cells. The major role in GVHD play the alloreactive clones of donor T cells leading to the host tissue damage. Selective allodepletion (SD) is a strategy to eliminate host-reactive donor T-cells from hematopoietic stem cell allografts to prevent graft-versus-host disease (GvHD) while conserving useful donor immune functions. *Aims.* We have selectively depleted host-reactive donor T cells from peripheral blood mononuclear cell (PBMC) *ex vivo* using an anti-CD25 immunotoxin. *Methods.* We have used irradiated peripheral blood mononuclear cells (PBMC) from cancer patients and healthy donor PBMC as responder cells in primary mixed leukocyte reaction (MLR).To prepare GVL/GVT-specific T-cells, alloreactive T-cells in primary MLR were depleted with anti-CD25 IT. The remaining T-cells had insignificant alloreactivity in secondary MLR. Allodepleted donor cells were then repeatedly stimulated using purified leukemia/tumor cells from the same cancer patient. Leukemia/tumor-reactive donor T-cells were purified immunomagnetically on the basis of INF-g production. *Results.* 20 MLRs (10 with acute myeloid leukemia cells, 3 with acute lymphocytic leukemia cells and 7 with multiple myeloma cells) were performed. Selective depletion of alloreactive donor T-cells with anti-CD25 IT led to significant depletion (99.2-100%, median 99.7%). Graft-versus-leukemia (GVL) effect of donor T-cells was well preserved (1.1-9.6%, median 7.46%) of donor T cells were GVL-reactive, while the graft-versus-host (GVH) reactivation of donor cells was negligible (0 - 0.23%, median 0.14%) even after repeated stimulation with patient's non-leukemic PBMC. *Summary*. In conclusion, it is possible to selectively deplete donor alloreactive T-cells with anti-CD25 IT. In both cases of patients with leukemia and multiple myeloma, a strong GVL reactivity was noticed in allodepleted donor T cells.

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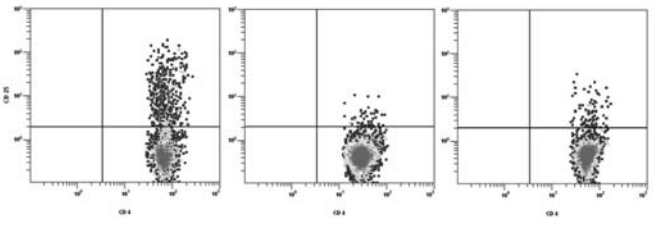

Figure 1. Flow cytometry of CD^+CD25^+ T cells in MLR.

0603

PROPHYLACTIC VACCINATION WITH TUMOR - DERIVED MICROVESICLES AS A WAY TO PREVENT TUMOR DEVELOPMENT

M. Majka, A. Mordel

Jagiellonian University Medical College, CRACOW, Poland

Background. Despite improvement in current treatment modalities, cancer is still very often an incurable disease. Tumor cells are often refractory to chemotherapy and irradiation and bulk tumors can not be radically resected. The ideal way of preventing tumor development will be through strengthening an immune response before tumor develops and spreads. This means to *teach* immune system to recognize tumor antigens and prepare immune cells to destroy tumor. *Aims.* The major objective of this project was to develop an efficient way of preventing occurrence of tumor by activation of the immune system through vaccination with tumor - derived microvesicles (TDMV). *Methods.* TDMV were isolated from murine melanoma (B78) and colon cancer (CT26) cell lines. Syngeneic mice (C57/Bl6 and BALB/c, respectively) were injected 3 times in weekly intervals with TDMV (30 μg/mice). One month after last injection mice were transplanted i.v. with 5×10^5 tumor cells. After 30 days half of mice were killed and number of lung foci was scored. Rest of mice was used to assessed the overall survival. *Results.* Number of lung foci in animals injected with TDMV was significantly smaller in comparison with controls and in some animals immunized with TDMV lung foci were absent. Moreover, the overall survival of animals injected with TDMV before tumor challenge was greatly better than in control groups. These results were obtained for both types of tumor. In order to characterized the cell populations responsible for the observed phenomenon we phenotyped peripheral blood obtained from immunized and non-immunized animals. We observed change in TCR repertoire, however these results requires further evaluation. Moreover, in immunized mice new population appeared on the cytogram. We are currently characterizing these cells. Summary: Our results suggest that TDMV from melanoma and colon carcinoma cell lines are able to induce immune response toward syngeneic tumors and thus prevent tumor growth. Based on these observations we hypothesize that TDMV express various tumor associated antigens and that these antigens are able to stimulate the anti-tumor immune responses and in a consequence rejection of the tumor.

Hogdkin's lymphoma

0604

PREDICTIVE VALUE OF EARLY PET DURING SALVAGE CHEMOTHERAPY IN RELAPSING/REFRACTORY HODGKIN'S LYMPHOMA (HL) PATIENTS

L. Castagna, S. Bramanti, M. Balzarotti, E. Todisco, B. Sarina, A. Anastasia, M. Magagnoli, A. Nozza, R. Mazza, L. Giordano, A. Chiti, A. Santoro

Istituto Clinico Humanitas, ROZZANO, Italy

Background. FDG-PET performed before high-dose chemotherapy (HDC) seems to predict the outcome. Few data have been reported about the value of FDG-PET performed early during salvage treatment. *Aims*. To evaluate the predictive role of early PET during salvage chemotherapy in relapsed/refractory HL patients. *Methods*. From November 2003 to May 2007, we reviewed 24 patients with relapsed or refractory HL treated with 4 courses of IGEV (ifosfamide, gemcitabine, and vinorelbine). FDG-PET was performed at relapse/progression, after the 2nd (PET2) and 4th (PET4) course. No progressive patients received one or two cycles of HDC with autologous stem cell support. The progression free survival (PFS) by PET2 and PET4 results was calculated. Univariate analysis, was performed considering PET2 and PET4 results, german prognostic score, age, stage at diagnosis and at relapse and response to first line treatment. *Results*. Median age was 33 years (range 15-51). 25% of patients were refractory after first line treatment and 75% had relapsed (50% within 12 months). PET2 evaluation was negative in 58% and positive in 42%. PET4 analysis became negative in 18 patients (75%) and was persistently positive in 6 patients (25%). All patients with a negative PET2 remained negative and 2 patients converted from PET2 positive to PET4 negative. 8/10 patients with positive PET2 relapsed after HDC. The 2 patients had converted to negative PET4, are in continuous remission. All patients with negative PET2 maintained complete remission after HDC. 1-year PFS and OS for all patients were 67 % and 90%, respectively (Figure 1). PFS was 92% *vs* 28%, for negative and positive cases by PET2, respectively (p<.001), and 78 % and 33%, for negative and positive cases by PET4, respectively (p=.002). In univariate analysis, at a median follow up of 11 months, only PET2 and PET4 significantly influenced the PFS (p= 0.01). *Conclusions*. This small retrospective study suggest that PET2 results significantly affect the outcome after HDC. These data have to be confirmed in a more consistent cohort of patients. If confirmed, PET2 positive patients should receive alternative treatment in controlled prospective studies.

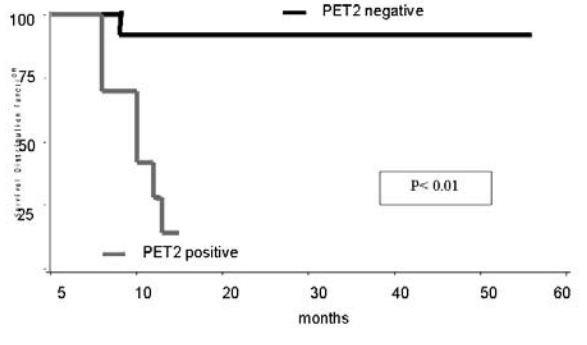

Figure 1. PFS by PET2 positive and negative.

0605

PHASE II STUDY OF THE HISTONE-DEACETYLASE INHIBITOR ITF2357 IN RELAPSED/REFRACTORY HODGKIN'S LYMPHOMA PATIENTS

V. Bonfante, S. Viviani, C. Fasola, F. Crippa, A. Marchianò, P. Valagussa, A.M. Gianni

Istituto Nazionale Tumori, MILAN, Italy

Background. Although great progress has been made in the last decades in the treatment of Hodgkin's lymphoma (HL), new therapeutic agents are still needed, in particular to treat patients (pts) relapsing or progressing after salvage chemotherapy. ITF2357 (Italfarmaco)is a new hydroxamate histone deacetylases inhibitor that has inhibitory activity in the production of pro-inflammatory cytokines, as well as cytotoxic activity both *in vitro* on several human tumor cell lines and *in vivo* in pts with hematologic malignancies. *Methods.* To evaluate the efficacy, safety and tolerability of daily 100 mg oral doses of ITF2357 in 3 four-week cycles, a phase II open label non randomised study is ongoing at the Istituto Nazionale Tumori of Milan as third-line or higher treatment of heavily pretreated, relapsed or refratory, HL pts. *Results.*From May 2007, 15 out of 23 planned pts have been enrolled. Characteristics at start of ITF were as follows: median age 26 years (range:21-54), Nodular Sclerosis histology 93%, advanced stage 73%, B symptoms 40%, extranodal±nodal involvement 47%, >3 involved sites 47%, median number of prior therapy lines 4 (range 2-4). Thirteen pts (87%) had a prior autologous transplant and 4 of them received an additional allogeneic transplant. The median mumber of ITF 2357 cycles administered was 2 (range 1-3).All fifteen pts completed at least one cycle of therapy and were evaluable for response. Nine pts (60%) had stable disease by CT scan that was associated with a significant reduction in FDG-PET uptake in 7 pts (47%) lasting a median of 3 months (range: 1-5). Six pts had progressive disease. Toxicity included: grade 1 leukopenia in 30%, grade 2 thrombocytopenia in 33%, fatigue in 50%, grade 1 diarrhea and/or abdominal pain in 40%; prolongation of Qtc prompting transient drug discontinuation in 20%. *Conclusions*. Preliminary results in this series of very heavily preatreated HL pts showed that oral ITF 2357 has antitumor activity and a good safety profile. The drug warrants additional studies, alone and in combination, as salvage treatment for HL.

0606

REDUCED-INTENSITY CONDITIONING (RIC) ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) IN RELAPSED OR REFRACTORY HODGKIN'S DISEASE (HD): DISEASE STATUS AT TRANSPLANT IS A MAJOR FACTOR FOR OUTCOME

T. Gastinne, J. Delaunay, B. Mahe, V. Dubruille, N. Blin, S. Ayari, S. Le Gouill, T. Guillaume, P. Chevalier, P. Moreau, J.-L. Harousseau, M. Mothy

University Hospital of Nantes, NANTES, France

The use of RIC prior to allo-SCT in adult patients with hematological malignancies has significantly increased over the last years. Indeed, the introduction of RIC regimens can allow a decreased incidence of early transplant-related mortality (TRM) while preserving the immune graft versus tumor effect. Thus, the use of RIC allo-SCT can represent an attractive treatment modality for those high risk patients usually not eligible for standard myeloablative allo-SCT. The aim of this analysis was to assess the outcome of 15 patients who received RIC allo-SCT for HD in a single centre. The median age was 27.5 (range, 19-62) years. Three patients (20%) were in complete response (CR), whereas 12 had a more advanced disease at time of allo-SCT [9 partial responses (PR), 3 progressive diseases (PD)]. Of note, all patients have already received and failed prior autologous transplantation. The conditioning regimen included Fludarabine 120 mg/m^2, busulfan 4 mg/kg, and ATG in 13 patients, whereas 2 patients received Fludarabine (90 mg/m^2) and low dose TBI (2 Gy.). All patients received CsA for GVHD prophylaxis, combined with MMF in 6 cases.

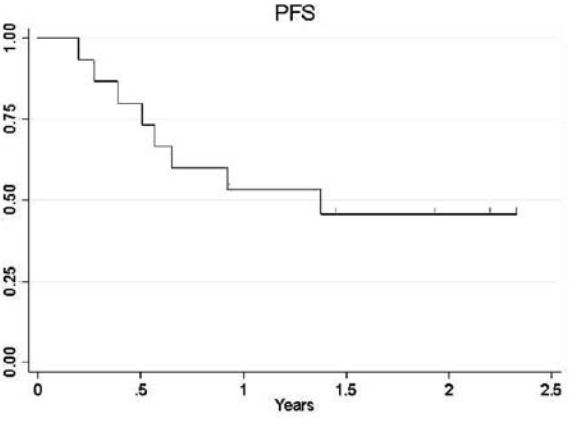

Figure 1. Progression Free survival.

The median CD34$^+$ cell dose was 5.6×10^6/Kg (range, 1.88-20.2). Neutrophil engraftment (ANC>500/µL) and platelets recovery (>50000/µL)

occurred in all the patients at a median of 19 (range, 0-21) and 10 (range, 0-37) respectively. The overall incidence of grade II-IV acute GVHD was 67% (6 grade II and 4 grade III-IV). 14 patients were evaluable for chronic GVHD for an overall incidence of 33% (all limited). With a median follow-up of 2.3 (range, 0.9-5.3) years, 7 patients (47%) had relapsed or progressed at a median of 7 months after allo-SCT. Patients transplanted in CR or PR had a 58% PFS *vs* 0% in those with PD. In all, 2 patients died of TRM (GVHD, n=1; other, n=1; TRM=13%). The Kaplan-Meier estimates of OS and PFS (Figure 1) were 84% and 46% at 2 years, respectively. In all, we conclude that RIC allo-SCT is a potentially feasible and efficient therapy for relapsed HD, with a relatively low rate of TRM. However, disease progression is still a matter of concern, especially in those patients not responding at time of allo-SCT, warranting prospective efforts to define early interventions to enhance the immune graft versus tumor effect.

0607

A NOVEL T(4;9)(Q21;P24) FUSES SEC31A TO JAK2 IN NODULAR-SCLEROSIS HODGKIN LYMPHOMA

K. Van Roosbroeck,[1] I. Lahortiga,[1] J. Cools,[1] P. Vandenberghe,[1] P. Marynen,[1] J. Delabie,[2] C. De Wolf-Peeters,[2] I. Wlodarska[1]

[1]Department of Human Genetics, Catholic University of Leuven, LEUVEN; [2]Department of Pathology, Catholic University of Leuven, LEUVEN, Belgium

Background. Molecular mechanisms underlying the pathogenesis of classical Hodgkin lymphoma (cHL) are poorly understood. Although no characteristic chromosomal translocation has been identified in cHL, gain and amplification of the 9p24 region harbouring JAK2 has been observed in up to 50% of cHLs. JAK2 encodes a protein tyrosine kinase (PTK) that plays a key role in the JAK/STAT signalling pathway. Chromosomal translocations and gain-of-function mutations involving JAK2 occur in several haematological malignancies. *Aims.* This study aimed at the molecular characterization of a novel t(4;9)(q21;p24) found in a case of nodular-sclerosis HL (NSHL). *Methods*. FISH, including MFISH, was used to identify chromosomal aberrations found in the reported patient and to map both breakpoints of the t(4;9). Subsequent molecular analysis using cDNA-based nested PCR followed by sequencing, was performed to confirm the FISH findings. Screening of series of HL cases was performed by PCR and/or FISH. *Results*. The t(4;9)(q21;p24) was found in a case of NSHL documented by a few abnormal metaphases. FISH with BAC clones flanking JAK2/9p24 demonstrated involvement of this gene. The 4q21 breakpoint was identified using a BAC-walking interphase FISH strategy. This extensive interphase FISH study narrowed down the 4q21 breakpoint to a 450 kb region harbouring three candidate partner genes: SEC31A, LIN54 and PLAC8. Further interphase FISH with fosmid probes flanking all three genes mapped the breakpoint to the region of SEC31A. This gene is ubiquitously expressed in human cells and is known to play a role in ER-to-Golgi vesicular transport. Further molecular studies led to the identification of a SEC31A-JAK2 in-frame fusion transcript in which exon 24 of SEC31A is fused to exon 17 of JAK2. The SEC31A-JAK2 fusion protein is likely to function as a constitutively activated tyrosine kinase, due to SEC31A-mediated oligomerization of JAK2. The transforming capacity of this fusion protein will be studied in IL3-dependent Ba/F3 cells. To determine the incidence of the t(4;9) in cHL, we are screening series of cHL cases using both FISH and cDNA-based nested PCR. So far, one out of 25 analyzed cases showed a SEC31A-JAK2 fusion with breakpoints identical to the index case. The remaining cases revealed recurrent gains/amplifications of JAK2. *Summary and Conclusions*. In summary, we identified and molecularly characterized the novel t(4;9)(q21;p24) in cHL resulting in a SEC31A-JAK2 fusion. This is the first recurrent JAK2-associated translocation in HL. Although aberrant expression of various PTKs including JAK2 has already been documented in cHL, our results indicate that at least in some cHL cases, this aberration can be driven by a chromosomal translocation. The findings of frequent gains of JAK2 in cHL suggests that not only structural but also numerical aberrations of JAK2, possibly leading to aberrant PTK activation, are playing a role in the pathogenesis of cHL.

0608

DOSE-INTENSITY OF ABVD AS AN IMPORTANT PROGNOSTIC FACTOR FOR FIRST LINE HODGKIN DISEASE: AN ANALYSIS OF 107 CONSECUTIVE PATIENTS TREATED IN A SINGLE INSTITUTION

E. Nicolas-Virelizier, H. Ghesquieres, S. Dussart, B. Favier, F. Lachenal, P. Biron, T. Gargi, C. Sebban

Centre Léon Bérard, LYON, France

Background. ABVD (Adriamycin, bleomycin, vinblastine and dacarbazine) is the world standard chemotherapy regimen for first line Hodgkin disease (HD). *Aims.* however, the delivery in clinical trials or in standard practice varies considerably and there are no strong data about the impact of dose intensity on the outcome. *Methods.* We reviewed all the 107 newly diagnosed HL patients treated in first line in our institution by full dosage of ABVD from 1996 to 2006 in order to assess the prognostic value of dose intensity on long-term outcome. Secondary prophylaxis with GCSF was made when absolute neutrophil count (ANC) was $<1.5\times10^9$/L to avoid a treatment delay or when a febrile neutropenia occurred. *Results.* Median age was 31 years, 44% had III or IV Ann Arbor stages, 56% had B symptoms, 21% had a bulky disease, 60% received radiotherapy after 3 to 6 courses of ABVD. G-CSF was administered in 65% of the cases.7% of patients had a febrile neutropenia. Five year overall survival (OS) survival and 5-year event free survival (EFS) were respectively 93% and 86%. Median dose intensity (mg/m2/week) for each drug were 12 for A (expected 12.5), 4.3 for B (expected 5), 2.9 for V (expected 3) and 180 for D (expected 188). Mean total dose intensity for the 4 drugs was superior to 90% in 61 patients (group 1: 57% of cases) and ≤0% in 46 patients (group 2:43% of cases).Patients in group 1 were significantly younger and had less often bone marrow involvement. 66% achieved CR after 3 or 4 induction courses in group 1 *vs* 50% in group 2. There is a trend for a best 5-y EFS for group 1 patients: 93% *vs* 77% (p=0.06) and a significant best 5y OS of 98% in group 1 *vs* 87% in group 2.(p=0.03). *Conclusions.* As DI seems to influence the outcome, ABVD must be delivered at full dosage and without any delay. The place of GCSF in this setting remains questionable as reported in other similar studies.

0609

THE INFLUENCE OF THE POLYMORPHISM OF GLUTATHIONE S-TRANSFERASE PI 1 (GSTP1) GENE IN HODGKIN'S LYMPHOMA RISK AND PROGRESSION

G. Lourenco, I. Neri, V. Sforni, R. Kameo, I. Lorand-Metze, C. Lima

State University of Campinas, CAMPINAS, Brazil

Background. Hodgkin's lymphoma (HL) is a heterogeneous malignancy, and little is known about the aetiology of this disease. The enzymes from the glutathione S-transferase (GST) system catalyse the conjugation of electrophilic molecules of numerous carcinogenic chemicals and products of oxidative stress to glutathione, reducing them to less toxic levels. The GSTP1 gene is a member of the GST system. A single nucleotide polymorphism in the GSTP1 gene causes the substitution of isoleucine to valine at amino acid codon 105 (Ile105Val). The frequency of the genotypes is different in distinct populations (Ile/Ile: 46-72%, Ile/Val: 28-43% and Val/Val: 00-15%). The valine allele is associated with a decreased activity of the enzyme compared with isoleucine allele. In addition to detoxification, the GSTP1 may also influence apoptosis, cell proliferation and survival. The GSTP1 wild-type expressing fibroblast cells increased the colony-forming efficiency of cells in stress conditions in comparison to GSTP1 allele variant expressing cells in a previous study. The association of the GSTP1 genotypes and the risk of developing HL are not yet fully clarified. *Aims.* The aim of this study was to evaluate the influence of the GSTP1 Ile105Val polymorphism for HL risk in individuals of the southeastern region of Brazil. *Methods.* For this purpose, genomic DNA from peripheral blood of 110 HL patients (median age: 27, range: 14-82, male: 57, female: 53, Caucasian: 96, Black: 14) and 110 gender and racially matched controls (median age: 51, range: 25-59, male: 60, female: 50, Caucasian: 94, Black: 16) were analyzed using polymerase chain reaction followed by enzymatic digestion. The differences between the groups were calculated by chi-square or Fischer exact test. Logistic regression analysis was used to obtain age, gender and ethnic origin adjusted odds ratios (ORs). *Results.* Controls' samples were in Hardy-Weinberg equilibrium for the GSTP1 Ile105Val locus (X2=1.56, p= 0.21). In contrast, patients' samples did not confirm the Hardy-Weinberg expectations at this locus (X2= 12.88, p<0.001). The frequency of the GSTP1 wild-type genotype (59.1% *vs* 37.3%, p= 0.005) was higher in HL patients than in controls. Individuals with the wild-type genotype had

an increased risk for disease than those with the variant allele (OR: 2.77; 95% CI: 1.36-5.63). Similar frequencies of the GSTP1 genotypes in patients stratified by age, gender, ethnic origin and bulky disease were also seen in this study. In contrast, a high frequency of the GSTP1 wild-type were observed in patients with tumours of stages III+IV in comparison with to tumours of stages I+II (39.1% *vs* 20.0%, p=0.03) using the multivariate analysis. *Conclusions.* These results suggest that the wild-type allele of the GSTP1 gene is linked to an increased the risk and higher aggressiveness of the HL in our cases, but it should be confirmed by further studies with larger cohorts of patients and controls. We hypothesised that this fact may be attributed to its effect on favouring tumour cell survival.

Supported by FAPESP

0610

DELIVERY OF FULL DOSE ABVD FOR HODGKIN'S LYMPHOMA WITHOUT GROWTH FACTORS AND REGARDLESS OF NEUTROPENIA DUE TO TREATMENT: A SAFE, EFFICACIOUS AND COST SAVING PRACTICE

J. Nangalia

Norfolk and Norwich University Hospitals NHS Trust, NORWICH, UK

Background. ABVD (Adriamycin, Bleomycin, Vincristine, Dacarbazine) chemotherapy induced neutropenia during treatment for Hodgkin Lymphoma (HL) is frequently encountered and the use of granulocyte colony-stimulating factor (G-CSF) to maintain dose-intensity is common practice, both outside of and within clinical trials, despite little evidence to suggest that this is necessary. At our institution, it has been the standard practice to proceed with ABVD chemotherapy in all patients without delay, dose reductions, or the use of growth factors regardless of the absolute neutrophil count (ANC) on the planned day of treatment as long as there is no associated anaemia or thrombocytopenia. *Aims.* We examined the safety and efficacy of administering full dose ABVD regardless of isolated neutropenia due to treatment without the use of growth factor prophylaxis and reviewed the current evidence for this practice. *Methods.* In a retrospective, single institution analysis, we reviewed the outcome of 24 patients with early and advanced stage HL treated with full dose ABVD chemotherapy (263 treatment deliveries) without the routine use of G-CSF or prophylactic antibiotics over a three year period. ABVD chemotherapy was administered regardless of the Absolute Neutrophil Count (ANC) on the day of planned treatment delivery, provided there were no other cytopenias. *Results.* The median ANC on all ABVD treatment days was 0.90×10^9 per litre. No cytopenias were noted prior to commencing chemotherapy. Subsequently, 50% of treatment deliveries were given to patients with an ANC less than 1.0×10^9 per litre and on 20% of treatment days, the ANC was less than 0.5×10^9 per litre. 4 patients required drug omissions (Vincristine or Bleomycin) due to non-haematological side effects. 0.76 percent of treatments were complicated by febrile neutropenia. All patients recovered from neutropenia following completion of chemotherapy with no cases of myelodysplasia. The median follow up period was 16 months (range 4 - 36 months). All 22 patients that have completed treatment are in remission, with one patient refractory to ABVD requiring salvage therapy. *Conclusions.* We conclude that it is safe and effective to administer full dose ABVD chemotherapy without prophylaxis with G-CSF, irrespective of the ANC on the planned treatment day. Furthermore, for the 24 patients treated in this manner, we estimate a saving of £60000 in pharmaceutical and nursing expenditure related to G-CSF, equating to a saving of £2000 per patient. Future clinical trials need to investigate the significance of isolated neutropenia during ABVD treatment and the need for G-CSF in this context.

0611

PROGNOSTIC SIGNIFICANCE OF GLUTATHIONE-S-TRANSFERASE (GST) GENOTYPES IN PATIENTS WITH ADVANCED HODGKIN' DISEASE (HD) ENROLLED IN THE HD2000 GISL TRIAL.

M. Gentile,[1] C. Mammì,[2] L. Marceselli,[3] F. Merli,[4] N. Cascavilla,[5] S. Luminari,[3] C. Stelitano,[6] M. Musso,[7] E. Iannitto,[8] F. Angrilli,[9] M. Petrini,[10] A. Riezzo,[11] S. Romito,[12] G. Quarta,[13] G. Partesotti,[14] S. Molica,[15] A. Fracasso,[16] G. Polimeno,[17] M. Lombardo,[9] M. Russo,[18] F. Ilariucci,[19] A. La Sala,[5] S. Pozzi,[3] V. Callea,[6] R Scalone,[7] C. Laganà,[2] M. Federico,[3] F. Morabito[1]

[1]Ematologia di Cosenza, COSENZA; [2]Unità Operativa di Genetica Medica, Azienda Ospedaliera di Reggio Calabria, REGGIO CALABRIA; [3]Dipartimento di Oncologia ed Ematologia Università di Modena, MODENA; [4]Dipartimento di Ematologia, Ospedale Santa Maria Nuova., REGGIO EMILA; [5]UO di Ematologia e Trapianto di Midollo Osseo, SAN GIOVANNI ROTONDO; [6]Unità Operativa di Ematologia, Azienda Ospedaliera Reggio Calabria, REGGIO CALABRIA; [7]Unita Operativa di Oncoematologia, Ospedale La Maddalena, PALERMO; [8]Divisione di Ematologia, Ospedale Policlinico, PALERMO; [9]Dipartimento di Oncologia, Ospedale Santo Spirito, PESCARA; [10]Dipartimento di Oncologia, Sezione Ematologia, Ospedale Santa Chiara, PISA; [11]Divisione di Ematologia, Ospedale 'S. Nicola Pellegrino', TRANI; [12]DIpartimento di Oncologia Medica, CAMPOBASSO; [13]Divisione di Ematologia, Presidio Ospedaliero A. Perrino, BRINDISI; [14]Divisione di Medicina, Ospedale Civile, SASSUOLO; [15]Dipartimento di Oncologia ed Ematologia, Azienda Ospedaliera Pugliese-Ciaccio, CATANZARO; [16]Unità Operativa di Medicina Interna, Presidio Ospedaliero di Matera, MATERA; [17]Servizio di Ematologia, Divisione di Medicina, Ospedale 'Miulli', ACQUAVIVA DELLE FONTI; [18]Unità Operativa di Ematologia, Ospedale San Vincenzo, TAORMINA; [19]Dipartimento di Ematologia, Ospedale Santa Maria Nuova, REGGIO EMILIA, Italy

Background. Polymorphisms in detoxification enzymes of the GST family have been associated with risk and prognosis of several solid tumors and some haematologic malignancies, including HD. *Aims*. To assess the prognostic significance of GSTM1 and GSTT1 deletions, and of GSTP1 Ile105Val polymorphism on toxicity, response to therapy and progression free survival (PFS) in patients with HD. *Methods*. Deletions of GSTM1 and GSTT1 were studied using a multiplex PCR technique, while the GSTP1 Ile105Val polymorphism was analyzed using the PCR-restriction fragment length polymorphism (RFLP) technique. 140/307 patients with untreated and advanced HD enrolled in the HD2000 GISL trail, an Italian multicentre randomized study designed for comparing the efficacy of ABVD *vs* BEACOPP *vs* CEC, were included in this study. The associations between GST genotypes and toxicity and outcome were analyzed. *Results*. The frequency of GST deletions in 140 HD patients was as follows: GSTM1 null 53% (74/140), GSTT1 null 21% (30/140). For the GSTP1 genotype, there were 10 (7%) patients homozygous for the 105Val/105Val genotype, 54 (39%) heterozygous (105Ile/105Val), and 76 (54%) homozygous for 105Ile/105Ile GSTP1 genotype. A comparable distribution of GSTM1 and GSTT1 deletions and of GSTP1 polymorphism was observed in the three therapy arms. Patients with homozygous deletion of the gene for GSTM1 had a significantly higher probability of achieving a complete response (CR) than those with undeleted genotype (92% *vs* 78%; p=0.03), while GSTT1 and GSTP1 genotypes failed to demonstrate any significant predictive value. GSTM1-null genotype maintained the prognostic impact even when cases were adjusted for IPS score and therapy arm. In fact, at the exact logistic regression model, patients with an undeleted GSTM1 genotype had a lower CR rate when compared with patients with null genotype (OR for risk of not achieving CR, 1 *vs* 3; p=0.04). At univariate analysis, we observed a significantly higher incidence of WHO grade III-IV anemia in both homozygous for genotype 105Val/105Val GSTP1 and heterozygous patients than in those homozygous for the 105Ile/105Ile GSTP1 (p=0.035). At multivariate analysis, homozygous status for genotype 105Val/105Val GSTP1 and heterozygous status remained significantly related to the occurrence of severe anemia episodes (OR adjusted for IPS score and therapy arm: homozygous for 105Ile/105Ile GSTP1 cases *vs* heterozygous cases: 1 *vs* 9.1; p=0.037; heterozygous cases *vs* homozygous for 105Val/105Val GSTP1: 9.1 *vs* 11.4, p=0.022). Patients with different genotypes for the 3 GST genes showed a similar occurrence of WHO grade III-IV neutropenia and thrombocytopenia. After a median follow-up of 41 months, PFS was not influenced by the genetic polymorphisms in the GSTT1, GSTP1 and GSTM1. Finally, we analyzed the effect of combinations of GST genotypes on prognosis.

Patients with undeleted GSTM1 and GSTT1 genes had a similar PFS when compared with those with one or both GST deletion (p=0.333). *Conclusions*. The results of the first 140 patients enrolled in the HD2000 trial suggest that the analysis of genetic polymorphisms in the GSTT1, GSTP1 and GSTM1 allows to distinguish HD cases with a higher probability of achieving CR (GSTM1 null) and patients with a higher incidence of severe anemia (105Val/105Val GSTP1 and 105Ile/105Val GSTP1), but it does not allow to stratify prognostically patients with different outcome. However, additional cases and a longer follow-up are required to provide definitive conclusions.

0612

PET/CT FINDINGS IN PATIENTS WITH HODGKIN'S LYMPHOMA (HL) AFTER ABVD COMBINATION CHEMOTHERAPY: CLINICAL AND PROGNOSTIC SIGNIFICANCE

T.P. Vassilakopoulos,[1] S. Masouridis,[1] M.K. Angelopoulou,[1] S.I. Kokoris,[1] S. Sahanas,[1] C. Kalpadakis,[1] E.M. Dimitriadou,[1] P. Tsirkinidis,[1] M. Moschoyiannis,[1] P. Tsaftaridis,[1] Z. Galanis,[1] E. Variamis,[1] A. Gouliamos,[1] V. Prassopoulos,[2] L. Gogou,[2] R. Eftimiadou,[2] I. Andreou,[2] K. Datseris,[3] C. Papavassiliou,[2] G.A. Pangalis[1]

[1]National and Kapodistrian University of Athens, ATHENS; [2]Depts of Radiotherapy, Radiology and Nuclear Medicine, HYGEIA Hospital, ATHENS; [3]Dept of Nuclear Medicine, Evangelismos Hospital, ATHENS, Greece

Background. Approximately 30% of patients (pts) with HL fail primary ABVD chemotherapy (CHT) or relapse after an initial remission. Furthermore many pts have residual masses, but do not progress in the long-term. PET scan is a functional imaging technique, which can detect the presence of viable tumor post treatment. Mid-CHT and post treatment PET results appear to highly affect prognosis. The predictive value of post-CHT PET findings in patients scheduled to receive additional radiotherapy (RT) is not clearly established. *Aims.* To retrospectively analyze PET/CT findings after the end of ABVD and determine their impact on the risk of subsequent progression using a treatment policy, which incorporated complementary RT in pts with stage I/II disease. *Methods.* Between Dec 2004 and June 2007, 125 pts were treated with 4-8 cycles of ABVD, representing the total HL pt population in our Unit: 78 underwent PET/CT after the end of ABVD, 36 were not evaluated with PET/CT (mainly due to cost issues and availability limitations), one died early and 10 experienced early disease progression detected by conventional methods prior to PET/CT. 9/10 pts with early progression had stage IIB/III/IV disease. All 78 pts who underwent PET/CT had achieved CR/CRu or PR with ABVD. *Results.* The median age of the 78 pts was 27.5 years (15-78), 58% were males, 95% had classical HL and 70% had clinical stages (CS) I/II. PET/CT was negative in 52/78 pts (67%) and positive in 26 (33%). Patients with indeterminate results (positivity exclusively detected in atypical, unexpected, not previously involved sites) were included in the PET(+) group. Among PET(-) pts, 51/52 remained progression free for a median of 13 months (1-32) from the end of ABVD: 38/52 pts, all CS I/II, received RT at a median dose of 2940 cGy, while 14 CS III/IV pts did not receive RT. Among 26 PET(+) pts, 19 received RT at a median dose of 3940 cGy, 5 were simply followed without further treatment, 1 progressed rapidly and 1 declined RT. After a median follow-up of 13 months (1-31), 7/26 pts experienced disease progression. The 18- month progression free survival was 98% for PET(-) and 63% for PET(+) pts (p=0.0007). For CS I/II pts these figures were 100% *vs* 57% (p=0.0004), while for CSIII/IV pts they were 90% *vs* 88% (p=0.72). SUMMARY/*Conclusions.* A negative PET/CT result after ABVD was associated with excellent outcome within the 18 initial months of observation. Pts with positive PET/CT were in increased risk of progression, but most of them had not progressed at the time of the analysis. A post-chemo positive PET/CT was more predictive in earlier stage pts, but, following RT, >50% of PET(+) pts remained progression free 18 months after ABVD. In contrast, most advanced stage pts who failed primary ABVD did so during treatment or soon after its completion, prior to PET-based restaging. Longer follow-up is needed to accurately assess the positive predictive value of PET/CT after ABVD and the potential modulatory effect of subsequent RT.

0613

CHEMOTHERAPY OR CHEMOTHERAPY PLUS RADIOTHERAPY IN EARLY HODGKIN'S LYMPHOMA ? FINAL RESULTS OF A RETROSPECTIVE ANALYSIS OF A REGIONAL ITALIAN EXPERIENCE

M. Clavio,[1] F. Olcese,[1] E. Rossi,[1] M. Spriano,[1] F. Ballerini,[1] L. Canepa,[1] I. Pierri,[1] S. Aquino,[1] R. Varaldo,[1] M. Cavaliere,[2] A. Manna,[3] V. Secondo,[4] O. Racchi,[5] E. Balleari,[1] S. Napoli,[6] A.M. Carella,[1] R. Ghio,[1] M. Gobbi[1]

[1]Hematology Inst., GENOVA; [2]Savona Hospital, SAVONA; [3]La Spezia Hospital, LA SPEZIA; [4]Galliera Hospital, GENOVA; [5]Villa Scassi Hospital, GE SAMPIERDARENA; [6]Sanremo Hospital, SANREMO, Italy

Background and Aims. Six-eight courses of ABVD represent the standard treatment for patients with advanced stage Hodgkin's Lymphoma (HL). Radiotherapy, on the other hand, forms the basis of conventional therapy for early stage HL patients. The observation of late risks of developing second tumours and coronary heart disease after radiotherapy has led to a reduced utilization of involved or extended field radiotherapy in the front line treatment. There is increasing evidence that early stage HL patients are likely to be cured by 3-6 courses of ABVD, with radiotherapy being utilized only on limited residual disease. With the aim of comparing outcome of early stage HL patients treated with chemotherapy only or with the association of chemotherapy and radiotherapy, we retrospectively reviewed clinical features, therapy and long term outcome of 139 stage I-II HL patients diagnosed and followed in oncohaematologic divisions of Liguria (Italy) from 1995 to 2007. *Methods and patients.* 63 patients (45%) received a median of 6 courses of ABVD (CT group) and 76 patients (55 %) were treated with chemotherapy (a median of 3 courses of ABVD or Stanford V regimen) plus involved field or extendend field-radiotherapy (CT+RT group). The two therapeutic groups were statistically comparable for median age (31 years and 30 years, for CT and CT+RT, respectively), male/female ratio, histology (nodular sclerosis in 83% and 83%; lymphocyte predominance in 11% and 9%, mixed cellularity in 6% and 8%, respectively), stage distribution (stage II in 87% and 85%, respectively), B symptoms (42% and 38%) , bulky disease (26% and 21%). Median follow up was different (50 and 90 months for CT and CT+RT groups, respectively). *Results*. In the CT group 54 patients achieved CR (86%) and 9 obtained PR (14%) after first line therapy. Four out of the 9 partial responders achieved CR after high dose therapy (HDT), so that 58 patients overall (92%) achieved CR. In the CT+RT group 73 patients achieved CR (96%) and 3 obtained CR after high dose therapy (HDT), so that the final CR rate was 100%. HDT was employed as salvage therapy of partial responders in 9(14%) and 3(4%) patients, in the two therapeutic groups, respectively. All relapses occurred in the first 48 months after the completion of therapy, 7 in the CT group (12%) and 9 in the CT+RT group (12%). At 60 months 88% and 89% of patients treated with CT and CT+RT are alive and relapse free, respectively. Two (3 %) and 3 (4 %) patients have died and median survival is 40 months (8-144) and 87 months (12-149), respectively. A second neoplasia has been diagnosed in 4/76 (5%) patients treated with CT + RT. Coronary heart diseases were similar in the two groups. *Conclusions*. The retrospective analysis of our series shows that in the vast majority of stage I-II HL patients long term control of disease may be achieved with a limited utilization of radiotherapy. The addition of radiotherapy might furthermore increase the risk of developing a second neoplasia.

0614

CHEST X-RAY(CXR) AND THORACIC CT SCAN EVALUATION OF MEDIASTINAL LYMPHADENOPATHY IN PATIENTS WITH HODGKIN'S LYMPHOMA REVISITED

M.P. Angelopoulos, M. Angelopoulou, G. Pangalis, A. Gouliamos, M. Siakantaris, S. Kokoris, E. Dimitriadou, M.-C. Kyrtsonis, P. Tsaftaridis, E. Variamis, C. Kalpadakis, S. Sahanas, S. Masouridis, T. Vassilakopoulos

National and Kapodistrian University of Athens, ATHENS, Greece

Background. Bulky disease, especially in the mediastinum, is considered an adverse prognostic factor for early stage Hodgkin's Lymphoma (HL). Its significance is not clearly defined in advanced disease. Bulky mediastinal masses also constitute an indication for additional radiotherapy. However the definition of bulky mediastinal disease is not uniform, when based on CXR findings. Furthermore the relationship between CXR-defined and CT-defined bulky disease has not been adequately investigated. *Aims.* To evaluate the relationship among various CXR- or

CT-based definitions of mediastinal bulky disease in patients with HL. *Methods.* We retrospectively evaluated CXR and thoracic CT findings in 199 patients with HL involving the mediastinum. Bulky mediastinal masses were evaluated by determining the madiastinal mass ratio (MMR) in posteroanterior CXR films taken in maximal inspiration, by two *Methods.* According to Method 1, MMR1 was defined as the ratio between the maximal transverse diameter of the mass to the internal transverse diameter of the thorax at the level of the T5-6 interspace. According to Method 2, MMR2 was defined as the ratio between the maximal transverse diameter of the mass and the maximal internal transverse diameter of the thorax, usually close to the diaphragm. CXR-bulk, according to either method, was defined as MMR1 or MMR2 ≥0.33. CT scans were measured at the level where the mediastinal mass was appearing in its maximal diameter and taking posteroanterior and transverse measurements. According to CT findings, bulky disease was defined at the presence of a mass ≥7 cm (CT-bulk7) or ≥10 cm (CT-bulk10). *Results.* MMR1 and MMR2 were ≥0.33 in 57% and 32% of the patients respectively. According to CT findings, 59% and 26% had bulky disease at the cutoff of 7 cm and 10 cm respectively. There was a significant correlation between MMR1 or MMR2 and the maximal diameter of mediastinal lymphadenopathy in CT (Spearman's rho 0.638 and 0.623 respectively, $p<0.001$). Bulky MMR1 correlated better with CT-bulk7 (concordance rate 76%) than with CT-bulk10 (concordance rate 65%). On the contrary bulky MMR2 correlated better with CT-bulk10 (concordance rate 82%) than with CT-bulk7 (concordance rate 65%). DISCUSSION: Different definitions of mediastinal bulky disease result to substantially different patient classification. MMR2 correlated better than MMR1 with the Cotswolds definition of bulky disease (cutoff set at 10 cm by CT). The use of different approaches may affect the prognostic significance attributed to bulky mediastinal disease.

0615

POSITRON EMISSION TOMOGRAPHY (PET) PERFORMED BEFORE ALLOGENEIC TRANSPLANTATION HAS A PROGNOSTIC ROLE IN PATIENTS WITH RELAPSED AND CHEMOSENSITIVE HODGKIN LYMPHOMA OR AGGRESSIVE NON-HODGKIN LYMPHOMA

A Dodero,[1] R. Crocchiolo,[2] F. Patriarca,[3] L. Castagna,[4] F. Ciceri,[2] N. Frungillo,[5] R. Fanin,[3] S. Bramanti,[4] R. Miceli,[6] A. Assanelli,[7] R. Milani,[5] F. Crippa,[8] P. Corradini[5]

[1]Istituto Nazionale dei Tumori, MILANO; [2]Department of Hematology, Ospedale San Raffaele, MILANO; [3]Department of Hematology, University of Udine, UDINE; [4]Department of Hematology, Istituto Clinico Humanitas, MILANO; [5]Department of Hematology, Istituto Nazionale dei Tumori, MILANO; [6]Department of Medical Statistics, MILANO; [7]Deparment of Hematology, Ospedale San Raffaele, MILANO; [8]Department of Nuclear Medicine, Istituto Nazionale dei Tumori, MILANO, Italy

Background. Positron emission tomography (PET) scan using 18-fluorodeoxyglucose [18F-FDG] has a recognised prognostic value in patients (pts) with Hodgkin Lymphoma (HL) or aggressive Non-Hodgkin Lymphoma (HG-NHL) receiving chemotherapy or autologous stem cell transplantation (SCT). *Aims.* We retrospectively assessed the prognostic role of PET scan before reduced-intensity conditioning allogeneic SCT. *Methods.* Between 2000 and 2007, 82 consecutive pts with HG-NHL or HL, responding to salvage therapy, were evaluated with a PET scan before allografting. Presence (PET-pos) or absence (PET-neg) of abnormal 18F-FDG uptake was correlated to progression-free survival (PFS) and overall survival (OS).Interpretation of PET scan was obtained with visual assessment alone by a nuclear medicine physician (evaluation of maximal SUV in PET-pos cases is ongoing). *Results.* Median age of pts was 36 years (range, 17-68 years). Histologic subtypes included: 38 HG-NHL [B phenotype (n=25), T phenotype (n=12), other (n=1)] and 44 HL. Forty-seven pts (57%) were allografted from a HLA-identical sibling donor, 16 from a haploidentical donor and 19 from an unrelated donor. Sixty-eight pts (83%) failed autograft, the median number of prior regimens was 3 (range, 1-6). PET scans were performed at a median of 30 days prior to allograft: 41 out of 82 pts were PET-pos [HG-NHL (n=18), HL (n=23)] whereas 41 were PET-neg [HG-NHL (n=20), HL (n=21)]. Pts with PET-pos or PET-neg scans were well balanced in terms of diagnosis, previous treatments, and type of donor. At a median follow-up of 30 months (range, 6 - 86 months), 54 pts are alive and 28 died [toxicity n=12, disease n=16]. Overall, the estimated 3-year PFS in pts with PET-neg or PET-pos scans were 68% (95% CI, 49%-81%) *vs* 30% (95% CI, 15-47%), respectively ($p<0.003$). For HG-NHL pts, the estimated 3-year PFS was 70% for PET-neg as compared to 41% for PET-pos ($p<0.02$) whereas for HL pts, the estimated 3-year PFS was 68% as compared to 17%, respectively ($p=0.05$). A statistically significant higher cumulative risk of relapse was observed in pts with PET-pos scan before allograft as compared to the PET neg scan (53% *vs* 21%, $p<0.022$). The estimated 3-year OS in pts with neg or pos PET scans were 77% (95% CI; 60-87%) *vs* 41% (95% CI; 24-57%), respectively ($p< 0.002$). *Conclusions.* Our study shows a better PFS and OS for pts being PET neg before allografting. PET scan should be incorporated in pre-transplant work-up to validate our findings prospectively.

0616

IS EARLY INTENSIFICATION USEFUL FOR PET2+ HL PATIENTS?

R. Sancetta,[1] C. Fraulini,[1] L. Rigacci,[2] B. Puccini,[2] P. Pregno,[3] U. Vitolo,[3] E. Brusamolino,[4] M. Gotti,[4] M. Magagnoli,[5] M. Balzarotti,[5] A.M. Carella,[6] E. Rossi,[6] A. Gallamini,[7] T. Chisesi[1]

[1]O.C. Umberto I, VENEZIA MESTRE; [2]SOD Ematologia, Azienda Ospedaliera Universitaria Careggi, FIRENZE; [3]SCDO Ematologia 2, AUO S. Giovanni Battista, TORINO; [4]Clinica Ematologica, Fondazione IRCCS Policlinico S. Matteo, Università di Pavia, PAVIA; [5]Dipartimento di Oncologia Medica ed Oncologia, Istituto Clinico Humanitas, ROZZANO (MI); [6]Divisione di Ematologia 2, Ospedale S. Martino, GENOVA; [7]U.O. di Ematologia, Az. Ospedaliera S. Croce e Carle, CUNEO, Italy

Background. As we can see from recent studies, PET2– in patients with HL is an important prognostic factor for survival. We therefore decided to evaluate retrospectively the outcome of HL pts with PET2+ according to Time to Treatment to analyze the feasibility of a prospective randomized study with or without early intensification with ASCT. *Methods.* Forty-two pts with Hodgkin Lymphoma (HL) and a PET2+, coming from different Italian Hematologic centres, were studied. Patients' characteristics: 17 M and 25 F, median age 36 yrs (range 17-77); Hystological types: 34 pts SN, 5 pts MC, 1 pt classical type, 1 pt LP and 1 pt PTS; 10 stage I-IIA and 32 stage IIB-IV; 19 pts had bulky disease. Nine pts (21%) have already undergone ASCT during 1st line or immediately after its end (*early*) and 4 pts (10%) will undergo *early* ASCT for presence of active disease documented by PET2+. Ten pts (24%) underwent ASCT after at least 3 months from the end of therapy; 19 pts (45%) did not receive any intensification of therapy with ASCT because they were either in CR (10 pts, 53%) at the end of 1st line therapy, or too old (2 pts, 10%), or were treated with an additional line of therapy (5 pts, 27%), or received no further therapy at all (2 pts, 10%). *Results.* all the 9 pts (status at transplant: 6 pts PR, 2 pts PD and 1 pt NR) who have already received *early* ASCT are alive and 6/9 (66%) are in CR, 3/9 (34%) are in PD. 10 pts were transplanted as salvage therapy: 3/10 (22%) are in CR, 1/10 (11%) is in PR, 1/10 (11%) is in MR, 2/10 (22%) are in PD, 3/10 (34%) died in PD. The 19 pts who did not have an intensification of therapy with ASCT are alive: 14/19 are in CR (74%), 2/19 (10%) is in PR, 3/19 (16%) are in PD. The characteristics of the three groups of pts were homogeneous in terms of clinical features and risk factors. *Conclusions.* our preliminary data show that the *early* ASCT has an advantage in terms of achievement of remission. We will need a large randomized study to determine if it is really mandatory for all pts with PET2+. (On behalf of Intergruppo Italiano Linfomi IIL)

0617

LEVELS OF CIRCULATING DNA IN THE PLASMA AT DIAGNOSIS ARE ASSOCIATED WITH CLINICAL CHARACTERISTICS AND PROGNOSIS IN PATIENTS WITH HODGKIN AND NON-HODGKIN LYMPHOMAS

S. Hohaus

Università Cattolica S. Cuore, ROME, Italy

Background. Increased levels of circulating nucleic acids (DNA and RNA) with a high fractional concentration of tumor DNA have been observed in the plasma of patients with a variety of human cancers of epithelial origin, and correlated to clinical characteristics and prognosis. *Aims.* To correlate levels of cell-free circulating DNA at diagnosis with clinical characteristics and prognosis in patients with lymphoma. *Methods.* We studied 142 patients with lymphomas (45 pts with Hodgkin lymphoma (HL), 63 pts with diffuse large B cell NHL (DLBCL), 24 pts with follicular, and 10 pts with mantle cell NHL) and 41 healthy individuals. DNA was extracted from plasma collected at diagnosis using the QIAamp DNA Blood MiniKit (Qiagen, UK) and DNA levels were determined using a quantitative PCR for the beta-globin gene. Associations with patient characteristics and event-free survival (EFS) were analysed using standard statistics (STATA 10). *Results.* DNA plasma levels ranged from 3 to 35 ng/mL in healthy controls (mean, 13.9 ng/mL). Compared

to controls, levels of circulating DNA were significantly higher in patients with Hodgkin lymphoma (mean 43.4 ng/mL), and patients with DLBCL (91.6 ng/mL) and mantle cell NHL (74.1 ng/mL), while plasma DNA levels in patients with follicular NHL were not significantly different from controls (22.9 ng/mL). Advanced stage of disease (stage III/IV), presence of B symptoms, LDH levels above normal range, and age >60 years were associated with increased levels of plasma DNA (p=0.009; <0.0001; <0.0001; 0.04, respectively). Increased LDH levels and B symptoms continued also in the multivariate logistic regression analysis to be associated with high DNA levels. In NHL, patients with high risk age-adjusted IPI (2/3) had higher levels of plasma DNA (p=0.001), while in HL only a trend for an association between elevated plasma DNA levels and an IPS score >2 was observed (p= 0.08). Plasma DNA levels above the normal range (>35 ng/mL) were associated with an inferior event-free survival in patients with HL (p=0.0005) and DLBCL (p=0 007). Including other risk factors for inferior EFS in univariate analysis (advanced stage and LDH) into a multivariate Cox analysis, stratified for lymphoma type and treatment, the plasma DNA level was an independent risk factor for inferior EFS (hazard ratio 3.5, 95% CI 1.2-10.1). *Conclusions.* Our study suggests that circulating levels of DNA in the plasma may become a new biomarker with prognostic impact also in patients with lymphomas.

0618

DHAOX AS SALVAGE THERAPY IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN'S AND NON HODGKIN'S LYMPHOMAS

L. Rigacci,[1] A. Fabbri,[2] B. Puccini,[3] U. Vitolo,[4] M. Lenoci,[2] S. Mappa,[3] I. Chitarrelli,[2] A. Levis,[5] F. Lauria,[2] A. Bosi[3]

[1]Azienda Ospedaliero Universitaria Careggi, FLORENCE; [2]Department of Hematology Azienda Ospedaliero Universitaria, SIENA; [3]Department of Hematology Azienda Ospedaliero Universitaria Careggi, FLORENCE; [4]UOA Ematologia, TORINO; [5]UO Ematologia, ALESSANDRIA, Italy

Background. Standard salvage chemotherapy for lymphomas has not been established. DHAP has been one of the most effective and utilized regimens. *Aims.* This study was designed to assess the efficacy and safety of substituting cisplatin with oxaliplatin in the DHAP regimen for patients (pts) with relapsed or refractory Hodgkin's and non Hodgkin's lymphoma on outpatients basis. *Methods.* Sixty-five pts with median age of 51 years (range 33-75) entered this study. Histological subtypes were 39 diffuse large B cell, 3 grade III follicular lymphoma and 23 Hodgkin's disease. Pts relapsed after first line or salvage therapy (ABMT) or primary refractory were treated at three weekly intervals with Oxaliplatin (120 mg/m^2 day 1), Cytarabine (2000 mg/m^2 days 2,3) and Dexamethasone (40 mg days 1 to 4). Fourty-five pts were programmed to receive peripheral blood stem cells transplantation and 20 did not. All pts with Hodgkin's disease were treated with 2 cycles of DHAOx to test chemosensibility and all responders were programmed to perform high dose therapy and autologous transplantations. *Results.* The overall response rate (RR) was 75% (49/65) including 29 complete remission (CR) and 20 partial remission (PR). Fifthy-six pts were treated with DHAOx as second line, 9 pts were treated with DHAOx as third line therapy (4 were relapsed after ASCT). Thirty-eight out 45 (84%) pts programmed to high dose therapy obtained at least a partial response and were treated with PBSCT. Twenty-two pts were primary refractory and four obtained a complete response 7 a partial response and 11 had a rapid progression. In a univariate analysis chemosensitive disease and PS 0-1 at salvage therapy were significantly correlated with response to therapy. The majority of pts experienced severe haematological toxicity despite the use of hemopoietic growth factors, none of them required hospitalisation. No grade 3-4 extra-hematological toxicity was reported, and in particular we did not observe any significant renal and neurological toxicity. During a median overall survival (OS) period of 15 months (range 2-51 months) 22 pts died (34%). Probabilities of 1-year progression free survival (PFS) and OS were 34% and 41% respectively. If we consider only chemosensitive pts (43), after DHAOx, the PFS and OS were 53% and 60% respectively. The two factors that significantly affect OS were response to therapy and chemosensitive disease. *Conclusions.* DHAOx is a novel combination for the treatment on outpatients basis relapsed or refractory pts with Hodgkin's and non-Hodgkin's lymphomas. It has a clinically significant activity in chemosensitive pts with an acceptable toxicity profile that makes this regimen attractive before high-dose chemotherapy.

0619

ASSOCIATION OF SERUM SELENIUM AT PRESENTATION, PATIENT CHARACTERISTICS AND OUTCOME IN HODGKIN AND FOLLICULAR LYMPHOMA

J.M. Stevens,[1] R. Waters,[2] C. Sieniawska,[3] A. Rohatiner,[1] J. Fitzgibbon,[1] S.P. Joel,[1] T.A. Lister[1]

[1]St Bartholomew's Hopsital, LONDONWEST SMITHFIELD; [2]Centre for Medical Statistics, Oxford University, OXFORD; [3]Trace Elements Laboratory, Southampton NHS Trust, SOUTHAMPTON, UK

Introduction. Selenium is a trace element, available from dietary sources and essential for human health. Its role as a chemomodulatory agent is under investigation. Our group has recently published *in vitro* data demonstrating chemosensitisation of B-cell lymphoma lines exposed to chemotherapy in combination with low-dose selenium (Cancer Res 2007; 67: 10984). Moreover, serum selenium level at presentation has been shown to correlate with response to therapy and overall survival in *aggressive* non-Hodgkin lymphoma (J Clin Oncol 2003; 21: 2335). *Aims.* To evaluate associations of serum selenium levels, patient characteristics and outcome in patients presenting with newly diagnosed follicular lymphoma (FL) and Hodgkin lymphoma (HL). *Methods.* Univariate and multivariate analysis of age, gender, stage, presence of B-symptoms, albumin, haemoglobin were performed in 111 patients with FL (47% male, median age 54, range 23, 86 years, presenting to St Bartholomew's Hospital (SBH) 1981-2000, median follow-up 15 years) and 156 patients with HL (63% male, median age 30, range 16, 77 years presenting 1982-2004, median follow-up 12 years) to identify correlations with serum selenium at presentation. Patients were selected on the availability of relevant serum sample. Serum selenium was measured by inductively-coupled plasma mass spectrometry and categorised as *low* or *normal/high* according to the laboratory normal range (70.3 µg/L to 157.9 µg/L). *Results.* 29 (26%) patients with FL, and 82 (53%) with HL had low serum selenium level. On univariate analysis higher stage, low Hb and low albumin correlated with low serum selenium level in both FL and HL; on multivariate analysis, Hb retained significance in both FL and HL (p=0.002 and p=0.001 respectively); albumin retained significance in FL (p=0.001) and the presence of B symptoms in HL (p=0.01). In terms of outcome, on univariate analysis overall survival was better in both FL and HL in patients presenting with normal serum selenium level (p=0.02 and p=0.05 respectively); on multivariate analysis, patients with FL and low selenium levels had a trend towards worse outcome (Hazards ratio 1.7 95% CI 0.98, 3.0 p=0.06). In HL, univariate analysis of response to 1st treatment showed a better response (CR v PR, stable disease or progression) for patients with higher selenium levels (odds ratio 1.17 95% C.I. 1.03, 1.31 p=0.01 for each 10mg/L increment in Se) however on multivariate analysis, stage was the only significant predictor for response (p=<0.0001). *Summary and Conclusions.* On univariate analysis, patients with FL and HL who present with low serum selenium have a worse overall survival and patients with HL have a worse response to chemotherapy. In patients with FL there is a trend, on multivariate analysis, towards poorer survival in patients with low serum selenium (p=0.06). The mechanism of this association is uncertain, however these results, when considered together with the *in vitro* data, suggest that a trial of selenium supplementation at the time of chemotherapy at least in patients with FL, is warranted.

0620

INTERMEDIATE DOSE GEMCITABINE - CISPLATIN COMBINATION CHEMOTHERAPY (GEMCIS) WITHOUT TREATMENT DELAY FOR CYTOPENIA - A NEW STANDARD IN PRE-AUTOGRAFT REMISSION INDUCTION FOR RELAPSED OR REFRACTORY HODGKIN'S DISEASE?

T. Todd, S. Raj, D. Camilleri, G. Stafford, G. Bulusu, G. Follows, M. Williams, R. Marcus

Cambridge University Hospitals NHS Foundation Trust, CAMBRIDGE, UK

Background. 10-20% of patients with Hodgkin's disease will fail to respond to first line therapy or will relapse. The treatment of choice in most cases is salvage chemotherapy followed by autologous stem cell transplant. A number of salvage regimens are in use with reported complete response (CR) rates of 17-49%, they have considerable toxicity, including treatment related fatality, and hospitalization may be required either to administer the therapy or manage complications. Alternative regimens with higher response rates and low toxicity which permit autologous transplantation are required. Gemcitabine, a pyrimidine analogue, and cisplatin in combination have been shown to be effective in various solid tumours and non-Hodgkin's lymphoma but there are only two small studies which have included patients with relapsed or refractory Hodgkin's disease. The two regimens used (gemcitabine-dexamethasone-cisplatin GDP and the higher dose gemcitabine-methylprednisolone-cisplatin GEM-P) differed but both had low complete response rates (17-20%), a key determinant of the effectiveness of autograft, and high rates of treatment delay or dose reduction (30-78%), an important determinant of response rates. The higher dose GEM-P study did not proceed to autograft in most patients with Hodgkin's disease. Neither study (combined n=41 Hodgkin's disease patients) reported treatment related death. *Aims*. To evaluate the response rates, autograft feasibility, tolerability, cost of, and survival following, a new gemcitabine-cisplatin regimen designed to proceed without treatment alteration for cytopenia and followed by autograft. *Methods.* The records of all patients (n=17) with refractory or relapsed Hodgkin's disease treated with GemCis between October 2005 and June 2007 either at, or in collaboration with, our institution were reviewed. All patients received 2 or 3 cycles of GemCis, consisting of gemcitabine 1000mg/m^2 on days 1, 8 and 15, cisplatin 70mg/m^2 on day 1, dexamethasone 20mg daily on days 1-4. All patients received pegylated filgrastim on day 8 and packed red cell or platelet transfusions as necessary but treatment was neither delayed nor dose reduced for cytopenia. Treatment repeated every 28 days for 2 or 3 cycles then almost all patients underwent peripheral stem cell harvest followed by autografting with BEAM conditioning. Response was assessed by computed tomography (CT) in all patients and by positron emission tomography (PET) in 15 patients. *Results*. All patients were evaluable. Eleven patients achieved complete response (65%) and 5 achieved partial response, overall response rate 94%. No patient had dose reduction or delay, neutropenic fever or hospital admissions and there were no treatment related deaths. Three patients required platelet transfusions, two required packed red cell transfusions and three required both with one or more cycles. 15 patients were planned for autograft, all were successfully harvested, and all engrafted (median 12 days to engraftment). After a median follow up of 20 months 15 (94%) of patients are still alive. Our cost analysis indicates that GemCis is cheaper than the most commonly used alternative in the UK, ESHAP. *Summary and Conclusions*. GemCis is a well tolerated, outpatient, salvage regimen for relapsed or refractory Hodgkin's disease which does not inhibit stem cell harvest or engraftment and achieves high pre-transplant CR rates.

Inherited anemias: sickle cell disease and red cell membrane disorders

0621

COMPARISON OF THE EOSIN-5-MALEIMIDE FLOW CYTOMETRIC METHOD WITH OSMOTIC FRAGILTY TEST USED IN DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS

P. Bianchi,[1] E. Fermo,[1] C. Vercellati,[2] A.P. Marcello,[2] A. Zanella,[1] A. Cattaneo,[2] W. Barcellini[2]

[1]Fondazione IRCCS OMPMARE, MILANO; [2]Fondazione IRCCS Ompmare, MILANO, Italy

Introduction. Hereditary Spherocytosis (HS) is a rare disease caused by defects of red cell membrane proteins (spectrin, ankyrin, band 3 and band 4.2) and associated with hemolytic anaemia of variable degree. The diagnosis is based on clinical history, blood smear examination, and red cell osmotic fragility tests whose sensitivity have been reported to range from 48 to 95%. A flow cytometric test based on the fluorescence of red blood cells after incubation with eosin-5-maleimide dye (EMA-binding test) has been recently described for the diagnosis of hereditary spherocytosis. *Aims*. The aim of the study was to compare the sensitivity of the EMA binding tests with the most common osmotic fragility tests for diagnosis of HS, in particular: NaCl osmotic fragility test on both fresh and incubated blood, standard glycerol lysis test, acidified glycerol lysis test (AGLT) and pink test. *Methods*. Fifty-eight consecutive patients previously diagnosed as HS were studied by EMA binding test and standard methods listed above. As controls, 145 healthy blood donors and 26 patients with other haemolytic anaemias (11 unknown anemia, 2 AIHA, 3 pyruvate kinase deficiency, 3 hereditary elliptocytosis, 2 PNH, 1 CDAI and 4 CDAII patients) were studied. EMA binding test results were expressed as percentage of the fluorescence reduction compared to the mean fluorescence of the five normal controls (as reported by Girodon *et al.*, 2007). Patients were considered positive with a decrease in fluorescence of 10% *Results*. The overall results are reported in the table. EMA binding test resulted positive only in 2/145 healthy blood donors, giving a specificity of 98%; with regard to non HS patients, it was positive only in CDA II. *Conclusions*. In conlusion, our data confirm previous studies showing that EMA binding is an effective tool for the diagnosis of HS and CDAII. It has a good sensitivity and specificity and it is useful in particular when small amount of blood is needed.

	Positive pts/Total HS	Sensitivity %	Positive pts/total non HS
GLT	32/58	55	2
AGLT	54/58	93	12
NaCl fresh	37/56	66	2
NaCl incubated	45/56	80	3
Pink test	48/57	84	7
EMA binding	53/58	91	4

Figure 1.

0622

USEFULNESS OF TWO NEW METHODS - FLOW CYTOMETRY (FC) AND CRYOHEMOLYSIS (CH) TESTS - FOR THE DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS (HS): COMPARISON WITH STANDARD SCREENING TESTS

L. Crisp,[1] C. Donato,[2] B. Venegas,[1] E. Chamorro,[3] A. Solari,[1] D. Vota,[3] D. Gammela,[1] G. Miguez,[1] A. Schvartzman,[2] D. Vittori,[3] S. Caldarola,[1] G. Alfonso,[1] A. Nesse[3]

[1]Hospital Nacional A.Posadas, BUENOS AIRES; [2]Consultorios de Hematología Infantil, BUENOS AIRES; [3]Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, BUENOS AIRES, Argentina

Background. CH and FC tests are recently developed methods for the diagnosis of HS. Their usefulness seems to be better than standard screening methods. No experience concerning these tests has been reported in our country. Furthermore, the prevalence of each protein deficiency underlying HS in argentine patients is unknown. *Aims.* a) To compare usefulness of CH and FC with standard tests; b) To establish the prevalence of each protein deficiency in Argentina. *Methods.* Nineteen patients (aged 3 months to 77 years) previously diagnosed with HS by standard tests, 20 asymptomatic family members, and 106 normal controls have been included so far. Patients, family members, and controls were studied by complete blood cells count, blood smear, osmotic fragility (OF), autohemolysis (AH), CH, eosin-5'-maleimide FC, and denaturing electrophore-

sis (SDS-PAGE); OF, AH, and SDS-PAGE were not performed in 72 normal controls. For FC, the reduction of the mean channel fluorescence (MCF) reading and the increase of the coefficient of variation (CV) were obtained using mean values for simultaneously performed normal controls. The cut-off values for CH and FC were determined through the analysis of receiver operating characteristics (ROC) curves. For FC and SDS-PAGE, each assay was set up together with 6 normal controls. *Results.* In patients, percentages of positive tests were: OF 79%, AH 59%, CH 78%, FC 89%, and SDS-PAGE 58%. Only 6 (32%) were positive for all the tests. Considering only CH and FC, at least one of them was positive in 100% of patients. For CH, the mean normal value was 1.47±0.56%, and the established cut-off value was 2.8%; mean value in patients was 8.55±8.45%. The CH showed sensitivity of 78% and specificity of 96%. Results of FC were evaluated either through MCF decrease or CV increase: the established cut-off values were 19% for MCF (sensitivity 74%, specificity 97.5%), and 12% for CV (sensitivity 74%, specificity 97.5%). No correlation was demonstrated between MCF and CV changes. In 2 patients, test was positive only through the CV change. In 6 cases (5 of them splenectomized), MCF changes were greater than CV changes. A cut-off value of 25% for each parameter determined a specificity of 100%. FC was positive in 7 of 8 patients without demonstrable protein deficiency (with CH positive in 3); in the remaining one, only CH was positive. SDS-PAGE detected the following deficiencies: spectrin (6), ankyrin (5), protein 4.1 (2), protein 4.2 (1). Seven patients showed a single deficiency whereas 4 showed 2 deficiencies. Band 3 deficiency was demonstrated in one asymptomatic family member in whom CH was the only positive test. *Conclusions.* CH and FC showed high specificity and sensitivity for diagnosis, similar to OF and higher than SDS-PAGE. Main advantages of CH and FC over standard tests are smaller amount of sample required and shorter performing time; moreover, CH is a very simple test not requiring special equipment. The simultaneous readings of MCF and CV improve the diagnostic value of FC compared with CH. The observed predominant spectrin deficiency agrees with reports from other Latin American countries.

0623

PARADOXICAL HYDROXYUREA STIMULATION OF PRO-INFLAMMATORY CYTOKINE GENES EXPRESSION IN ENDOTHELIAL CELLS: RELEVANCE TO SICKLE CELL DISEASE

S. Laurance,[1] O. Dossou-Yovo,[1] A. Benecke,[2] F.X. Pellay,[3] E. Verger,[1] R. Krishnamoorthy,[1] J. Elion,[1] C. Lapoumeroulie[1]

[1]INSERM, U763-Université Paris 7 Denis Diderot, PARIS; [2]Institut des Hautes Etudes Scientifiques, BURES-SUR-YVETTE; [3]Systems Epigenomics-Interdisciplinary Research Institute, LILLE, France

Background. Hydroxyurea (HU) is the only drug with a demonstrated clinical benefit for sickle cell disease (SCD) patients by reducing the frequency of vaso-occlusive crises, acute chest syndromes and transfusion requirements. Initially, HU was administered to induce HbF expression but there is no short term correlation between the observed clinical benefit and the expected increase of HbF. Although HbS polymerisation is the basis of SCD pathophysiology, the endothelium plays an essential role in vascular occlusion as increased adherence of circulating cells to vascular endothelial cells (VEC) and abnormal vascular tone regulation have been clearly documented. Our laboratory investigates the effect of HU on VEC. We have previously shown that HU modulates, *in vitro*, the expression of endothelial genes implicated in SCD since they encode for adhesion molecules (VCAM-1, ICAM-1) and for a powerful vasoconstrictor (ET-1). ET-1 down-regulation by HU was also confirmed by an *in vivo* study on a paediatric cohort. *Aims*. To evaluate the impact of HU on human VEC, we have engaged a systematic screening for HU endothelial target genes based on a pangenomic analysis. *Methods*. The impact of HU on the transcriptome profile of a human VEC line derived from bone marrow microcirculation (TrHBMEC) has been evaluated after a 24h and 48h HU treatment period combined or not with a treatment by pro-inflammatory cytokines to simulate SCD pro-inflammatory context. Micro-arrays set up to test 29098 genes expression were analysed on an Applied Biosystems platform. *Results*. Subtraction profiles (HU-treated *vs* non-treated) lead to the identification of 2,448 new potential target genes. Interestingly, mRNAs of several pro-inflammatory cytokines are up-regulated by HU, including RANTES, MCP-1, MCP-2, MIP-3a, IL-1a, IL-1b, IL-6, and IL-8. The stimulation factor varies from 2 and 30 fold, as determined by real-time PCR. mRNA levels for the anti-inflammatory cytokines IL-4 and IL-10 are not modified. These results are surprising because chronic inflammation is a characteristic feature of SCD. Thus, in addition to TrHBMEC, real-time PCR and ELISA experiments were carried out on primary cultures of two other VEC types: HUVEC (Human Umbilical Vein Endothelial Cells, macro-circulation) and HPMEC (Human Pulmonary Micro-circulation Endothelial Cells). Whatever the VEC type and the vascular bed, HU stimulates gene expression and secretion of these factors under basal and pro-inflammatory conditions. The four interleukins are known to be elevated in SCD patients, but our study is the first to point out the potential implication of chemoattractant cytokines in this context. *Summary and Conclusions*. *In vivo* stimulation of IL-1a and IL-6 by HU has already been reported in a rat model (Navarra *et al.* J Pharmacol Exp Ther. 1997;280: 477-82). Still, increased levels of these pro-inflammatory factors seem to be paradoxical compared to the beneficial effect of HU in SCD. Interestingly, Navarra *et al.* also reported that HU-stimulated cytokines induce a strong activation of the hypothalamo-hypophyseal-adrenocortical axis. A tempting hypothesis is that HU favorably alters the subtle balance between the SCD-induced pro-inflammatory stress and the natural anti-inflammatory response in SCD patients.

0624

PREDICTORS OF NOCTURNAL OXYHAEMOGLOBIN DESATURATION IN CHILDREN WITH SICKLE CELL DISEASE

J. Kirkham,[1] O. Wilkey,[2] D.K.M. Hewes,[1] J.P.M. Evans[1]

[1]UCL Institute of Child Health, LONDON; [2]North Middlesex hospital, LONDON, UK

*Background.*Nocturnal oxyhaemoglobin desaturation (NOD, mean saturation <96% on overnight pulse oximetry) appears to be a predictor of central nervous system events and frequent pain in children with sickle cell disease (SCD). The risk factors are poorly understood. *Aims*. To undertake a secondary analysis of potential risk factors (age, genotype, haematocrit, previous chest crisis, comorbid asthma or central nervous system disease and upper airway infection) for NOD in the East London cohort. Method Of an unselected hospital cohort of children, 69 (median age 7.8 (range 1-16.5) years; 39 male) had overnight pulse oximetry at home between 1990 and 1995. Those over the age of 6 and any with CNS events were invited to have magnetic resonance imaging (MRI) and angiography (MRA). Clinical details, including emergency department attendances and hospital admissions, were obtained from the hospital notes for the period preceding the overnight study. A sleep questionnaire was administered to a subset. Logistic regression was used to look at predictors of NOD. *Results*. For mean overnight saturation (SpO2), the range was 84-99.7% (median 96.1%) and values were significantly lower in patients with HbSS (median 95.4, range 84-99.7%)

than in those with HbSC (median 97.9, range 94.8-98.9%) or Sβ thalassaemia (median 97.8, range 93.5-99.5%). 25 children (36%) had mean saturation <96%. 13 had dips in saturation suggesting OSA, of whom 10 also had mean saturation <96%. 36 completed the sleep questionnaire, of whom 30 snored (14 sometimes, 3 often, 13 always); there was no evidence that NOD was associated with concurrent snoring (Fisher's exact test, p=0.8). Of 11 children with abnormal overnight studies who had repeat overnight pulse oximetry after adenotonsillectomy, 8 were still abnormal. In univariate logistic regression, genotype (OR 9.00, 95% ci 1.08, 74.7; p=0.04), haemogloin at the time of the study (odd ratio, OR 0.64 95% confidence intervals, ci 0.47, 0.87; p=0.005), greater age at first Casualty attendance for an upper airways infection (p=0.02) and abnormal MRA (OR 4.5, 95%ci 1.24, 16.3; p=0.02) were associated with OD and there were trends for white cell count at the time of the study (OR 1.10, 95% ci 1.00, 1.21; p=0.06) and infarction on MRI (OR 3.14, 95% ci 0.67, 14.6; p=0.15). There was no effect of age at sleep study, haemoglobin F, doctor-diagnosed asthma, number of admissions with chest infection or any other measure of complication rate. Both haemoglobin at the time of the study (OR 0.68, 95% ci 0.48, 0.95; p=0.02) and age at first upper airway infection (OR 1.44, 95% ci 1.04, 1.99; p=0.02) remained in the multiple logistic regression model. *Summary and Conclusions* Nocturnal oxyhemoglobin desaturation is common in patients with HbSS, but is difficult to predict clinically and may not improve with adenotonsillectomy. Prospective studies of aetiology should commence in infancy and ideally include serial measurements of cerebral as well as lung function. If NOD is confirmed as a predictor of complications in SCD, treatment options might include hydroxyurea or overnight continuous positive airways pressure.

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0625

OVERNIGHT OXYHAEMOGLOBIN DESATURATION PREDICTS ABNORMAL TRANSCRANIAL DOPPLER IN SICKLE CELL ANAEMIA

J. Kirkham,[1] P. Telfer,[2] R.S. Bucks,[3] D.K.M. Hewes,[1] B. Kaya,[2] M. Prengler,[1] I. Dundas,[2] R. Lane,[1] S. Carr,[2] J.P.M. Evans[1]

[1]UCL Institute of Child Health, LONDON, UK; [2]Royal London hospital, LONDON, UK; [3]University of Western Australia, PERTH, Australia

Background. Snoring appears to be associated with high internal carotid/middle cerebral artery velocities on transcranial Doppler (TCD) in the general paediatric population[1] but there are few data comparing overnight oximetry in those with normal or abnormal TCD. Snoring and low oxyhaemoglobin saturation (SpO2) are common in sickle cell anaemia (SCA) but any association with abnormal TCD has received little attention. *Aims.* to conduct a secondary analysis of sleep and TCD data from the East London cohort of children with SCA to determine whether lower overnight oxyhaemoglobin saturation is associated with conditional or abnormal TCD independent of haemoglobin and markers of inflammation and haemolysis. *Methods.* Children with SCA in the East London cohort had overnight pulse oximetry and regular TCD scans. 65 had sleep studies as part of an unselected cohort studied between 1991 and 1993 (only 6 of whom had no history of snoring) and the remainder were undertaken in children with clinical evidence of sleep related breathing disorders (SRBD) as part of clinical care. The most recent steady state haematology was obtained from the medical notes. Mean and minimum overnight SpO2 and the haematological variables were compared in those with standard risk, conditional (>170 < 200 cm/s) or abnormal (>200 cm/s) TCD. *Results.* 148 children (90; 61% boys; median [range] age 6 [1-23] years) had overnight oximetry, of whom 137 also had TCD; 115 TCDs were standard risk, 15 were conditional and 7 were abnormal. In multivariable binary logistic regression, lower mean overnight SpO2 was associated with conditional or abnormal TCD (p<0.01). Age was not a significant predictor of TCD abnormality in this model. Overnight minimum SpO2 data were available for 123 of the 137 children. However, adding this to the model did not significantly improve the fit (p=.399). There were TCD category differences in Haemoglobin, χ^2 (2) = 7.07, p=0.029 and in Haematocrit, χ^2 (2) = 9.21, p=0.010 (which were highly correlated, r = 0.92), but not in White cell count, χ^2 (2) = 4.03, p=0.133, Platelets, χ^2 (2) = 1.85, p= 0.396, or in Haemoglobin F, χ^2 (2) = 4.71, p= .095. There were also no TCD status differences in Reticulocytes, χ^2 (2) = 3.37, p=0.185. Given that haemoglobin and haematocrit were so highly correlated, haemoglobin was entered into the model at Step 2, with mean overnight SpO2 at Step 1. Steady state haemoglobin did not significantly add to the prediction of TCD category (p=0.246). *Summary and Conclusions.* TCD velocity >170 cm/sec, an intermediate endpoint which predicts a high risk of stroke in SCA, is commoner in snoring children with SCA and sustained and intermittent overnight oxyhaemoglobin desaturation, independently of haematocrit. There is no evidence for an interaction with white cell or reticulocyte count. Early management of oxyhaemoglobin desaturation and SRBD in SCA might prevent the development of cerebrovascular disease and/or haemodynamic insufficiency secondary to high cerebral blood flow, thereby reducing the risk of stroke.

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0626

BONE MINERAL DENSITY IN CHILDREN WITH SICKLE CELL DISEASE (SCD)

M de Montalembert,[1] E. Chapelon,[1] V. Brousse,[1] J.C. Souberbielle,[1] J.L. Bresson,[1] M. Garabedian,[2] M. De Montalembert[1]

[1]Hopital Necker, PARIS; [2]Hopital Saint-Vincent de Paul, PARIS, France

Background. SCD is characterized by chronic haemolysis and recurrent painful crises. Children may also experience slow growth and delayed puberty. Low bone mineral density (BMD) has been repeatedly reported in SCD adults, but the prevalence of this complication is not known in children. *Aims*. We assessed BMD in a cohort of SCD children and looked for a correlation between its value and age, gender, SCD severity, growth and pubertal development, vitamin D concentration, calcium intake, and markers of bone turnover. *Methods*. Fifty-three children (45 SS, 4 SC, 4 Sb-thalassaemia), 27 females, and 26 males, mean-age: 12.8±2.4 y (9-19 y) were enrolled between 2002 and 2006. They originated from: West and Central Africa: 40, Caribbean Islands: 9, North Africa: 3, Brazil: 1.We assessed height, weight, sexual maturation (Tanner), number of hospitalized painful crises and of transfusions in the 3 preceding years, calcium intake, steady-state haemoglobin (Hb) and leukocyte count, calcemia, phosphatemia, calciuria/creatinuria, 25-OH D, 1-25-OH D and PTH concentrations, osteocalcin, urinary deoxypyridinolin (DPD) and C-terminal component of pro-collagen type I (CTX). BMD was assessed using a dual X-ray absorptiometry (DXA) scanner. *Results*. In the whole population, mean lumbar spine z-score was -1.1±1.3 (-3.9-1.8), females having non-significantly lower values (-1.42±1.29) than males (-0.77±1.26). In prepubertal children, lumbar spine z-score was lower in females (-1.7±0.7) than in males (-0.43±1) (p: 0.01). BMD was not correlated to hospitalization and transfusion episodes, Hb, leukocytes. Hb and leukocyte count were inversely correlated (p: 0.02) and leukocytosis was correlated to the number of hospitalizations (p<0.0001). 72% of patients were vitamin D deficient (25-OH D<10 ng/mL) and 38% had a secondary hyperparathyroidism (PTH>46 pg/mL). BMD was not related to calcium intake, vitamin D status, osteocalcin, and markers of bone resorption. As osteocalcin and CTX were normal or low in patients, an increased bone resorption was unlikely to explain low BMD. Mean circulating concentrations of IGF-I measured in 7 children were low (134±98 ng/mL). *Conclusions*. A slight decrease of BMD was observed in SCD children, as soon as prepuberty, more marked in females. This low BMD was not related to vitamin D deficiency and could most probably be related to abnormal bone formation process. Future research should focus on the mechanisms involved, in order to try to prevent osteoporosis in adult SCD patients.

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UGT1A1 GENE VARIATIONS & GALLSTONES IN SICKLE CELL DISEASE PATIENTS FROM SULTANATE OF OMAN

V. Pathare,[1] S. Alkindi,[1] S. AlZadjali,[1] V.K. Panjwani,[1] D. Dennison,[1] R. Krishnamoorthy[2]

[1]Sultan Qaboos University, MUSCAT, Oman; [2]INSERM, U763, PARIS, France

Background. Sickle cell disease (SCD) is a congenital hemolytic anemia with increased red cell destruction, and variations in the UDP-glucuronosyltranferase1A1 (UGT1A1) enzyme activity can lead to hyperbilirubinemia and its complications. Several polymorphisms in the UGT1A1 gene have been reported to be associated with decreased enzyme activity. *Aims.* The aim of this study was to define the underlying molecular genetic basis of reduced expression in UGT1A1, leading to increased serum total bilirubin concentration in Omani sickle cell anemia patients and correlate the same with gallstones in these patients. *Methods.* The study enrolled a total of 248 SCD patients (192 SS homozygotes; 7 SD heterozygotes; 2 SC heterozygote; 56 Sbeta+ Thal double heterozygotes), with a median age of 22 years (22.1 + 9.1; Mean + SD). 129(52%) were males. Serum samples for biochemical investigations were analyzed using standard laboratory techniques. Total and unconjugated serum bilirubin measurements were obtained after an overnight fast in steady state. UGT1A1 gene was studied for the promoter region A(TA)nTAA configurations and several other polymorphisms that have been reported to reduce the enzyme activity namely -3440C>A; -3401T>C; -3729T>G; -3154G>A & +211G>A. *Results.* Amongst 248 patients analyzed, 100(40%) were homozygous for (AT)6 UGT1A1 allelle;114(46%) were heterozygous for (AT)6 and (AT)7 alleles and 23(9%) were homozygous for the (AT)7 allele. Mean serum bilirubin was significantly higher in the homozygous (AT)7 group as compared to the (AT)6 group(47.7 v/s 23.4; p<0.001, student's t-test). Furthermore, the mean serum bilirubin concentrations were also higher in -3440AA homozygotes, -3279GG homozygotes, and -3154AA homozygotes respectively when compared to the relative wild alleles. Amongst the patients with gallstones, the mean serum bilurubin was significantly higher, than in patients without gallstones (36.2 v/s 23.4; p<0.01 student's t-test). The frequency of cholethiasis was significantly higher in patients with (TA)7/(TA)7 than (TA)6/(TA)6 [p<0.01; chi square test]. Furthermore, the -3154AA homozygotes were significantly over represented in patients with gallstones. (p<0.01; chi square test] *Summary and Conclusions.* In conclusion, apart for the UGT1A1 (AT)7 homozygosity; -3154AA homozygosity and -3729GG homozygosity, were significantly associated with raised total bilirubin levels, with the former two, also associated with gallstones in the Omani patients with SCD. However, this study did not find any statistically significant association between -3440 C>A, -3401T>C and +211G>A polymorphisms and the bilirubin levels in these SCD patients.

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ENALAPRIL THERAPY IN CARDIAC REMODELLING OF SICKLE CELL DISEASE PATIENTS

C. Lima, O. Ueti, A. Ueti, K. Franchini, F. Costa, S. Saad

State University of Campinas, CAMPINAS, Brazil

Background. The moderate to severe anaemia associated with sickle cell disease (SCD) causes a high output state that can lead to cardiac hypertrophy followed by cardiac enlargement and congestive heart failure. Angiotensin-converting enzyme inhibitors (ACEi) are able to decrease remodelling in patients with cardiac dysfunction. *Aims.* Since the cardiac effects of ACEi in SCD patients are unknown, it was the aim of the present study. *Methods.* Nine adult patients with SCD in attendance at the Haematology and Haemotherapy Centre, who presented microalbuminuria, were enrolled for enalapril treatment. The dose of enalapril was 5mg, given once a day. Nine SCD patients without microalbuminuria, matched according to diagnosis, age, and levels of haemoglobin (Hb), haematocrit (Ht) and foetal haemoglobin (F Hb), did not receive enalapril and were considered as controls. None patient or control was in chronic transfusion. Cardiac evaluation was performed before the study entry and once a year in treated patients and after 36 months of follow-up in controls. Mean blood pressure (MBP) was calculated as the diastolic blood pressure plus one third of the difference between systolic and diastolic pressure. Echocardiography was performed using an ATL UltraMark 4 machine (Advanced Technology Laboratories, Bothel, Washington, USA), with 3 to 5-MHz Doppler transducers. Left ventricular (LV) dimensions and mass were assessed from 2D guided M-mode tracings, according to the American Society of Echocardiography's recommendations. M-mode measurements were averaged from 5 cycles. LV end-systolic, end-diastolic, and stroke volumes were calculated with the use of Sympsons's method. Echocardiograms were acquired and results were recorded and revised by 2 qualified and independent echocardiographers, blinded to patient condition. The inter-observer correlation coefficient for echocardiographic measurements was 0.84. *Results.* Median age, baseline values of Hb, Ht, F Hb and MBP were similar in treated and untreated patients. The average values of echocardiogram measurements were similar in both groups and within the range of clinically normal values, at the beginning of the observational period. At 36 months of follow-up, MBP was lower than the baseline value only in the treated group. Significant increases in LV mass and mass index, posterior LV wall thickness in end-diastole, interventricular septal wall thickness in end-diastole, and aortic root dimension values were seen in untreated, but not in enalapril treated patients. No major changes were seen in LV systolic diameter, diastolic dimension and ejection fraction, and left atrial diameter, in both groups, along the observational period. No significant correlation was detected between the data obtained by echocardiography and levels of Hb, Ht F Hb, MBP, albuminuria and glomerular filtration rate using the Spearman coefficient. At the end of the study, no symptoms or signals related to cardiac failure were found in any of the enrolled patients. *Conclusions*. The results found in study suggest that long-term treatment with enalapril has beneficial effects on cardiac remodelling of SCD patients. However, a large trial concerning the response to enalapril in these patients should be carried out to clarify this issue.

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SIX YEARS FOLLOW-UP OF HYDROXUREA TREATMENT IN YOUNG ALGERIAN PATIENTS WITH SICKLE CELL DISEASE

M. Bradai,[1] F. Talbi,[2] F. Lamraoui,[1] S.A. Oukrif,[1] F.Z. Ardjoun[3]

[1]Institute of Medicin of Blida, BLIDA; [2]Institute of Medicin of Algiers, ALGIERS; [3]Istitute of Medicin of Algiers, ALGIERS, Algeria

Background. Sickle cell anaemia (SCA) is of frequent occurrence in Algerian population and presents with variable clinical manifestation and complication. Hydroxyurea (HU) is the first widely used treatment to have an impact on the severity of SCA and associated with reduced mortality (Steinberg MH and *et al.*, Blood 2000; 96:485a). *Aims.* This work aimed to confirm long term clinical efficiency of HU in previously severely ill Algerian patients with SCA. Patients: Since 2000 forty-for children and adolescent patients severely affected with SCA were given HU (14 to 27 mg/kg/day), 20 male/24female, median ages: 15years (range: 4-25), 29 homozygote (SS), 14S/βthalassemia and 1 SC disease patient (with haemolysis delayed syndrome). 11 patients (8SThal and 3SS) underwent splenectomy before inclusion. 35with history of>3stroke or >1 acute chest syndrome (ACS)/year, 6 with severe anaemia (3 with complex allo immunisation), 2 with priapism and 1 with nephrotic syndrome and renal insufficiency (creatinemia: 19mg/mL). Foetal haemoglobin (HbF) has been monitored every 3 months, determined by HPLC using Variant 2. *Results.* Annual rates of vaso-occlusive crisis, ACS, transfusion administration and days in hospital, all decreased significantly (p<0,001). Mean duration of HU treatment was 69 (range: 38-94 months) Stopped Reed blood transfusion was obtained in 31 patients, sporadic transfusions (less 3/years in the others). 3 episodes of acute priapism was noted in 1 patient caused sexual powerless. Recurrent stroke occurred in 1 patient after 21 months of HU. Splenic regeneration was observed in 3 patients. Recurrent acute splenic sequestration observed in 3 patients whom underwent splenectomy. Progressive symptomatic osteonecrosis were observed during HU treatment in 2 patients (SThal) aged 12 and 15 years. One patient had sarcoidosis and was treated with steroid occasioned pain crisis and need regular exchange transfusion during 6 months. Haematological *Results.* HU induced an increase in HbF levels in all patients out 2, only one patient with renal failure had no significant increase of HbF(3 to 5%); Rise HbF values averaged 20 percent after 12 months of treatment; Median Hb increase after one year of HU was 1,5g/dL(7,1±1,0 to 8,6±1,5) . Reduction of reticulocytes, neutrophiles and platets was also observed. No serious acute toxicity was seen, only one patient had very low platet (25000/μl) count symptoms, HU was discontinued and platet count returned to normal within 3 weeks. Similar effects were observed in patients with SS an S/β-THL. All patients gained weight; growth and sexual development progressed normally. During this period there were 2 reported deaths, 1 caused by progressive renal failure, after 18 months of treatment with HU and erythropoietin, the second cause of death was sepsis. For all others patients this efficacy is sustained. The compliance was excellent, just 1 patient is lost follow-up after 30 months of HU. *Conclusions*. Our data support the efficacy of HU in the management of severe forms of SCA in Algerian patients, may be a good alternative for the management of this disease, especially in countries where standards of care are often lacking.

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GENERALIZED RADIOLOGICAL ABNORMALITY ASSOCIATED WITH ACUTE NEUROLOGICAL PRESENTATIONS IN SICKLE CELL DISEASE

J. Kirkham,[1] P.B.D. Inusa,[2] K. Pohl,[2] M. Bynevelt,[1] T.C. Cox,[1] W.K. Chong,[1] D.E. Saunders,[1] M. Prengler[1]

[1]UCL Institute of Child Health, LONDON; [2]Evelina Children's, St Thomas' hospital, LONDON, UK

Background. Sickle cell disease (SCD) is the commonest cause of stroke in childhood with a recurrence rate of 10% despite regular prophylactic blood transfusion. Ninety percent of patients have radiological or pathological evidence of large vessel disease, most of whom present with acute hemiparesis and focal infarction on neuroimaging. However, patients presenting with neurological symptoms and signs after chest crisis have been reported to have generalised neuroradiological abnormalities, for example posterior leukencephalopathy[1] and acute demyelination.[2] As few cases have been reported, the pathophysiology and natural history remain obscure. *Aims.* To review the presentation and outcome for those with generalised radiological abnormality associated with acute neurological symptoms and signs and to compare them with those with focal abnormality. *Methods.* As part of a 10-year prospectively collected registry of children with SCD, we reviewed our experience with patients presenting acutely and found to have generalised or focal neuroradiological abnormality *Results.* Of 61 patients documented to have had an acute neurological presentation with focal signs, seizures or coma, 7 (11%) had generalized rather than focal abnormality on imaging within 3 days of presentation. All presented with seizures and reduced conscious level. Four (after chest crisis or facial infection) had generalised cerebral oedema, of whom 2 had bilateral borderzone infarction involving grey as well as white matter. These patients survived and reintegrated into mainstream school without significant motor disability; none has had a recurrence after follow-up of 4-10 years. Three, after chest crisis or nephrotic syndrome treated with Cyclosporin, had posterior leukencephalopathy radiologically; these patients recovered consciousness but one died of his renal disease. None of these patients had transcranial Doppler velocities >200 cm/sec either before (n=2) or during (n=4) the acute presentation. There was significantly increased chance of finding a prodromal illness in those with generalized radiological findings (7/7 *vs*18/54; chi-sq p=0.001). MRA abnormality was less likely in those with generalised radiological changes (3/7, 43%) compared with those with focal changes (49/54, 91%) (chi-sq; p=0.007). None had a recurrent neurological event (compared with 31/54 focal, chi-sq; p=0.005) after a follow-up of 4-10 years. *Summary and Conclusions.*

Our patients extend the neuroradiology associated with acute seizures and coma in SCD to include generalised cerebral oedema and bilateral borderzone infarction as well as posterior leukencephalopathy [1] and demyelination. The neurological outcome may be favourable if the patient survives the acute phase. The role of acute hypoxia and blood pressure abnormalities requires investigation.

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ELEVATED NUMBERS OF CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN SICKLE CELL DISEASE

E. Nur,[1] R.T. van Beem,[2] A.J. Duits,[3] E.J. van Beers,[1] H. C. de Boer,[4] B. J. Biemond,[1] J.J. Zwaginga[4]

[1]Academic Medical Center, AMSTERDAM, Netherlands; [2]Sanquin, AMSTERDAM, Netherlands; [3]Red Cross Blood Bank Foundation, CURAÇAO, Netherlands Antilles; [4]Leiden University Medical Center, LEIDEN, Netherlands

Background. Sickle Cell Disease (SCD) is characterized by chronic recurrent (a-) symptomatic microvascular vaso-occlusions that lead to local ischemia and vasculopathy. This vaso-occlusion-induced tissue ischemia may lead to a potent angiogenic response. Indeed, increased levels of angiogenic growth factors such as erythropoietin (EPO), Angiopoietin-2 (Ang-2) and Placental growth Factor (PIGF) have been reported in patients with SCD with further increments during painful crisis. Circulating endothelial progenitor cells (EPC) have been suggested to modulate neovascularisation and decreased numbers of EPC have been associated with worse outcome in patients with coronary artery disease. Previous studies have shown that EPO plays a role in EPC mobilization. *Aims.* To determine the numbers of circulating EPC in patients with SCD during steady state and painful crisis and to assess the association of EPC numbers with EPO levels and sickle cell-related organ damage. *Methods.* Numbers of circulating EPC (defined as KDR^+/ $CD34^+$ cells) and levels of EPO were measured in 65 consecutive asymptomatic (steady state) patients (30 males, age 19-54) and 41 painful vaso-occlusive events in 24 patients (10 males, age 19-54). *Results.* Numbers of circulating EPC were significantly higher in patients with vaso-occlusive painful events (median (IQR) 123 cells/mL (51 - 294)) as compared to SCD patients in steady state (median (IQR): 8.30 cells/mL (0.0 - 28.6); p= 0.0001). This was also found in a paired analysis in thirteen patients during painful crisis and steady state (median (IQR): 55.3 cells/mL (18.7 - 110) and 11.6 cells/mL (0.0 - 27.4) respectively; p=0.01). There was no correlation between the circulating EPC numbers and EPO levels or manifestations of organ damage. *Conclusions.* Acute vaso-occlusive painful events result in increased circulating EPC numbers. No correlation with EPO plasma levels was observed. Although increased circulating EPC numbers have been suggested to be involved in vascular repair and/or neovascularisation, no association between manifestations of sickle cell-related organ damage was observed.

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REDUCED CO2-INDUCED CEREBRAL VASOMOTOR REACTIVITY IN PATIENTS WITH SICKLE CELL DISEASE

E. Nur, Y. Kim, J. Truijen, E.J. van Beers, S.C.A.T. Davis, B. J. Biemond, J.J. van Lieshout

Academic Medical Center, AMSTERDAM, Netherlands

Background and objective. Sickle Cell Disease (SCD) is associated with high incidence of stroke and silent cerebral infarcts (SCI) resulting in significant morbidity and mortality. In young children these SCI are associated with poor educational and cognitive functioning. SCD is characterized by chronic hemolysis and endothelial dysfunction which are considered to play an important role in the reduced bioavailibility of nitric oxide (NO) in these patients. NO is a potent vasodilator and plays a major role in CO_2-induced vasomotor reactivity (VMR). Given the important contribution of CO_2-induced VMR to the cerebral blood flow (CBF) regulation and the high incidence of cerebrovascular events in SCD patients, we determined the CO_2-induced VMR in patients with SCD. *Methods.* 14 patients with homozygous HbSS SCD and 9 healthy race-, age- and gender-matched controls underwent trans-cranial Doppler (TCD) and End-tidal CO_2 tension ($PETCO_2$) measurements during breathing of both room air and air with 5% CO_2. CO_2-induced VMR was expressed by the relative change in TCD-measured mean blood flow velocity in middle cerebral artery (MCA Vmean) per mmHg change in $PETCO_2$. Associations with markers of hemolysis and plasma levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase, were assessed. *Results.* Patients with SCD had a significantly lower CO_2-induced VMR (3.55 (2.95 - 4.80) compared to healthy controls (5.58 (4.14-6.45), p=0.017). VMR was not related to markers of hemolysis. A significant association was found between CO_2-induced VMR and ADMA plasma levels. *Conclusions*. Patients with SCD have an impaired cerebral chemoregulation (reduced CO_2-induced VMR) which may result in an impaired cerebral blood flow and could be one of the contributory factors to cerebrovascular events observed in these patients. The negative association between VMR and levels of ADMA indicates that a reduced NO production due to inhibition of NO-synthase, rather than NO-scavenging by cell-free heme, plays the predominant role in reduced NO-bioavailability in SCD patients.

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SCREENING FOR HAEMOGLOBINOPATHY IN NORTHERN GREECE - EPIDEMIOLOGICAL SURVEY

S. Theodoridou, M. Alemayehou, P. Perperidou, V. Aletra, T. Karakasidou, P. Lazaridou, T. Vyzantiadis, A. Manitsa, A. Stamna, L Papayiannis

Hippokration Hospital, THESSALONIKI, Greece

Greece is a Mediterranean country with a high frequency of thalassaemia and haemoglobinopathies. Beta thalassaemia (β-thal) carrier frequency is approximately 8%, while 1.5% of the population is heterozygous for the HbS gene. Since 1974 a nation wide program for Thalassaemia prevention has been implemented in our country. The aim was to educate and to increase awareness of the public concerning these hereditary anaemias, to screen couples for haemoglobinopathies, to give genetic counselling and perform prenatal diagnosis. Due to the increased number of immigrants over the last 20 years, especially people from Albania, the former Soviet Union, the middle East and south East Asia, the number of affected individuals and the spectrum of mutations have both increased and somehow changed. The above mentioned geographic areas, excluding the former Soviet Union, are regions with a high frequency of carriers for the thalassaemia and other haemoglobinopathy genes. It is estimated that currently 10% of the population in Greece are immigrants, and of these, most of them (~58%) are Albanians. Natives and immigrants are screened free of charge for haemoglobinopathies, counselled and prenatal diagnosis carried out through the Population screening and prenatal diagnosis program that is performed by the National Centre for Thalassaemia in Athens / Greece and the various Prevention Units throughout the country. We report the results of the Thalassaemia / Haemoglobinopathy prevention program in Northern Greece, over a 22-year period (1987-2007). A total of 80,401 subjects were screened for haemoglobinopathies in our Thalassaemia Prevention Unit which covers the regions of central and western Macedonia, in northern Greece, with a population of around 2.5 million. Currently we use the Cation Exchange HPLC variant system (Biorad), electrophoretic techniques both at alkaline pH on cellulose acetate and at acid pH on citrate agar, sickling test, and tests for HbH inclusion bod-

ies. Biosynthesis of haemoglobin and DNA techniques are also performed on selected cases. The frequency of β-, α-, σβ-thalassaemia carriers was found to be 8.6%, 1.47%, and 0.33%, respectively. The prevalence of sickle cell carriers was 1.06 % and carriers for haemoglobinopathy Lepore was 0.13%. Few cases of haemoglobinopathy H (--/-α) (0.05%), and heterozygotes for HPFH (0.048%), ∏bO-Arab (0.04%), HbC (0.01%), and HbD (0.031%) were also encountered, in addition to very few cases of structural haemoglobin variants such as HbE, HbE Saskatoon, Hb Setif, and Hb Osu-Christianborg. A limited number of compound heterozygotes such as β-thal/HbD Punjab, β-thal/Hb Lepore, β-thal/σβ-thalassaemia, α-thal/HbS, and HbS/HbD Punjab were also detected. According to our data over the last five years, each year about 25 couples undergo prenatal diagnosis for clinically severe haemoglobinopathies. The birth rate of children in our country born with thallasaemia major or sickle cell syndromes is 1 to 2 new cases per year compared to the expected 250 cases which would have been born without preventive measures. It should be noted that, children born with severe Thalassaemia Major or Sickle Cell disease were due to couples that failed to use the Thalassaemia Prevention Program or due to refusal of termination of the pregnancy of a sick foetus, for one reason or other. Our conclusion is that, wide spread population screening for thalassaemia, in a country like Greece with a high frequency of the thalassaemia gene and that of other haemoglobinopathies, and the implementation of prenatal diagnosis in at risk couples, have both decreased the incidence of children born with Thalassaemia Major and Sickle Cell disease. This Thalassemia screening program has proved successful in our case as it has in many other countries that have implemented it.

0634

MID-TERM EFFICACY OF SUBTOTAL SPLENECTOMY IN YOUNG CHILDREN WITH HEREDITARY SPHEROCYTOSIS

R.Y.J. Tamminga,[1] P.M.A. Broens[2]

[1]University Medical Centre Groningen, GRONINGEN; [2]University Medical Centre, Division of Paediatric Surgery, GRONINGEN, Netherlands

Background and Aims. Severe hereditary spherocytosis (HS) can be treated with splenectomy. However, in young children, there is a considerable risk for life threatening postsplenectomy infections. Therefore, we investigated the mid-term efficacy of subtotal splenectomy (STSP) for HS in our hospital. *Methods.* Patients were eligible for STSP, if they had a severe phenotype at a young age (interferance with normal development, requirement of blood transfusions). They were operated upon between 2002 and 2005. All children were preoperatively vaccinated for pneumococci and received postoperatively prophylactic antibiotics at least until a functional splenic remnant was demonstrated by ultrasound and scintigraphy 3 months after the procedure. *Results.* Included were 10 children (6 male), mean age 7.1 years (range 5.5 - 10.9). Mean follow-up: 4 years (range 3-6). No peroperative complications occurred. One patient (5.5 years) did develop regeneration of the splenic remnant within 4 months after STSP and underwent re-operation with total splenectomy. At evaluation 3 months after surgery, in one patient (6.6 years) no spleen was visible on ultrasound and also no activity could be shown on scintigraphy; one other patient (5.9 years) had a patent splenic vessel on ultrasound, but no activity could be demonstrated on scintigraphy. The seven remaining patients had a clearly visible splenic remnant with sufficient blood flow on ultrasound and noticable splenic activity on scintigraphy. None of these children developed serious infections, despite cessation of antibiotic prophylaxis after demonstrating a functional splenic remnant. After STSP, in these 7 children, the haemolysis was decreased resulting in normal daily activities comparable to their healthy peers. In them, during the >3-year follow-up a small increase in haemolyis was shown (3 months postoperatively: mean Hb 7.8 mmol/L with 31 *reticulocytes, total bilirubin 21 μmol/L; 3 years postoperastively: mean Hb 7.0 mmol/L with 50* reticulocytes, total bilirubin 19 μmol/L). However, clinically the children are still performing as their schoolmates and no re-operation is contemplated in any of these children. *Summary*. STSP is a feasible procedure for young children with severe HS. In 1/10 regeneration necessitated re-operation with total splenectomy, in 2/10 degeneration of the splenic remnant resulted in asplenia, in 7/10 normal daily living was achieved for >3 years, without the need for transfusions or antibiotic prophylaxis. *Conclusions*. STSP in stead of total splenectomy should be considered in young children with severe HS in order to achieve a normal live and preserve splenic function.

0635

PULMONARY HYPERTENSION IN CHILDREN AND YOUNG ADULTS WITH SICKLE CELL DISEASE: LACK OF RESPONSE TO HYDROXYUREA AND EVIDENCE FOR FAMILIAL CLUSTERING

M. Hayek,[1] H. Dahoui,[1] F. Bitar,[2] S. Muwakkit,[1] I. Dabbous,[1] M. Arabi,[2] P. Nietart,[3] M. Abboud[1]

[1]Children's Cancer Center of Lebanon, BEIRUT, Lebanon; [2]Department of Pediatrics, American University of Beirut Medical Center, BEIRUT, Lebanon; [3]Department of Biostatistics, Bioinformatics and Epidemiology, Medical University, CHARLESTON, USA

Background. Pulmonary hypertension (PHTN) in patients with sickle cell disease (SCD) is associated with early mortality during adulthood. *Aims.* To determine the prevalence of PHTN and identify factors associated with this complication among children and young adults followed at the Children's Cancer Center of Lebanon. *Methods.* Transthoracic Doppler echocardiography was performed during steady state at the time of the initial visit and then yearly. PHTN was defined as a tricuspid regurgitant jet velocity (TRV) of 2.5m/s or higher. From June 2004 to February 2008, 90 patients were studied. Correlation of TRV ≥2.5 m/s with age, mean corpuscular volume (MCV), fetal hemoglobin (HbF),serum lactate dehydrogenase level (LDH), Reticulocyte count (RC) and hydroxyurea use was performed. *Results.* Twenty eight of the 90 (31.1%) patients were found to have PHTN. Patients with TRV ≥2.5 m/s had higher MCV (p=0.02), higher LDH (p=0.004), higher RC (p=0.04) and a history of hydroxyurea use (p=0.02) compared to patients with TRV< 2.5m/s. There was no age difference between the patients (13.4±8.3 *vs* 12.3 ±7.6years p=0.3).The fetal hemoglobin was lower in patients with PHTN (15.2 *vs* 21 p=0.2). Both age and HbF results were not statistically significant. Ten of the patients with PHTN were tested for glucose-6-phosphate dehydrogenase (G6PD) deficiency and all had normal G6PD levels. Five patients with a normal initial TRV developed PHTN. The median age at the time of the first evaluation was 11.2 years, and abnormal TRV was detected after a median follow up of 24.2 months. All 5 had received hydroxyurea continuously during this period, at a mean dose of 19 mg/kg/day. They experienced significant clinical improvement, manifested by decreased painful crises, hospitalizations and need for transfusions, as well as increase in MCV and HbF levels. Among the 90 patients studied, there were 17 families with more than one sibling affected by SCD. PHTN was found in 11 of the 17 families and these families contributed 19 of the 28 patients with PHTN. In one consanguineous family 2 young siblings, ages 11 and 13 years and two maternal uncles, aged 24 and 26 years were found to have abnormal TRV. This familial clustering was highly statistically significant (p= 0.0001).V. *Conclusions*. PHTN is prevalent among children and young adults with SCD in Lebanon. In our population use of hydroxyurea did not seem to prevent the development of PHTN. The familial clustering of PHTN observed in our patients has not been previously described.

Multiple myeloma - Clinical II

0636

LENALIDOMIDE, MELPHALAN, PREDNISONE AND THALIDOMIDE (RMPT) FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA

A. Palumbo,[1] P. Falco,[1] G. Sanpaolo,[2] A. Falcone,[3] V. Ferderico,[4] L. Canepa,[5] M. Crugnola,[6] L. Baldini,[7] S. Caltagirone,[1] A. Larocca,[1] F. Gay,[1] V. Magarotto,[1] M.T. Petrucci,[3] M. Boccadoro[1]

[1]A.O.U. San Giovanni Battista, TORINO; [2]UO Emat. e Trapianto Cellule Staminali, IRCCS Casa Sollievo della Sofferenza, SAN GIOVANNI ROTONDO; [3]Italian Multiple Myeloma Network, GIMEMA, ITALY; [4]Dipartimento di Biotecnologie e Ematologia, Università La Sapienza, ROMA; [5]Clinica Ematologica, Osp. San Martino, Università di Genova, GENOVA; [6]Cattedra e UO Ematologia e Trapianto Midollo, Università degli Studi di Parma, PARMA; [7]Dipartimento di Ematologia e Oncologia, Osp. Maggiore, I.R.C.C.S., MILANO, Italy

Background. The oral combinations melphalan, prednisone, thalidomide or melphalan prednisone, lenalidomide have significant anti-myeloma activity in newly diagnosed multiple myeloma (MM) patients. In advanced MM, the very good partial response (VGPR) rates were 12% for MPT and 43% for the 4-drug combination bortezomib, melphalan, prednisone, thalidomide. *Aims.* These observations provide the rationale for evaluating the tolerability and efficacy of the 4-drug combination lenalidomide, melphalan, prednisone, and thalidomide (RMPT) as salvage treatment for advanced MM. *Methods.* Oral lenalidomide was administered at 10 mg/day on days 1-21, melphalan at 0.18 mg/kg on days 1-4, prednisone at 2 mg/kg on days 1-4, and thalidomide at 50-100 mg/day on days 1-28. The RMPT regimen was delivered every 28 days for 6 cycles and was followed by maintenance therapy with lenalidomide alone at 10 mg/day. Aspirin was given as a prophylaxis for thrombosis. *Results.* Forty-three patients, median age 69 years (range 47-80 years), with relapsed or refractory myeloma have been enrolled. Sixty-one percent of patients received RMPT as second line therapy, 39% as third line. Sixty-one percent of patients received prior autologous transplant, 19% thalidomide-based regimen and 16% bortezomib-based regimen, two patients received prior allogeneic stem cell transplant. After two RMPT cycles, 52% of patients achieved at least a partial response (PR). After a median of 4 courses, 91% of patients achieved a PR including 45% of patients who achieved at least a very good partial response (VGPR). The most frequent adverse event was haematological toxicity: 48% of patients experienced grade-3 neutropenia and 16% of patients grade-4 neutropenia, G-CSF support was required in 39% of patients. Grade-3 thrombocytopenia was recorded in 26% of patients and grade-4 thrombocytopenia in 10% of patients. One patient needed platelet transfusion. The most frequent non-haematological toxicities were infections (19%), mainly pneumonia and febrile neutropenia. Grade-3 neurological toxicity occurred in one patients. No thromboembolic events were recorded. *Conclusions*. Initial results showed that RMPT is an effective salvage regimen for relapsed/refractory myeloma. Neutropenia was the most frequent adverse event. No thromboembolic events were recorded with aspirin prophylaxis. An update of the trial will be presented.

0637

RESULTS OF A PHASE I/II TRIAL OF DEUTSCHE STUDIENGRUPPE MULTIPLES MYELOM , SHOWING EFFICACY AND SAFETY OF RAD REGIMEN (REVLIMID®, ADRIAMYCIN®, DEXAMETHASONE) IN RELAPSED/ REFRACTORY MULTIPLE MYELOMA

S. Knop,[1] C. Gerecke,[2] P. Liebisch,[3] M.S. Topp,[1] U. Platzbecker,[3] C. Vollmuth,[1] H. Einsele,[1] R. Bargou[1]

[1]Wuerzburg University Hospital, WUERZBURG; [2]Charité Campus Buch, BERLIN; [3]University Hospital, ULM, Germany

Background. Lenalidomide (Revlimid®; Len) has significant clinical activity in previously treated multiple myeloma (MM) patients (pts), with or without dexamethasone (Dex). The antimyeloma activity of Len and Dex might be further enhanced by the addition of Adriamycin®. *Aims.* We report data from a multicentre dose-finding phase I/II trial involving the RAD regimen (Revlimid®, Adriamycin®, Dex). *Methods.* Previously treated MM pts with measurable disease and no more than 3 prior treatment lines were enrolled. Pts were required to have adequate hematopoietic and organ function. Prior Len treatment was an exclusion criterion. RAD was administered for six 28-day cycles along with either aspirin 100 mg/day or low-molecular-weight heparin for prophylaxis of venous thromboembolism (VTE). Phase I was a dose-escalating study with increasing doses of either Len (10-25 mg/day) or Adriamycin (4-9 mg/m /day). In Phase II, the 5th dose level: granulocyte-colony stimulating factor support (G) 6 mg on day (d) 6; Len 25 mg d1 21; Adriamycin 9 mg/m / day d1-4; and Dex 40 mg d1-4 and 17-20 of each 28-day cycle (DL 5+G), was used. The European Group for Blood and Marrow Transplantation criteria were used to evaluate response. Cytogenetic analyses were performed by FISH. *Results.* Of 69 pts, 61 pts were evaluable for response assessment and divided into 2 groups: 20 pts from phase I, who received doses up to DL 5+G *vs* 41 pts on DL 5+G from both phase I and II. The median age across both groups was 65 (range, 46-77) years and 47% had received 2-3 prior anti-myeloma treatments, including autologous stem cell transplantation (SCT) (72%), allogeneic SCT (12%), bortezomib (57%), and thalidomide (20%). The maximum tolerated dose was not reached, even at DL 5+G. Overall response rate (ORR) for DL 1-4 was 60% including 5 pts (25%) with near complete response (nCR). ORR for 41 pts on DL 5+G was 85%, including 10 pts (24%) with immunofixation-negative CR and 24 pts (59%) with very good partial response. Median overall time to progression was 9.3 (1 to 25+) weeks and overall survival was 79%, with a median follow-up of 5 months for DL 5+G. Of 37 pts evaluable, 46% had 13 q deletion (del[13q]). Partial response (PR) or better was seen in 71% of pts with del(13q). Of 25 pts evaluable, chromosomal translocation (4;14) was present in 16% and 12% achieved PR. Six of 31 pts (19%), had del(17p) involving the tumor suppressor gene p53 with two pts achieving PR and an additional two, stable disease. Grades 3/4 infection occurred in 9.8% of pts and the incidence of VTE was 4.9%. No neuropathy was diagnosed *de novo*. Eight patients prematurely discontinued the trial due to catheter-related septicemia (2), thrombosis of basilar artery (1), prolonged pneumonia (1), and withdrawal of consent (4). *Conclusions*. The RAD regimen, incorporating 25 mg of Len for 21 of 28 days, demonstrates clinical efficacy and may be considered as a new combination regimen for heavily pre-treated myeloma pts. Toxicity was moderate and manageable. Furthermore, patients displaying high-risk cytogenetic abnormalities also achieved high responses.

0638

EFFECT ON SURVIVAL OF LENALIDOMIDE AND DEXAMETHASONE ASSOCIATED DEEP VEIN THROMBOSIS (DVT) IN RELAPSED MULTIPLE MYELOMA PATIENTS

M. Zangari,[1] L. Fink,[2] R. Knight,[3] T. Cavanaugh,[3] D. Weber,[4] R. Niesvizky,[5] G. Tricot[1]

[1]University of Utah, SALT LAKE CITY; [2]Nevada Cancer Institute Laboratory Medicine, LAS VEGAS; [3]Celgene Corporation, NEW JERSEY; [4]University of Texas, M.D. Anderson Cancer Center, HOUSTON; [5]Cornell University, New York Presbyterian Hospital, NEW YORK, USA

Background. Venous thrombo-embolism is a serious complication in patients with cancer. It is well recognized that patients who develop thrombo-embolic disease either at presentation or during the course of their malignancy have a poor prognosis. The effect on survival of lenalidomide and dexamethasone-associated DVT in relapsed myeloma patients was assessed. *Methods.* Patients with progressive multiple myeloma after at least one previous treatment, and with measurable disease were enrolled in a multi-center, double-blind, Phase III trial (Celgene MM009). Patients received 25 mg of daily oral lenalidomide or placebo from days 1 to 21 during each 28-day cycle. All patients also received 40 mg of oral dexamethasone on days 1 to 4, 9 to 12, and 17 to 20 (high dose dexamethasone). After the fourth cycle, 40 mg of dexamethasone was administered only from days 1 to 4. Treatment was continued until disease progression or unacceptable toxicities. Only the lenalidomide/dexamethasone arm of the study was analyzed. *Results.* A total of 177 patients were enrolled; the median age was 64 years, 60% were male, 64% were Durie-Salmon, stage III, 71% had beta-2 microglobulin level more than 2.5 mg/L. With a median follow-up of 26 months, 31 patients (17.5%) experienced a thrombo-embolic event. The baseline characteristics were balanced between patients with and without DVT, with the exception of female gender, which was more prominent in the non-DVT group. Previous lines of therapy including Thalidomide, Bortezomib, and stem cell transplantation were equally distributed. No negative effect of DVT on overall survival (p= 0.4) and time to progression (p= 0.7) was observed in this study. (Figure 1) *Conclusions.* As previously reported for thalidomide-associated DVT in newly diagnosed myeloma, patients treated with lenalidomide and dexametha-

sone at relapse, who developed a thrombo-embolic episode, do not experience a shorter overall survival or time to progression compared with those who did not have such a complication during treatment.

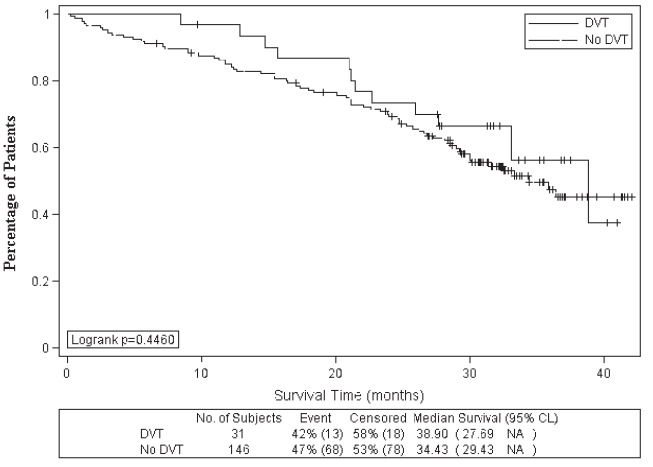

Figure 1. KM Curve for Overall Survival by DVT

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EXPANDED ACCESS PROGRAM (EAP) FOR LENALIDOMIDE PLUS DEXAMETHASONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

A. Palumbo,[1] F. Gay,[1] G. Buda,[2] T. Guglielmelli,[2] G. Perrone,[2] P. Corradini,[3] F. Benedetti,[2] M. Offidani,[2] D. Rossi,[2] A. Gozzetti,[2] L. Catalano,[2] L. Canepa,[4] F. Di Raimondo,[5] F. Patriarca,[2] M.T. Petrucci,[2] N. Di Renzo,[2] M. Galli,[2] F. Cavallo,[1] S. Bringhen,[1] M. Boccadoro[1]

[1]A.O.U. San Giovanni Battista, TORINO; [2]Italian Multiple Myeloma Network, GIMEMA, ITALY; [3]Divisione Ematologia, Istituto Nazionale Tumori, MILANO; [4]Clinica Ematologica, Osp. San Martino, Università di Genova, GENOVA; [5]Cattedra di Ematologia, Ospedale Ferrarotto, CATANIA, Italy

Background. Lenalidomide in combination with Dexamethasone was approved in the United States by the Food and Drug Association and in the European Union by the European Medicine Agency (EMEA) for the treatment of patients with relapsed or refractory multiple myeloma (MM). This expanded access program (EAP) was designed to make Lenalidomide available to patients without viable therapeutic alternatives and with a high likelihood of benefit, before marketing authorisation, pending final approval by Italian National Health Authorities. *Aims.* To provide Lenalidomide to MM patients with a high likelihood of benefit and to obtain additional safety data. *Methods.* Patients progressing after at least 2 cycles of anti-myeloma treatment or that have relapsed with progressive disease after treatment were eligible. Subjects received oral Lenalidomide (25 mg daily for 3 weeks every 4 weeks) plus Dexamethasone (40 mg on days 1-4, 9-12, 17-20 every 4 weeks for the first 4 cycles and on days 1-4 only for the following cycles). The treatment was delivered until disease progression, or study drug discontinuation for any reasons, or lenalidomide became commercially available for this indication. Daily anti-thrombotic prophylaxis with aspirin was recommended. For patients unable to tolerate aspirin, low molecular weight heparin or warfarin were recommended. Interim analysis was planned after the first 100 patients have received at least 2 cycles of treatment, to timely identify and report any possible unexpected toxicities. *Results.* Between August, 2007 and February, 2008, 221 subjects from 55 Italian Centers were enrolled in the study. Median age was 68 years (range 43-85 years) with 66% of patients aged ≥65 years, 52% were male and 54% had Karnofsky Performas Status ≥80%. Sixty percent of patients had IgG myeloma, 20% had IgA myeloma and 12% has BJ myeloma. Baseline prognostic features included serum levels of albumin <3.5 g/dL in 25%, β-2-microglobulin ≥5.5 mg/L in 17%, ISS stage III in 16%, Durie and Salmon stage III in 70%, creatinine ≥2 mg/dL in 6%, Protein C-Reactive ≥6 mg/L in 14% of patients. Median number of prior therapies was 3 (range 1-12) and median time from diagnosis was 5 years (range 1-21 years): 27% of patients received prior bortezomib based-regimens, 27% prior thalidomide based-regimens and 17% prior transplantation. Data from interim analysis will be available for the meeting. *Conclusions.* The EAP of lenalidomide plus dexamethasone in MM represents a model of how government, research institutions, healthcare providers and industry can work together to quickly provide treatment to subjects in need while a clearly active treatment regimen is awaiting approval.

0640

PHASE I TRIAL OF ORAL VORINOSTAT IN COMBINATION WITH BORTEZOMIB IN ADVANCED MULTIPLE MYELOMA

D.M. Weber,[1] S. Jagannath,[2] A. Mazumder,[2] R. Sobecks,[3] G.J. Schiller,[4] M. Gavino,[1] K. Meehan,[1] C. McFadden,[3] C. Chen,[5] J.L. Ricker,[5] S. Rizvi,[5] C. Oerth,[5] P. Brownell,[5] C. Sanz-Rodriguez,[6] M.A. Hussein[7]

[1]MD Anderson Cancer Center, HOUSTON, USA; [2]St. Vincent's Comprehensive Cancer Center, NEW YORK, USA; [3]Cleveland Clinic Foundation, CLEVELAND, USA; [4]UCLA School of Medicine, LOS ANGELES, USA; [5]Merck Research Laboratories, UPPER GWYNEDD, USA; [6]MSD, MADRID, Spain; [7]H. Lee Moffitt Cancer Center, TAMPA, USA

Background. Vorinostat is a histone deacetylase inhibitor that has demonstrated antiproliferative and proapoptotic activity alone and in combination with the proteasome inhibitor bortezomib in preclinical multiple myeloma (MM) models. In a Phase I study, vorinostat also demonstrated modest single agent activity in patients with relapsed or refractory MM. *Aims.* A Phase I trial of the combination of vorinostat and bortezomib was conducted to determine the maximum tolerated dose (MTD) and assess activity and safety. *Methods.* We conducted a Phase I trial of oral vorinostat (200 mg bid or 400 mg daily for 14 days [days 1-14]) in combination with bortezomib 0.7 or 0.9 mg/m^2 i.v. on days 4, 8, 11 and 15 or 0.9, 1.1, or 1.3 mg/m^2 i.v. on days 1, 4, 8 and 11. Cycles were repeated every 21 days for a maximum of 8 cycles until progressive disease (PD) or intolerable toxicity. Patients with active relapsed or refractory MM who had not received bortezomib in the preceding 3 months and with adequate hematologic, hepatic, and renal function, and ECOG performance status of 0-2 were eligible. The primary objective was to determine the maximum tolerated dose (MTD). Activity (utilizing EBMT criteria) and safety of the combination regimen were also assessed. *Results.* Twenty-four patients have been enrolled: median age, 61 years (range 45-76), median number of prior systemic therapies, 3 (range 1-14), prior therapy with bortezomib (6 patients). All 24 patients have received ≥1 dose and were evaluable for safety as of 11/1/07. Two patients experienced dose-limiting toxicities (DLTs): transient AST elevation (Cohort 3) and thrombocytopenia (Cohort 5). The MTD has not been reached. The most common drug-related toxicities of any grade were nausea (58%), thrombocytopenia (54%), diarrhea (50%), vomiting (50%), fatigue (42%), anemia (25%), and neutropenia (25%). Grade ≥3 drug-related adverse events were thrombocytopenia (38%, none associated with bleeding), fatigue (12%), neutropenia (12%, none associated with fever), nausea (8%), peripheral neuropathy (8%), vomiting (8%), diarrhea (4%), diverticulitis (4%), increased AST (4%), memory changes (4%), tremor (4%), and upper respiratory infection (4%). Eleven patients discontinued treatment, 3 due to PD (at 9.7, 3.5, and 1.7 months; after PR, SD, and SD, respectively), 7 due to adverse experiences [fatigue (2), nausea (2), diarrhea (1), diverticulitis (1), pneumonia (1)] (after 2-6 cycles), and 1 withdrew consent. Of 21 evaluable patients for efficacy as of November 1, 2007, all had measurable response or stable disease (5 had a partial response, 5 had a minimal response, and 11 stable disease). Among 6 evaluable patients previously treated with bortezomib, 2 achieved a partial response, 3 had a minimal response, and 1 had stable disease. Three patients at the highest dose level were not yet evaluable for response. *Conclusions*. Although accrual continues to determine the MTD, the combination of vorinostat and bortezomib is generally well tolerated and effective in this group of heavily pretreated patients with refractory/relapsed MM.

Table 1.

Cohort	*Vorinostat (mg)*	*Bortezomib (mg/m^2)*	*N*	*# of Cycles*	*DLTs*	*Best Response*
1	200 bid	0.7*	3	3, 3, 14	-	SD (2), MR
2	200 bid	0.9*	3	4, 4, 6	-	SD (2), PR
3	400 bid	0.9+	6	2, 2, 8, 9°, 10°, 10°	Transienet AST elevation	SD (3), MR, PR (2)
4	400 bid	0.9+	6	3, 4, 5°, 7°, 7°, 8°	-	SD (4), MR, PR
5	400 bid	1.3+	6	1°, 2, 2°, 4°, 6°, 6°	Thrombocytopenia	NE (3), MR (2), PR

MR, minimal response; NE, not evaluable; PR, partial response; SD, stable disease; *Days 1, 4, 8 and 11. ° Treatment cycle in progress.

0641

ROLE OF THALIDOMIDE ON VELCADE NEUROPATHY IN MYELOMA PATIENTS

T. Caravita,[1] A. Siniscalchi,[1] A. Spagnoli,[2] M.T. Petrucci,[3] F. Vincenzo,[3] P. Falco,[4] M. Offidani,[5] M. Rizzo,[2] A. Palumbo,[4] P. De Fabritiis[1]

[1]S.Eugenio Hospital, ROME; [2]Hematology, Policlinico and University "Tor Vergata", ROME;[3]Hematology, University La Sapienza, ROMA; [4]Hematology, St.Giovanni Battista Hospital, University, TURIN; [5]3Hematology, Ospedali Riuniti Università Politecnica, ANCONA, Italy

Background. Bortezomib (B) is the first proteasome inhibitor to be used in clinical practice and has been recently approved for second line treatment of multiple myeloma (MM) patients (pts). Several trials demonstrated that Bortezomib is relatively well tolerated; however, manageable non-hematologic and hematologic toxicity has been reported. The dose limiting toxicity is peripheral neuropathy (PN), reported up to 35% of the patient population. *Aims.* We retrospectively evaluated the incidence and severity of PN in 179 MM patients that received B as single agent or in combination. MATERIAL AND *Methods.* Informed consent was obtained from all the subject. Patients characteristics were as follows: median age was 66.7 years (range: 32-82) with 52% male; 55 patients were at the onset of the disease, 124 were pretreated. Median time from diagnosis to treatment with B was 25.4 months (range 0-111), median value of Beta2-microglobulin was 3.02 (0.2-20). Risk factors for PN included prior use of thalidomide in 68 patients (38%), vincristine in 75 patients (41.8%) and diabetes mellitus in 15 patients (8.8%). *Results.* Patients received bortezomib alone (8) or in combination with either dexamethasone (n=52), chemotherapy (n=114) or thalidomide (n=38). Overall, the response rate (>PR) was 86%. PN of grade >2 was observed in 73 pts (41%); grade 3-4 occurred in 32 (18%). Median time to the onset of bortezomib-related PN was 84 days (range, 10-449) after bortezomib initiation. In most cases (93%), patients had sensory symptoms, while 5 patients (7%) experienced both sensory and motor symptoms. Bortezomib-related PN led to therapy discontinuation in 31 pts (17%). For PN treatment, pts received mostly supportive therapy (analgesics, gabapentin, pregabalin, amitriptyline and vitamin supplements). Of the 31 patients with bortezomib-related PN that required discontinuation, resolution or improvement occurred in 16 (51.6%), at a median time of 140 days (range, 27-346) from B discontinuation. In the subset of relapsed/refractory patients, data analysis of PN risk factors (age, diabetes, sex, Beta2M, neurotoxic pre-treatment) showed for PN grade >3 a significant association with age >75 years (p<0.013) and thalidomde pre-treatment (p=0.048). However, in the subset of newly diagnosed MM pts, the risk of PN was greater in pts treated with association of drugs not including thalidomide (p=0.018). *Conclusions.* In our experience, PN is a relatively frequent side effect of bortezomib treatment, generally manageable by dose reduction or discontinuation and reversible in the majority of pts. Advanced age and pre-treatment with thalidomide represent the strongest PN risk factors. In up-front treatment, Thalidomide associated with B showed a protective factor on PN, probably due to its anti-inflammatory properties. Further studies are needed to better define the PN pathogenesis and develop optimal strategies to improve the B related PN management.

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PHASE I TRIAL OF VORINOSTAT PLUS BORTEZOMIB (BORT) IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA (MM) PATIENTS (PTS)

Z. Badros,[1] S. Philip,[1] R. Niesvizky,[2] O. Goloubeva,[1] C. Harris,[1] J. Zweibel,[3] J. Wright,[3] A. Burger,[1] M. Baer,[1] M. Egorin,[4] S. Grant[5]

[1]University of Maryland, BALTIMORE, MD; [2]Cornell University, NEW YORK; [3]CTEP, NCI, MARYLAND; [4]University of Pittsburgh, PITTSBURGH; [5]Medical Collage of Virginia, RICHMOND, USA

Background. vorinostat, an oral, histone deacetylase inhibitor, affects cell growth by modifying the transcription of cellular proteins such as histones, transcription factors, ubiquitin E3 ligases and stress response proteins (e.g. HSP90). *In vitro*, vorinostat showed synergistic cytotoxicity with the proteasome inhibitor Bort in MM cells by disrupting aggregates of the ubiquitin conjugated aggresomes, Pei *et al*. 2004. *Aims.* the aims of the study were to determine the maximum tolerated dose (MTD), pharmacokinetics (PK) and pharmacodynamic (PD) of vorinostat plus Bort combination in relapsed and refractory MM pts. *Methods.* Therapy included Bort 1.3 mg/ m^2 IV on days 1, 4, 8 and 11 and vorinostat dose escaltion between 100- 400 mg days 4-11. Five 3-Pt cohorts were evaluated at various dose levels. *Results.* twenty-three Pts were treated. Median age was 54 yrs (range 39-78). Median time from MM diagnosis to study entry was 5.3 yrs (range: 1.5-9 yrs). Isotypes included IgG (n=11), IgA (n=4), light chain (n=8). Fourteen Pts had complex karyotype. Median number of prior regimens was 7 (range 3-13); including autologous transplant (n=20), thalidomide (n=23) and lenalidomide (n=17). Nineteen pts had received a median of 2 (range: 1-5) Bort-based prior regimens; 9 were refractory and 10 had a response followed by progression. The median time from last therapy to study entry was 20 days (range: 15-39). Two pts had DLTs: grade 4 prolonged QT interval and grade 4-fatigue occurred in the 500 mg daily cohort. The MTD was SAHA 400 mg daily x 8 days plus Bort 1.3 mg/ m^2 days 1, 4, 8 and 11. Eight pts were treated at MTD. Grade 3-4 toxicities included myelo-suppression requiring transfusional support and growth factors, fatigue (n=11), diarrhea (n=5), atrial fibrillation (n=1), shingles (n=1), pneumonia (n=2). In 21 pts evaluable for response *cycle 2*, there was 2 VGPR and 7 PR (overall response rate of 42%), 10 pts had stable disease and 2 had PD. Dexamethasone was added in 4 pts after cycle 2; with no upgrade in response. The PK of vorinostat after a single oral dose were linear from 100-500 mg with mean AUC (0.7 + 0.45 to 4.4+ 0.07 mM/h), Cmax (0.3 + 0.14 to 1.2 + 0.06 mM) and Tmax (1.3 + 0.4 to 2.3 + 2.5 /h). Ten pts had CD-138+ cells isolated from bone marrow on day 1 and on day 11 of the first cycle; preliminary PD studies showed reduction of NFK-B, Bcl-2, bclxl, P21, XIAP compared to those with SD/PD. *Conclusions.* vorinostat administration after Bort was well tolerated in heavily treated MM pts. PK studies are similar to single agent. The regimen showed promising responses in Bort-refractory pts and will be evaluated in a phase II trial.

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Table 1. Response using standard criteria

		Response				
Prior Bortzezomib	No of Pts	VGPR	PR	SD	PD	NE
Naive	4	1	2	1		
Pre-treatment	10	1	2	5	1	1
Refractory	9		3	4	1	1

0643

PHASE II TRIAL WITH PLITIDEPSIN (APLIDIN) ALONE AND IN COMBINATION WITH DEXAMETHASONE (DEX) IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA; PROMISING ACTIVITY WITH MANAGEABLE TOXICITY

M.V. Mateos,[1] M.V. Mateos,[2] M.T. Cibeira,[3] J. Bladé,[3] P.G. Richardson,[4] F. Prosper,[5] A. Oriol,[6] J. de la Rubia,[7] A. Alegre,[8] J.J. Lahuerta,[9] R. García-Sanz,[1] J. Espinoza,[10] K. Mitsiades,[4] K. Anderson,[4] J.F. San Miguel[1]

[1]University Hospital of Salamanca, SALAMANCA, Spain; [2]Hematology, SALAMANCA, Spain; [3]Hospital Clinic i Provincial, BARCELONA, Spain; [4]Jerome Lipper Multiple Myeloma Center, Dana-Farber Cancer Institute, BOSTON, USA; [5]Clinica Universitaria, NAVARRA, Spain; [6]Hospital Germans Trials i Pujol, BADALONA, Spain; [7]Hospital La Fe, VALENCIA, Spain; [8]Hospital La Princesa, MADRID, Spain; [9]Hospital 12 de Octubre, MADRID, Spain; [10]PharmaMar SAU, Clinical R&D, COLMENAR VIEJO, MADRID, Spain

Introduction. Plitidepsin (Aplidin - APL) is a cyclic depsipeptide isolated from the marine tunicate, Aplidium albicans. *In vitro* studies have shown potent activity against multiple myeloma (MM) cell lines and fresh cells obtained from MM patients. APL was also active against cells resistant to conventional anti-MM agents and novel drugs (including bortezomib, thalidomide). A Phase I study explored 4 different schedules of administration and determined that muscle and liver (transaminases and/or alkaline phosphatase elevations) toxicities were dose limiting. Hematologic toxicity was not observed at the recommended Phase II dose of 5 mg/m^2. *Aim and Methods.* The aim of this non-randomized two-stage Phase II, multicenter, clinical and pharmacokinetic trial was to explore the activity of APL in refractory/relapsed MM patients. APL was administered at a dose of 5 mg/m^2 (administered over 3 hours by intravenous infusion) every 2 weeks. In the first stage, 16 evaluable patients (pts) were included. At least one response was required in order

to proceed with the second stage. Response was assessed by Blade criteria and toxicities assessed by NCI-CTC, v3.0. *Results.* Between June'04 and January'08, 52 relapsed/refractory MM pts were enrolled. Median age at time of inclusion was 65 years (range: 47-82). The median number of prior lines of therapy were 4 (range: 1-9): 60% had undergone autologous stem cell transplant, 58% thalidomide-based therapy and 48% prior bortezomib. Of 52 pts included in the study, 7 pts did not receive three or more doses of APL and per protocol were not evaluable for response but were evaluated for toxicity. Among the remaining evaluable 45 pts to. APL monotherapy 2 pts (4%) achieved PR , and 6 pts (13%) MR; in addition, disease stabilization was observed in 18 pts (40%). Eighteen pts progressed during APL treatment. In August 2005 the protocol was amended and the addition of Dexamethasone (Dex) (20 mg on days 1-4) was permitted in pts progressing after 3 cycles or with stable disease after 4 cycles. Accordingly, Dex was added in 15 pts including 8 with SD and 7 with PD.Among 15 pts evaluable for response with the combination APL plus Dex, 3 pts (20%) achieved PR and 2 pts (13%) MR; in addition, 8 pts (53%) remained in SD and 2 pts progressed. The most common G3-4 adverse events included fatigue in 4 pts (11%), serum creatine phosphokinase increase in 8 pts (24%), muscle toxicity (weakness, myopathy) in 4 pts (11%) and hepatic toxicity in 10 pts (28%). No significant APL-related hematologic toxicity or neuropathy was observed. *Conclusions*. APL as monotherapy has activity in heavily pretreated relapsed/refractory MM with 18% of pts achieving PR/MR and 40%stable disease ; response was improved with the addition of Dex, with an increase in PR/MR to 33%. APL was generally well tolerated, although caution in pts with hepatic, and muscle toxicity is warranted. Further combination studies exploring lower doses of APL with bortezomib, lenalidomide and dex are planned.

0644

BORTEZOMIB-RELATED PERIPHERAL NEUROPATHY: AN IMPORTANT BUT REVERSIBLE SIDE EFFECT OF THERAPY. A STUDY ON 105 MULTIPLE MYELOMA PATIENTS

S. Mangiacavalli,[1] A. Corso,[1] M. Varettoni,[1] L. Barbarano,[2] D. Petrò,[2] E. Morra,[2] M. Lazzarino[1]

[1]Fondazione IRCCS Policlinico S. Matteo, UNiversity of Pavia, PAVIA; [2]Ospedale Niguarda Ca' Granda, MILANO, Italy

Background. The proteasome inhibitor Bortezomib has proven to be active in multiple myeloma (MM) either in relapsed/refractory or untreated pts. Bortezomib-related peripheral neuropathy (PN), is characterized by pain and burning paresthesias. Neurological pain is the most common reason for drug reduction or discontinuation. *Aims.* To analyze the incidence and characteristics of PN in a cohort of 105 MM pts treated with Bortezomib plus Dexamethasone as up-front therapy (group A) or in relapsed/refractory disease (group B). *Methods.* Group A included 54 pts enrolled in a high-dose protocol (4 cycles of Bortezomib plus Dexamethasone followed by 2 DCEP with stem cell collection and single transplant, Tx). IRB approval and signed informed consent was obtained. Median age was 57 years (37-65); ORR (at least PR) after 4 Bortezomib cycles was 86% (CR 28%, nCR 22%, VGPR 20%, PR 16%), the median follow-up was 12.7 months (0.4-23.1). Group B comprised 51 relapsed/refractory pts consecutively treated: median age 59 years (38-67); median number of prior therapies 1 (1-6); 61% of pts had prior Tx, 78% thalidomide; 5 pts had grade 1 PN at baseline. Median number of Bortezomib cycles 5 (1-8); ORR was 56% (CR 4%, VGPR 35%, PR 17%), the median follow-up was 11.8 months (2-31). Neurological pain and paresthesias were graded according to NCI common toxicity criteria. Mann-Whitney test was used to compare numerical variables, Fisher exact and Chi-square tests for categorical variables. *Results.* Table 1 summarizes the characteristics of Bortezomib-related PN in group A and in group B. The incidence of PN in the two groups was similar (group A 52%; group B 59%, p=0.56), as was the severity (grade 3-4 PN 18% *vs* 16%, p=0.48) and the median time to PN occurrence (64 *vs* 67days, p=0.74). With a similar follow-up, the median time to pain disappearance was significantly shorter in group A with respect to group B (24 *vs* 99 days, p=0.007), while paresthesias resolved later than pain with a median time not significantly different between groups (group A 178, group B 130 days, p=0.15). In relapsed/refractory pts, previous therapeutic history (number of previous therapies, prior thalidomide, Tx) was correlated neither with the incidence nor with the severity of PN. In a multivariate logistic regression analysis (group, age, ORR), only age appeared to have a significant prognostic impact for PN (p=0.044). Although with a different timing, clinically relevant neurological symptoms always disappeared in both groups. Actually, at the time of this analysis, all painful neuropathies resolved. *Conclusions.* In this study the incidence of Bortezomib-related PN was higher than that reported in registrative studies but similar to other single-centre experiences. Incidence, severity, time to occurrence, and time to paresthesias resolution were similar in the two groups (untreated *vs* relapsed/refractory pts). Only time to pain disappearance was significantly shorter in untreated pts compared to relapsed/refractory (p 0.007), thus allowing the completion of assigned treatment. In all settings, however, painful neuropathy always resolved even if at different times. In this analysis, age was the only risk factor for PN development.

Table 1. Comparison of Bortezomib-related PN between untreated and relapse/refractory MM pts.

	Untreated (Group A)	Relapsed/ refractory (Group B)	p
Patients Number	54	51	
PN incidence	52%	59%	ns
NCI grade 3-4 PN incidence	18%	16%	ns
Median time to PN occurrence (days)	64 (41-133)	67 (29-184)	ns
Median time to neurological pain resolution (days)	24 (10-111)	99 (17-202)	p=0.007
Median time to dysesthesias resolution (days)	178 (29-357)	130 (17-276)	ns

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RESPONSE TO BORTEZOMIB RETREATMENT IS DETERMINED BY DURATION OF PRECEDING TREATMENT FREE INTERVAL - RESULTS FROM A RETROSPECTIVE MULTICENTER SURVEY

I. Hrusovsky,[1] B. Emmerich,[2] M. Engelhardt,[3] G. Hess,[4] M. Kornacker[5]

[1]St. Joseph Hospital, BREMERHAVEN; [2]Oncology Practice, MUNICH; [3]Department of Internal Medicine I, University Hospital, FREIBURG; [4]Medical Department III, University Hospital, MAINZ; [5]Ortho Biotech, Div. of Janssen-Cilag, NEUSS, Germany

Background. Bortezomib (Velcade) has demonstrated highest single agent response rates in anti-myeloma therapy. There are no data from preclinical studies to suggest that resistance develops from repeated treatment with bortezomib. It was therefore of interest to investigate, whether repeated bortezomib treatment is safe, feasible and efficient. *Aims.* We have retrospectively collected data from multiple myeloma patients who had previously responded to bortezomib (previous bortezomib treatment), presented with relapsed disease and who received bortezomib for a second time (retreatment). *Methods.* Treatment and retreatment during the course of this multicenter non-interventional survey (26866138MMY4014) had been on discretion of the treating physician according to prescribing information.

Table 1.

	Prev. bortezomib treatment	Retreatment
Overall response rate	100% (per protocol)	63.3%
Time to response (median)	3.2 months	3.0 months
Time to progression (median)	10.9 months	6.7 months
Duration of response (median)	6.3 months	4.5 months
Treatment free interval (med.)	6.6 months	4.1 months

	<= 6 months after prev. bortezomib	> 6 months after prev. bortezomib
CR/nCR after retreatment	9.1%	18.5%
PR after retreatment	40.9%	55.6%

Results. Data from a total of 19 centers and 65 patients were obtained: these patients had all received bortezomib and were eligible for safety analyses. Sixteen patients had to be excluded due to insufficient data documented, leaving 49 patients for further analysis. Patients had a mean age of 66 years and had been treated with a mean of 4 prior therapies before

receiving bortezomib for the first time. Mean cycle number for previous bortezomib therapy and retreatment was 5.0 and 4.4, respectively. The majority of patients (85.7%) received doses of 1.3 mg/m^2 body surface area. Concomitant dexamethasone was given in 38.8% of patients with previous bortezomib treatment, and in 61.2% with retreatment. Six patients (12.2%) received various anti-myeloma therapies between bortezomib treatment and retreatment. Efficacy data are summarized in the Table 1, revealing encouraging response rates for bortezomib retreatment. Subgroup analysis according to the duration of treatment free interval (TFI) after previous bortezomib therapy demonstrated a higher response rate when preceding TFI was > 6 months. For thirty-three (50.8%) patients a total of 107 adverse drug reactions (ADRs) were documented, the most frequent being thrombocytopenia and peripheral neuropathy. For six (9.2%) patients a total of 11 SADRs were documented. In 2 (3.1%) patients, these SADRs were life-threatening or disabling. At the time of analysis, 21 patients had died (32.8 %). *Conclusions.* This retrospective survey suggests that the safety profile of bortezomib retreatment is in line with the current SmPC of Velcade and that high remission rates can be achieved. A treatment free interval > 6 months after previous bortezomib treatment increases the likelihood of obtaining CR or PR.

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A PHASE I DOSE ESCALATION STUDY TO DETERMINE THE MAXIMUM TOLERATED DOSE OF CYCLOPHOSPHAMIDE WHEN GIVEN IN COMBINATION WITH DEXAMETHASONE AND LENALIDOMIDE IN RELAPSE/REFRACTORY MULTIPLE MYELOMA

S. Schey,[1] G. Morgan,[2] F. Davies,[2] K. Phekoo,[1] K. Ramasamy,[1] M. Jenner,[2] B. Hazel[1]

[1]*King's College Hospital, LONDON;* [2]*Royal Marsden Hospital, SURREY, UK*

Background. Lenalidomide is an oral immunomodulatory drug that has been shown to be effective for the treatment of relapsed/refractory myeloma. *In vitro* laboratory studies suggest that its action may be synergistic with a number of conventional chemotherapeutic agents. *Aims.* To assess the maximum tolerated dose and toxicity profile of cyclophosphamide when used in combination with lenalidomide and dexamethasone for patients with relapsed refractory disease. *Methods.* Multiply relapsed/refractory patients were entered into an open label dose escalation study to determine the maximum tolerated dose (MTD) of cyclophosphamide when given at increasing doses from 300 mg po up to the maximum planned dose of cyclophosphamide 700 mg po on days 1 and 8 of a 28 day cycle in combination with dexamethasone (20mg po, daily on days 1-4 and 8-11) and Lenalidomide (25mg po, daily on days 1-21). Informed consent was obtained from all patients.

Table 1.

Incidence of most commonly observed adverse events during cycle 1

Adverse Event	NCI CTCAE Grade	total number of patients experiencing this AE during cycle 1	total number of patients at 300mg (n=3) experiencing this AE during cycle 1	total number of patients at 400mg (n=6) experiencing this AE during cycle 1	total number of patients at 500mg (n=3) experiencing this AE during cycle 1	total number of patients at 600mg (n=3) experiencing this AE during cycle 1	total number of patients at 700mg (n=6) experiencing this AE during cycle 1
HAEMATOLOGICAL							
febrile neutropenia	3	1					1
neutropenia	2	3		2			1
neutropenia	3	3	1	1			1
neutropenia	4	2					2
anaemia	1	3		3			
anaemia	2	1					1
anaemia	4	1					1
thrombocytopenia	1	1		1			
thrombocytopenia	4	1					1
NON-HAEMATOLOGICAL							
cramps - intermittent	1	7		2	3	1	1
cramps - intermittent	2	1		1			
somnolence	1	7	1	2	1		3
somnolence	2	3	1				2
somnolence	3	1	1				
Lightheadedness	1	5			3	1	1

Response data was assessed on day 15, and day 28, and toxicity profiles were assessed weekly, and at the end of cycle one to determine the maximum tolerated dose of cyclophosphamide. Providing that no patients in any cohort experienced dose limiting toxicity (DLT), the subsequent cohort of three patients received an increased dose of cyclophosphamide. If one patient in a dose cohort experienced DLT, another three patients were enrolled at the same dose. If 2 or more of all 6 patients at that dose experienced DLT, the MTD would be determined as one cohort dose level below. If 1 or fewer of the 6 patients experienced DLT, three more patients were recruited into the subsequent, higher dose cohort, up to a maximum of 700 mg. DLT was based on the NCI criteria (v3.0), and defined as; Grade 4 haematological toxicity occurring during cycle 1 of treatment, or febrile neutropenia during cycle 1, or Grade 3/4 non-haematological toxicity during cycle 1 (excluding alopecia and nausea/vomiting unless occurring despite maximum anti-emetic use), or failure to start cycle 2 within 7 days of scheduled day due to treatment related toxicity. *Results.* 21 patients were enrolled into the study. 12 patients had previously received high dose melphalan, 20 patients thalidomide and 6 patients bortezomib. The median time from diagnosis to treatment initiation was 54 months (range 17-98). In all, five dose cohorts were enrolled; Cohort1 (300mg (n=3)), cohort 2 (400 mg, (n=6)), cohort 3 (500mg (n=3)), cohort 4 (600 mg (n=3)) and cohort 5 (700 mg (n=6)). The most common adverse events experienced during cycle 1 are shown in Table 1. No patients receiving doses of 300 to 600mg experienced dose limiting toxicity. 2 patients receiving 700mg qualified as having dose limiting toxicity (One patient with febrile neutropenia, pneumonia and syncope (all grade 3)), and another with grade 4 pancytopenia). *Conclusions.* We have therefore established the MTD of cyclophosphamide in combination with lenalidomide and dexamethasone at 600 mg daily on days 1 and 8 of a 28 day cycle.

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MELPHALAN-DEXAMETHASONE IN PATIENTS WITH AL AMYLOIDOSIS NOT ELIGIBLE FOR HIGH-DOSE MELPHALAN THERAPY

S.O. Schönland,[1] S. Dietrich,[2] U. Hegenbart,[2] T. Bochtler,[2] A.V. Kristen,[3] H. Goldschmidt,[4] A.D. Ho,[5] S.O. Schönland[2]

[1]*University of Heidelberg, HEIDELBERG;* [2]*Medical Department V, Amyloidosis Clinic, Universital Hospital, HEIDELBERG;* [3]*Medical Dept. III, University Hospital, HEIDELBERG;* [4]*Med. Dept. V, Myeloma Center, University Hospital, HEIDELBERG;* [5]*Medical Dept. V, University Hospital, HEIDELBERG, Germany*

Background. The most efficient therapy for patients (pts) with AL amyloidosis is high-dose melphalan (HDM) treatment with autologous stem cell support. However, the toxicity of HDM limits its feasibility to a minority of patients. An option for pts not eligible for HDM is standard dosed oral melphalan and dexamethasone (M-dex, Palladini *et al.*, Blood, 2004 and 2007). We report on 61 pts with AL amyloidosis who received i.v. M-dex from 2004 to 2007 and were evaluated in our Amyloidosis Clinic. *Methods.* The median age was 67 (range 45-78) years, the median number of involved organs was 3 (range 1-6). Criteria for ineligibility for HDM were advanced cardiac disease (NYHA stage III, n=36), age > 70 years (n=20), Karnofsky-Index, KI <70% (n=6), symptomatic pleural effusion (n=8) or other (n=3). M-dex was administered as follows: Melphalan 16 mg/m^2 day 1 intravenously, dose adjustment in case of impaired renal function, dexamethasone orally 40 mg day 1-4, every 28 days. Pts over the age of 70 years or with cardiac disease NYHA III received 20mg of dex. Pts with NYHA III received their first cycle as inpatients. Antibiotic prophylaxis was used days 1-21 after start of M-dex. Melphalan dosage was reduced in case of haematological toxicity > grade II. Response was assessed every 3 cycles. Informed consent was obtained from each patient.

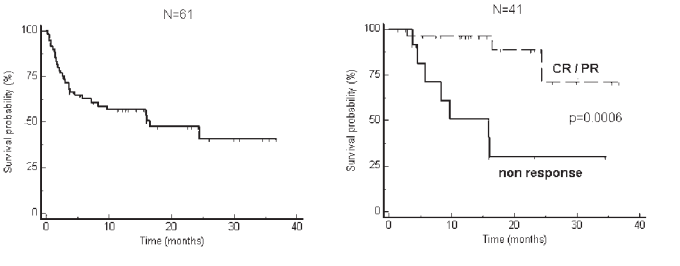

Figure 1. a. Overall Survival of patients receiving M-dex; b. Overall Survival according to haematoligica response.

Results. Median overall survival (OS) was 16.5 months for the entire group (Figure 1a). Median follow-up of patients alive (n=38; 62%) was 16 months. Pts received a median of 4 cycles (range, 1-12), 20 pts less than 3 cycles. Haematological toxicity was in the expected range (NCI grade, median 2, 0-4). However, non-haematological toxicity was high with a median NCI grade of 3 (range 0-5). Twenty-one patients (34%) died under therapy, however it was often not possible to distinguish between amyloid related (mostly cardiac) death and treatment related mortality. Overall, 41 pts were evaluable for haematological remission (HR). A complete haematological remission (CR) could be achieved in 7 pts (17%), a partial haematological remission (PR) in 22 pts (54%) and

12 pts did not respond. Achievement of HR was associated with superior OS (median not reached *vs* 15.9 months, p=0.0006, Figure 1b). In 13 pts (32%) HR was followed by organ response (OR), which was significantly correlated with each other (r=0.44, p=0.004). A median of three cycles (range 2-6) had to be given to achieve HR; it took a median of 5 months (range 2-15) to observe OR. Multivariate analysis with covariates age, KI, number of involved organs, troponin T, brain natriuretic peptide (NT-ProBNP), proteinuria and creatine clearance revealed that patients with elevated NT-ProBNP (p=0.001) and reduced KI (p=0.02) were at high risk to die under therapy. *Summary*. We could confirm that patients with haematological remission after M-dex have a prolonged survival. However, the rate of death under therapy is remarkably high in our study. We ascribe this to the inclusion of patients with very advanced disease. For these patients less toxic therapies which induce a rapid HR are warranted.

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MOBILISATION FAILURE IS PREDICTIVE OF POOR OVERALL SURVIVAL AFTER AUTOLOGOUS TRANSPLANTATION

K. Ramasamy, S. Mahmood, Z.Y . Lim, A. Mijovic, S. Devereux, A. Pagliuca, G.J. Mufti, S. Schey

Kings College Hospital, LONDON, UK

Background. High dose therapy (HDT) with autologous stem cell rescue improves event free survival (EFS) in patients with myeloma. In two large randomised trials supporting the use of HDT, 25% of patients assigned to high dose therapy failed to proceed to transplant. Heavily pre-treated patients treated with alkylating agents may lead to poor mobilisation of peripheral blood stem cells. It is reported, 12-20% of patients fail to mobilise adequate PBSC numbers (< 2×10^6 CD34/kg) at first mobilisation. There are no significant differences in overall survival (OS) or engraftment kinetics between patients transplanted with PBSC obtained from single mobilisation or multiple lines of mobilisation. It is less clear, what is the fate of those who subsequently successfully mobilise or undergo autologous transplantation (ASCT) with suboptimal CD34 dose *Aims.* We wanted to compare OS in patients who successfully mobilised at first attempt and patients who failed first mobilisation after prior therapy. *Methods.* We analysed data on 180 myeloma patients referred between 1993- 2006 for an ASCT. Cyclophosphamide (1.5g/m^2) and G-CSF (5mcg/kg) was used as first line mobilisation regimen and high dose G-CSF (16-24mcg/kg) alone as second line. In patients who failed first PBSC collection a repeat attempt at PBSC collection after a 6 week delay and/or a bone marrow harvest was performed. We followed up patients who failed first mobilisation and subsequently successfully mobilise or undergo ASCT with suboptimal CD34 dose. OS was calculated from the date of transplant and statistical analysis was performed using SPSS 14.0 software.

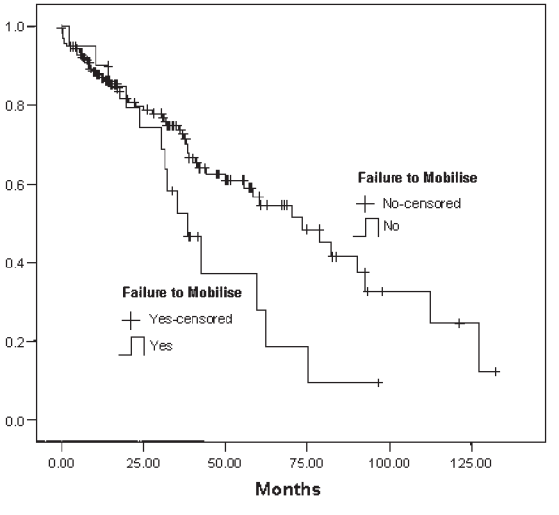

Figure 1. Overall Survival - Mobilisers & Failed Mobilisers

Results. 32/180 (17.7%) failed mobilisation at first attempt. 8/32 mobilisation failures had a previous ASCT at our centre and the median number of lines of prior therapy was 2 (Range 1-4). 23/32 were at their first best response and 9/32 were at their second best response. 4/35 patients had prior melphalan therapy and 5/35 patients had thalidomide therapy prior to mobilisation, with no patients receiving both. 14/32 (44%) of patients who failed first mobilisation were successfully mobilised at the second attempt. BMH was performed in 8/32 patients and 4/32 patients needed more than 2 lines of mobilisation to augment the stem cell dose. During follow up of 20/32 went on to subsequently receive an autologous transplant, 10 with suboptimal cell dose. Median OS of 156 patients who successfully mobilised at first attempt and had an ASCT was 73.5 months (51.5-95.4) and 20 patients who failed first mobilisation and had an ASCT was 38.5 months (27.1-49.8). Using Log Rank test, there is a statistically significant difference between survival in the two cohorts (p=0.033). *Conclusions.* Despite being heavily pre-treated there is a significant reduction in OS in the cohort of patients, who failed first mobilisation. We are investigating this group of patients as there is recent data suggesting patients who mobilised poorly had cytogenetic features suggestive of myelodysplasia at follow up. As it is currently unclear whether this group of patients are biologically distinct and hence their poor survival, efforts to preserve stem cell pool and improve stem cell yield should be given due consideration.

0649

RESPONSE TO RE-TREATMENT ON RELAPSE MULTIPLE MYELOMA PATIENTS PREVIOUSLY TREATED WITH BORTEZOMIB

A. Rubio-Martinez, V. Recasens, B. Soria, M.A. Montañes, R. Rubio-Escuin, P. Giraldo

Miguel Servet University Hospital, ZARAGOZA, Spain

Background. Bortezomib has been shown to be effective in multiple myeloma (MM), but there is limited experience in response to re-treatment. *Aims.* To evaluate the efficacy and safety of Bortezomib in an every day clinical use in refractory/relapsed consecutive MM patients treated with Bortezomib since December 2003 to January 2008 in a single institution. *Patients and Methods.* 47 patients with relapsed/refractory MM receiving Bortezomib alone (1.3 mg/m^2 on days 1,4,8,11 in a 21-day course) as second or more line of therapy. The response was evaluated according EGBMT criteria (Bladé J, Samson D, Reece E *et al.*), IFE was performed in cases with complete remission. Patients without response after 4 courses and patients that relapsed after reached CR or PR with Bortezomib alone, a combination of bortezomib + dexamethasone (BD) or bortezomib+melphalan+prednisone (BMP) were administered. Adverse effects were registered. *Results.* 47 patients (males 41.8%), mean age 67.3 years (34-89), over 65 years (51.1%). Bortezomib was administered as second line: 14 (32.5%), as third or more: 29 (67.4 %). Overall response: 31 (77.5%); CR+PR: 29 (72.5%); MR: 2 (5.0%); CR: 16 (40.01%); CR-EIF negative: 11 (27.5%); failure: 9 (22,5%), mean courses to reached response: 3.6. No relation to response and presence or not chromosomal aberrations. At 42 months on follow-up, of 40 valuable patients, 18 (45%) are in stable response without therapy. Seven patients (16.3%) do not reached response after 4 courses and received a Bortezomib combination therapy, 15 patients (31.9%) were relapsed after a mean of 22 months follow-up in stable response. 3 patients(20%) receiving Bortezomib alone again, 7 patients (46.6%) a combination of BD and 5 patients (33.3%) received BMP. *Responses*. 4 patients obtain a new CR in a Bortezomib re-treatment schedule (1 CR-EIF-), 8 PR, 3 Failures; infectious complications (35%), Adverse events: thrombocytopenia 40% (grade III: 20), fatigue 25%, peripheral neuropathy 37.7%, constipation 35%, diarrhoea 22.5%, ZHV 15%, non documented infection 35%, fever 12.5%, hypotension 5%, leucopenia grade 3 15%. Only 3 patients (7.5%) need disrupted therapy by toxicity. No more adverse events were observed in patients treated with bortezomib in combination. *Comments*. In our experience Bortezomib, induces new response in relapsed patients previously treated with the drug. The safety is good with tolerable adverse.

0650

VAMP/THACYDEX: VELCADE® (BORTEZOMIB), ADRIAMYCIN, MELPHALAN AND PREDNISONE ALTERNATING WITH THALIDOMIDE, CYCLOPHOSPHAMIDE AND DEXAMETASONE AS A SALVAGE REGIMEN IN RELAPSED MULTIPLE MYELOMA PATIENTS: A SPANISH MYELOMA GROUP (GEM/PETHEMA) STUDY

E. Colado,[1] M.V. Mateos,[1] M.J. Moreno F. De Arriba,[2] J. De la Rubia,[3] P. Iniesta,[4] M.C. Viguria,[5] R. Garcia-Sanz,[1] J. Olazabal,[1] J.F. San Miguel[1]

[1]Hospital Universitario de Salamanca, SALAMANCA; [2]Hospital Morales Meseguer, MURCIA; [3]Hospital La Fé, VALENCIA; [4]Clinica Virgen de la Vega, MURCIA; [5]Hospital de Navarra, PAMPLONA, Spain

Background. Multiple Myeloma (MM) remains an incurable disease. Therefore, new treatments approaches are needed in order to improve the outcome of these patients. Bortezomib and Thalidomide are effective in MM patients, including those with adverse cytogenetics (CG) but, although combination therapy can result attractive, toxicity could be increased. Based on this background, an alternating regimen consisting on two highly effective schedules could overcome MM drug resistance without an increase in toxicity. *Aims.* To evaluate the efficacy and toxicity of a sequential treatment with VAMP (Velcade®, Bortezomib; Adriamycin; Melphalan; and Prednisone) a followed by ThaCyDex (Thalidomide; Cyclophosphamide; and Dexametasone) in refractory/relapsed MM patients. Patients and *Methods.* Treatment schedule consisted on 6 alternating cycles of VAMP (Bortezomib 1,3mg/msq IV days 1,4,8 and 11; Melphalan 9mg/msq po, days 1-4; Prednisone 60mg/msq po, days 1-4; and conventional or liposomal Adriamycin 40 or 30mg/msq respectively on day 1 of a 28 day cycle) alternating with ThaCyDex (Thalidomide 200mg/d po day 1-28; Cyclophosphamide 50mg/d po, days 1-28; and Dexametasone 40mg/d po, days 1-4). After 6 cycles, responding patients, received the previous schedule, every other month as a consolidation therapy. *Results.* Until February 2008, 17 patients have been included in a multicenter trial, with a median age of 63 years (Range 48-81). 12 patients (70.5%) had previously received polychemotherapy followed by autologous stem cell transplantation. 6 patients (35%) had previously received Bortezomib based therapy, and one patient (6%) had previously received IMID based therapy. After a median of 6 cycles, 16 patients have received at least one cycle of treatment, and therefore, they are evaluable for efficacy. 6 patients (37%) obtained a negative immunofixation Complete Response (CR), 2 patients (12.5%) nCR, 3 patients (18.7%) partial response, which makes an ORR of 69%. In addition, 5 patients (31%) remained stable disease. Seven patients had high risk cytogenetic abnormalities [t(4;14) and/or delRB], and CR was obtained in 3 patients (42%) and nCR in 1 patient (14%). Moreover, allogeneic stem cell transplantation was performed in two of these high risk patients, as they were effectively rescued by this salvage regimen. Two patients progressed in the maintenance treatment. Toxicity was manageable, being haematologic events the most frequently reported. 6 patients (42%) developed ≥G3 thrombocytopenia and 6 patients (42%) neutropenia. Infection ≥G3 occurred in 3 patients (23%). Despite the combination of two drugs with neurologic toxicity, the use of them in alternated schedule resulted in that only 3 patients (23%) developed Peripheral Polyneuropathy, none of them ≥G2. *Conclusions.* Preeliminary results show that alternating VAMP/ThaCyDex is a highly effective salvage regimen in relapsed/refractory MM patients, including high risk subgroup with adverse cytogenetic abnomalities. Haematologic toxicity was the most frequent adverse event, while Peripheral Polyneuropathy incidence was low, despite the use of two neurotoxic drugs. A second analysis will be performed in May 2008 and results will be updated.

0651

PRETREATMENT WITH THALIDOMIDE AND BORTEZOMIB DOES NOT INFLUENCE RESPONSE TO LENALIDOMIDE IN RELAPSED MYELOMA. PRELIMINARY RESULTS FROM PATIENTS INCLUDED IN DUTCH COMPASSIONATE NEED PROGRAMME

E. Kneppers,[1] H.M. Lokhorst,[1] C. Eeltink,[2] R. Raymakers,[3] R. Schaafsma,[4] P. Sonneveld,[5] E. Vellenga,[6] S. Wittebol,[7] P. Wijermans,[8] S. Zweegman[2]

[1]UMC Utrecht, UTRECHT; [2]VUMC, AMSTERDAM; [3]UMC St Radbout, NIJMEGEN; [4]Medisch Spectrum Twente, ENSCHEDE; [5]Erasmus MC, ROTTERDAM; [6]UMC Groningen, GRONINGEN; [7]Meander MC, AMERSFOORT; [8]HagaZiekenhuis, DEN HAAG, Netherlands

Background and methods. The clinical data on efficacy and toxicity of lenalidomide, a highly effective structural analogue of thalidomide with a different side effect profile, mainly come from patients treated in prospective clinical studies. Selection of patients and specialized clinical care might influence these clinical data. Here we report preliminary data of 42 patients with relapsed or refractory multiple myeloma who were treated in a compassionate need programme. The complete dataset of 105 patients treated in community centers will be available in June 2008. Treatment consisted of lenalidomide 25 mg given on day 1-21 of a 28-day cycle, in combination with dexamethason in most cases 40 mg on day 1-4 and 15-18. Patients were treated until disease progression, unacceptable toxicity or until a maximum of 8 courses were given. In 15 patients, after 6-8 courses, lenalidomide 10 mg maintenance therapy without dexamethasone was given. All patients received VTE prophylaxis with acetylsalicylic acid, 80-100mg/daily. *Results.* The mean age of the patients was 60.3 years. A median of 4 (range 1-7) previous lines of anti-myeloma therapies was given, including thalidomide in 98%, bortezomib in 78%, an autologous transplant in 73% and an allogeneic transplant in 40% of patients. Forty patients had stage II/III disease, 2 patients had stage I disease. The overall response rate was 83.3%. There were 2 complete responses (CR, 4.8%), 11 very good partial responses (VGPR, 26.2%), 19 partial responses (PR, 45.2%) and 2 minimal responses (MR, 4.8%). Median progression free survival (PFS) was 10 months and median overall survival (OS) has not been reached yet. The patients received a median of 6 cycles (range 1-8), with a median of 3 (range 1-6) cycles before maximal response. Nine patients are still on maintenance therapy. Fifteen (36.6%) patients discontinuated their lenalidomide treatment before they yielded their planned number of cycles: 4 patients because of progressive disease (PD), 2 patients died (no PD, 1 patient died of acute GvHD grade 4), 4 patients stopped because of toxicity and 1 because of suspicion of GVHD induction by lenalidomide. Six patients (14.3%) reported non-hematological toxicity > grade 3, two of them had a grade 3 neuropathy. Hematological toxicity > grade 3 was experienced by 13/42 patients (31.7%), infections in 6/42 patients (14.6%). Although thrombotic prophylaxis was given, 3 (7.3%) patients developed a thrombo-embolic event. We noticed a flare-up of GvHD while on lenalidomide in 7/17 (41.2%) patients who previously underwent allogeneic stem cell transplantation (allo-SCT) and donor lymphocyte infusions (DLI), which was fatal in 1 patient. It is not clear yet if this GvHD flare-up was due to a direct immune stimulating effect of lenalidomide or was part of the usual side effects of Allo-SCT and DLI. *Conclusions.* This preliminary analysis confirms the high effectiveness and feasibility of lenalidomide in combination with dexamethasone in heavily pretreated multiple myeloma patients in a compassionate need programme.Thalidomide and bortezomib pretreatment does not seem to influence sensitivity to lenalidomide.

0652

THALIDOMIDE-DEXAMETHASONE (THALDEX) AS SALVAGE THERAPY FOR MULTIPLE MYELOMA PATIENTS RELAPSING AFTER HIGH DOSE THERAPY AND AUTOPBSCT

G. Charlinski, M. Barwicka, E. Wiater, J. Dwilewicz-Trojaczek, W. Wiktor-Jedrzejczak

Medical University, WARSAW, Poland

Background. High dose therapy with melphalan and autoPBSCT induce remissions in multiple myeloma but are not able to cure this disease. Eventually, all patients relapse and require further treatment. One possibility suitable particularly for patients who had not been earlier treated with thalidomide is a combination of this drug with dexamethasone. *Aims.* To retrospectively assess the effectiveness of such treatment in a cohort of patients treated in a single center. The end points of the study were: response, EFS, OS and toxicity. *Methods.* Forty nine multiple myeloma patients (23F/26M) with a median age of 54 years (range 33-69) were included into the analysis between February 2003 and October 2007. Twenty five patients were IgG, 11-IgA, 2-nonsecretory, 11-light chain. Clinical stage according ISS: 22 pts in stage 1, 11-stage 2, 16-stage 3. Patients have been initially treated with VAD protocol (vincristin, adriamycin, dexamethasone) followed by cyclophosphamide mobilization and then by tandem high dose melphalan with autoPBSCT. Relapsing patients were treated according to the following protocol: thalidomide 200 mg/day po. continuously until any sign of progressive disease or relapse, and dexamethasone 40 mg po. for 4 days every 3 weeks. Fifteen patients received enoxaparin and 34 pts acetylsalicylic acid prophylaxi. Response was evaluated using the International Myeloma Working Group Uniform Response Criteria. Moreover, overall survival (OS) and event free survival (EFS) was assessed. *Results.* Altogether 2 patients (4%) achieved complete response (CR), 7 patients (14.3%)

very good partial response (VGPR), 23 patients (47%) partial response (PR), 17 patients (34.7%) stable disease (SD). Time to response was 6 weeks. Duration of treatment TD was 14.6 months (median), (3-48.6). The median EFS was 24 months (14-41.3). The median OS has not been reached. The major adverse events of THALDEX were: peripheral neuropathy-9 pts, constipation-8 pts, somnolence-4 pts, deep-vein thrombosis-1 patient. *Conclusions.* THALDEX seem to be effective in multiple myeloma patients relapsing after high dose therapy and tandem PBSCT and well tolerated

0653

PROGNOSTICATION OF RESPONSE TO BORTEZOMIB, PROGRESSION FREE AND OVERALL SURVIVAL IN RELAPSED/REFRACTORY MYELOMA PATIENTS

M.C. Kyrtsonis,[1] T.P. Vassilakopoulos,[2] S. Sachanas,[2] G. Panagoulias,[3] V. Bartzis,[3] K. Anargyrou,[2] M. Dimou,[3] T. Tzenou,[3] S. Masouridis,[2] C. Kalpadakis,[2] E. Dimitrakopoulou,[3] S.I. Kokoris,[2] E. Dimitriadou,[2] M.P. Siakantaris,[2] M.K. Angelopoulou,[2] P. Panayiotidis,[3] G.A. Pangalis[2]

[1]University of Athens, ATHENS; [2]Dpt of Hematology, Athens Med. School, Laikon Hosp, ATHENS; [3]1rst Dpt of Propedeutic Int. Medicine, Athens Med.School, Laikon Hosp., ATHENS, Greece

Background. Bortezomib produces high response rates in patients with relapsed or refractory MM. However, the duration of response varies among patients with obvious consequences on overall survival (OS) after treatment. *Aims.* To evaluate the prognostic factors of response to bortezomib, progression free survival and OS in in a series of relapsed/ refractory MM patients with a long follow-up. Patients and *Methods.* 76 relapsed/refractory MM patients were treated with Bortezomib at standard doses and schedule, in our Department. Sixty-seven percent were males, 64% were 65 years old or more, 66% had IgG, 21% IgA, 1% IgD, 12% light chain MM 36% were in stage Durie-Salmon (DS) III and 57% in ISS 3. Thirty-nine received the drug as 2nd line treatment, while the other 37 were in the 2nd or more relapse. The median time from diagnosis to Bortezomib administration was 26.2 months in all patients; 12.3 in those treated as 2nd line and 54.5 months in the others. 64 (84%) patients responded (including 37% CR and nCR). Median time to response was 1.4 months (range 0.4-5.77). The 2-year PFS was 41% with a median time to progression (TTP) of 8.4 months. The 2-year OS was 59%. Median survival after Bortezomib administration was 27.5 months and 31 of 76 patients died. Variables predicting PFS and OS were performance status (PS) (p=0.0016 and 0.0001 respectively), DS staging (p=0.014 and 0.01 respectively), elevated serum LDH (p< 0.0001 for both), Hb (p=0.0097 and 0.0025 respectively), PLT (p=0.0009 and <0.0001 respectively), LC MM (p=0.007, significant for PFS only). The quality of response influenced both PFS and OS. In multivariate analysis, three parameters remained important adverse factors for both PFS and OS: serum LDH levels, PS equal to 2 or more and PLT below 140×10^9/L. The 2-year PFS and OS were 60% and 84% respectively in the presence of none of the above 3 adverse factors, 25% and 45% respectively in the presence of 1 factor and 0% for both in the presence of 2-3 factors (p<0.0001). *Conclusions.* Three easily assessable variables, LDH, PS equal to 2 or more and PLT below 140×10^9/L, predict response to Bortezomib, PFS and OS.

0654

THE EFFICACY AND SAFETY OF PAD REGIMEN (BORTEZOMIB, DOXORUBICIN, DEXAMETHASONE) IN THE TREATMENT OF PLASMA CELL LEUKEMIA

M. Kraj, R Poglod, T Szpila, K. Warzocha

Institute of Hematology and Transfusion Medicine, WARSAW, Poland

Bortezomib (formerly PS341), shows encouraging results in patients with relapsed and newly recognized multiple myeloma, applied with dexamethasone shows additive benefit. *In vitro* bortezomib demonstrates potent synergy with cytotoxics including anthracyclines. Treatment of multiple myeloma with PAD regimen gives 95% responses, including 30% CR/nCR (Oakervee Br J Haematol 2005; 129: 755). Some reports have suggested that bortezomib is effective in inducing responses in patients with primary or secondary plasma cell leukemia (PCL) (Musto *et al.* Blood 2006; 108: 3546a; Alegre *et al.* Haematologica 2005; 90 (s 1): 152; Morris *et al.* Haematologica 2005; 90 (s 1): 153). We evaluated 4 patients with PCL diagnosed between January, 2003, and May, 2007, who had received bortezomib for the treatment of their disease (2 females, 2 male in age 50, 50, 57, 73 years, respectively). Three patients had primary and 1 secondary PCL. Two patients had previously received 2 to 4 lines of chemotherapy, including thalidomide (1). Two patients were treated at diagnosis. Bortezomib was given acc. to standard schedule of 1.3 mg/sqm days 1, 4, 8, 11, with an interval of 10 days between cycles. Three patients received doxorubicin and dexamethasone in combination with bortezomib (PAD regimen). In the first patient with primary PCL (with bone marrow plasma cell rate - 80%, absolute peripheral blood plasma cell count - 3.7×10^9/L, IgGÏ serum monoclonal protein - 8.5 g/dL and osteolysis) bortezomib was administered twice as an induction therapy and was re-administered in relapse. A near-complete remission (disappearance of circulating and bone marrow plasma cells, disappearance of M-component at electrophoresis, but positive immunofixation) was achieved subsequent to induction PAD treatment. In this patient herpes zoster and neurological grade 2 toxicity was observed. Following cyclophosphamide 4.9 g and G-CSF, peripheral blood stem cells were succesfully (6.5×10^6 CD34$^+$ cells/kg) harvested. After Melphalan 200 mg/m^2 peripheral blood autologous stem cell transplantation (PBSCT) was performed. Time to neutrophil >0.5×10^6 engraftment was 20 days and time to platelet > 0×10^6 engraftment was 17 days. PBSCT led to complete remission which lasted 7 months. Partial remission was achieved subsequently to relapse retreatment with PAD. At present, the patient is further on bortezomib therapy, in partial second remission, 22 months after diagnosis of PCL. In the second case of primary PCL with renal failure requiring hemodialysis a partial response was achieved after 3 cycles of PAD treatment. Nine months since PCL diagnosis the patient in partial remission still remains on bortezomib therapy. The third patient with recurrent primary PCL died of progressive disease after completing 2 cycles of bortezomib. In the fourth patient with secondary PCL bortezomib therapy was interrupted after one cycle due to severe neurological toxicity. Two last patients survived respectively 46 and 2 months, since PCL diagnosis. We suggest, bortezomib in combination with other agents may be considered as an initial treatment of primary PCL. PAD regimen is effective and does not prejudice peripheral blood stem cell collection or subsequent engraftment.

0655

SAFE AND EFFECTIVE MOBILIZATION OF STEM CELLS IN MULTIPLE MYELOMA FOLLOWING PRIMING BY HIGH-DOSE CYCLOPHOSPHAMIDE AND BORTEZOMIB

G. Mikala

St Istvan & St Laszlo Hospital of Budapest, BUDAPEST, Hungary

Contamination of the stem cell graft by clonogenic myeloma cells is a known cause of relapse after high-dose chemotherapy followed by autologous stem cell transplantion. In this disease, cytostatic priming preceding stem cell collection has not been proven to delay relapse, nevertheless, improved tumour control with novel agents may reasonably be expected to improve outcome. In this study, we analyzed the results of CD34$^+$ stem cell collection that followed priming by cyclophosphamide (3 g/m^2, day 1), bortezomib (1.3 mg/m^2, days 1 and 4), and G-CSF (target and median of 10 ug/kg from day 3). A historic control group of similarly cyclophosphamide primed (without the inclusion of bortezomib) myeloma patients are also presented for comparison. 35 consecutive myeloma patients were treated with the Cyclo/Vel protocol. Stem cell harvest was done after median of 10 (7-19) days (control group of 25 Cyclo patients, median 10 (8-17) days). At this point, peripheral blood CD34$^+$ cell count had a median of 133/uL (24-498) (control group 156/uL (11-696)). Securing graft volume sufficient for one transplantation was achieved in 100% of the patients. A single apheresis procedure yielded sufficient number of stem cells in 74% (26/35) of our cases (72% (18/25) for the control group). Average stem cell yield was 10.4×10^6/kg (3.9-27.6) and 13.2×10^6/kg (2.3-48.5) for the control group. Inclusion of bortezomib in our protocol did not result in any meaningful additional toxicity. 34/35 transplantations were already done with the collected stem cells; no unusual toxicity and no change in engraftment parameters were observed. Our data indicate that inclusion of bortezomib in the priming protocol does not significantly alter stem cell mobilization parameters in myeloma patients as compared to a historic control and does not carry an increased risk. Moreover, it may provide better tumour control and may influence our long-term results.

Multiple myeloma - Clinical III

0656

PAD (BORTEZOMIB/ADRIAMYCIN/DEXAMETHASONE) REGIMEN IS VERY EFFECTIVE IN HIGH RISK, NEWLY-DIAGNOSED MYELOMA, REDUCES DICKKOPF- 1 AND ABNORMAL BONE RESORPTION AND NORMALIZES IMPAIRED ANGIOPOIETIN-1/-2 RATIO

E. Terpos,[1] I. Baltathakis,[2] K. Anargyrou,[1] S. Delimpasi,[2] E. Kastritis,[3] A. Christoforidou,[4] K. Tsionos,[1] K. Tsatalas,[4] E. Nikiforakis,[2] M.A. Dimopoulos,[3] N. Harhalakis[2]

[1]251 General Air Force Hospital, ATHENS; [2]Department of Hematology, Evangelismos General Hospital, ATHENS; [3]Department of Clinical Therapeutics, University of Athens Medical School, ATHENS; [4]Department of Hematology, Democritus University of Thrace School of Medicine, ALEXANDROUPOLIS, Greece

Background and Aims. Bortezomib has significant activity in multiple myeloma (MM). Its efficacy is increased with the addition of dexamethasone and doxorubicin *in vitro*, thus providing the rationale for combination regimens with these agents. The aim of this study was to evaluate the efficacy and safety of PAD regimen (bortezomib, doxorubicin, dexamethasone) in high-risk, newly diagnosed, MM patients and evaluate its effect on bone remodeling and angiogenesis. *Patients and Methods.* The inclusion criteria included newly diagnosed MM, ISS 2/3 disease or del13q detected by FISH. Patients received four 21-day cycles of PAD: bortezomib 1.3 mg/m^2 on days 1, 4, 8 and 11; dexamethasone 40 mg on days 1-4 and 8-11; bolus doxorubicin 9 mg/m^2 on days 1-4. All patients received monthly zoledronic acid and prophylactic dose of cotrimoxazole and acyclovir. Following peripheral blood stem cell (PBSC) collection, eligible patients received high-dose melphalan with PBSC transplantation. Effect of PAD on angiogenesis was evaluated by measuring serum levels of VEGF, VEGF-A, angiogenin, angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), and basic fibroblast growth factor at baseline and on day 21 of cycle 4. Bone remodeling was studied by the measurement of a series of serum indices: i) osteoclast stimulators [soluble RANKL, and osteoprotegerin (OPG)], ii) osteoblast inhibitor dickkopf-1 (Dkk-1), iii)bone resorption markers [C-telopeptide of collagen type-I (CTX), and tartrate resistant acid phosphatase-5b (TRACP-5b)], and iv) bone formation markers [bone alkaline phosphatase (bALP), and osteocalcin] at baseline and on day 21 of cycle 4. All above molecules were also measured in 22 healthy controls of similar age and gender. *Results.* To-date, 32 patients (18M/14F, median age 61 years) completed 4 cycles of therapy: 17 (53%) had ISS stage 2 and 15 (46%) stage 3 disease. Del13q was detected in 15 patients. The majority of patients (n=18) had more than 3 lytic lesions and/or a pathological fracture in the plain radiography of the skeleton. The objective response rate was 90% (29/32 patients): CR 25%, vgPR 12% and PR 53%. Median time to response was 35 days. Grade 3/4 adverse events included infections (10 patients-31%; one died due to septicemia), lymphopenia (10-31%), thrombocytopenia (9-28%), neutropenia (6-18%), peripheral neuropathy (6-18%), fatigue (4-12%), and hyponatremia (4-12%). At baseline, MM patients had increased serum levels of Dkk-1, sRANKL, CTX, TRACP-5b, OPG, angiogenin, and Ang-2 compared with controls (p<0.01), while the ratio of Ang-1/Ang-2 was reduced (p<0.01). The administration of PAD resulted in a dramatic reduction of Dkk-1, sRANKL and bone resorption markers (p<0.01) and a borderline increase in bALP (p=0.07). PAD also produced a significant increase of Ang-1/Ang-2 ratio (p<0.01), which was normalized. No patient developed a skeletal related event during 4 cycles of therapy. Eleven patients (34%) had a PBSC collection; the median number of CD34$^+$ cells was 6×10^6/kg (range: 2.3-13×10^{6}cells/kg). *Summary and Conclusions.* PAD has significant activity in high-risk, newly diagnosed patients with MM, overriding del13q. This regimen reduces Dkk-1 resulting in a borderline increase in bone formation, while decreases sRANKL and bone resorption. Furthermore, PAD normalizes Ang-1/Ang-2 balance which is crucial for the process of angiogenesis in MM.

0657

DETERMINING THE EXTENT OF DISEASE WITH MAGNETIC RESONANCE IMAGING OF THE BONE MARROW (BM-MRI) IN PATIENTS WITH MULTIPLE MYELOMA.

S. Ailawadhi, A.N. Abdelhaleim, L. Derby, T.L. Mashtare, G. Wilding, K.C. Miller, R. Gottleib, S. Padmanabhan, R.A. Alberico, D.L. Klippenstein, K. Lee, A. Chanan-Khan

Roswell Park Cancer Institute, BUFFALO, USA

Background. Multiple myeloma (MM) remains an incurable cancer. Treatment is often initiated with progressive increase in tumor burden that correlates with clinical symptoms. Commonly used parameters to assess tumor burden include disease stage, presence of lytic bone disease, and beta-2 microglobulin (B2M). We investigated imaging of the bone marrow as a novel approach to quantify disease burden and correlated the extent of marrow infiltration determined on BM-MRI with these established parameters. *Methods.* Extent of marrow involvement was evaluated by BM-MRI. This technique included sagittal T1 and fast spin echo inversion recovery sequences of the cervical, thoracic and lumbosacral spine and coronal T1 and fast spin echo inversion recovery sequences of the sacrum and pelvic bones. Clinical staging was done as per Durie-Salmon (DS) and International Staging System (ISS) while lytic bone lesions were assessed by skeletal radiographs. To study statistical relationship between pairs of ordinal and/or nominal variables the Spearman correlation, Wilcoxon or Kruskal-Wallis test were used. Statistical assessment of observed differences in survival distributions was done using the log-rank test. The Bonferroni adjusted alpha level of .05/6 was used to perform pairwise comparisons. A 0.05 nominal significance level was used in all testing. Following staging system was defined for evaluation of the marrow involvement by BM-MRI: A (0%), B (< 10%), C (10%-50%), D (> 50%).

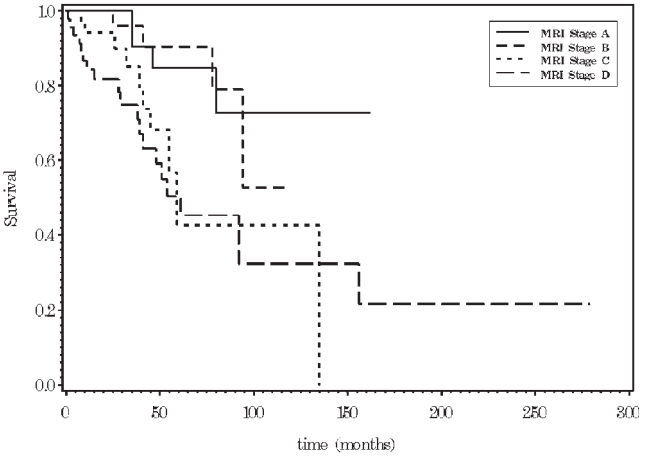

Figure 1. Survival curves for patients as per BM-MRI stages.

Results. We evaluated 170 consecutive patients (77 females and 93 males). Median age was 61 years (range 35-83). Number of patients in BM-MRI stages A, B, C and D were 31, 32, 35 and 46, respectively. Advance stage disease (> stage 1) based on DS or the ISS criteria was observed in 47.6% (n=81) and 53.3% (n=77) patients, respectively. Lytic bone disease was noted in 70.6% (n=120) patients. Estimated Spearman correlation coefficient between BM-MRI involvement and DS stage was 0.2795 (p=0.0006) demonstrating a significant association between BM-MRI involvement and DS stage. This correlation with clinical stage remained significant using the ISS system (p=0.0001). The correlation of marrow infiltration on BM-MRI was also significantly associated with the presence of lytic bone disease (p<0.0001) as well as the B2M levels (p<0.0001). There was no significant association between MRI involvement and patient age (p=0.7921) or the Ig type (p=0.8123). There was a significant difference in the survival distributions between the MRI staging groups for all patients (p=0.0031) and also when only newly diagnosed patients were analyzed (p=0.0106). *Conclusions.* BM-MRI is novel, non-invasive approach to quantify disease burden in patients with MM. Our investigation in a large cohort of patients demonstrates that the extent of marrow involvement determined by this method correlates

accurately with other conventional methods used to assess myeloma disease burden such as clinical stage, lytic bone disease or B2M. The BM-MRI stage is also predictive of survival in MM patients. We conclude that BM-MRI can be used to determine disease burden in patients with MM and can predict difference in overall survival based on variability in marrow infiltration.

0658

DICKKOPF 1 PROTEIN LEVELS IN PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS)

G.B. Lobreglio, A. Gatto, P. Capuani, P. Verardo, E. Chiuri, D. Turco, G. Pasanisi

A.O. Card G. Panico, TRICASE, Italy

Background. A rised bone resorption with an increased prevalence of vertebral fractures have been reported among patients with monoclonal gammopathy of undetermined significance.[1] The mechanisms of this skeletal imbalance are not well understood, but they are actively studied at the cellular, biochemical and molecular levels.[2] *Aims.* In this study we investigated if Dickkopf-1 (Dkk-1), a protein expressed by osteoblasts and osteocytes that acts as soluble inhibitor of the WNT signaling pathway and that is involved in the regulation of bone metabolism, might have a role in the development of skeletal involvement in MGUS. *Methods.* We evaluated the concentration of Dkk-1 with an enzyme immunoassay in the plasma of 116 patient with MGUS (55 IgG k, 31 IgG λ , 8 IgA k, 12 IgA λ; 8 IgM k; 2 IgM λ) and in 45 appropriately matched normal subjects; none of the patients with MGUS had vertebral fracture or apparent radiographic evidence of osteoporosis or osteopenia. *Results.* The results show significantly lower concentration of Dkk-1 among patients with MGUS (8.4±7.9 pmol/L) compared to normal matched subjects (25±12.9 pmol/L; $p<0.001$) *Summary and Conclusions.* These results suggest that the low expression of Dkk-1, an inhibitor of osteoblast differentiation, may counteract the role of osteoclast-activating factors (RANK-L, parathyroid hormone-related protein, interleukin1 and macrophage protein 1-alpha) and thus compensate the increased bone resorption with new bone formation.

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0659

BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE AS PRIMARY INDUCTION THERAPY FOR NEWLY DIAGNOSED MULTIPLE MYELOMA SIGNIFICANTLY DECREASES BONE RESORPTION WHILE SPARING BONE FORMATION AS COMPARED TO THALIDOMIDE-DEXAMETHASONE

P. Tosi, E. Zamagni, P. Tacchetti, G. Perrone, M. Ceccolini, A. Brioli, M. Pallotti, L. Pantani, A. Petrucci, M. Baccarani, M. Cavo

Seragnoli Institute of Hematology and Medical Oncology, BOLOGNA, Italy

Bone disease occurs in approximately 80% of patients with newly diagnosed multiple myeloma (MM) and is caused by the interaction of the neoplastic clone with bone marrow microenvironment, ultimately resulting in an altered balance between bone resorption and bone formation. It has been previously reported that therapies aimed at eradicating the myeloma clone could contribute to decrease bone resorption, even though bone formation remains impaired due to the use of high-dose steroids. It has been recently demonstrared, both *in vitro* and in animal models, that Bortezomib improves bone formation by stimulating osteoblasts. In order to assess whether this activity was retained also *in vivo*, we evaluated markers of bone resorption (serum crosslaps) and bone formation (serum osteocalcin-OC and bone alkaline phosphatase - BAP) in a series of newly diagnosed symptomatic MM patients who were enrolled in the *Bologna 2005* phase III clinical trial at our Center. By study design, after registration patients were randomized to receive three 21-days courses of induction therapy with either VTD (Bortezomib, 1.3 mg/m^2 on d 1, 4, 8, and 11, plus Dexamethasone, 40 mg on each day of and after Bortezomib administration plus Thalidomide 200 mg/d from d 1 to 63.) or TD (Thalidomide as in VTD and Dexamethasone 40 mg/d on d 1-4 and 9-12 of every 21-d cycle), prior to stem cell collection and double autologous stem cell transplantation. As of January 2008, 23 patients (16 male and 7 female, median age = 58 yrs) entered the substudy; of these, 13 and 10 patients were randomized in the VTD and TD arm, respectively. At diagnosis, both group of patients showed a marked increase in serum crosslaps (7433±2033pmol/L in the VTD arm and 12109±2549pmol/L in the TD arm) while both OC and BAP were reduced. After completion of the induction therapy, serum crosslaps were significantly decreased in both treatment groups (3030±463pmol/L in VTD arm, $p=0.04$; 4245±989pmol/L in the TD arm, $p=0.04$). In the TD group a significant further reduction in bone formation markers was also observed (49% reduction in serum OC and 20% in BAP, $p=0.03$ and 0.05 as compared to pre-treatment values); on the contrary, in the VTD arm both OC and BAP were not significantly decreased as compared to baseline values (13% and 9% reduction for OC and BAP, respectively). These data suggest that incorporation of Bortezomib into induction therapy counteracts the inhibitory effects of high-dose steroids on osteoblastogenesis, thus sparing bone formation. Evaluation of a larger series of patients will be presented at the meeting.

0660

THE BONE DEGRADATION MARKER CTX-I SHOWS UNIQUE PROPERTIES COMPARED TO NTX-I AND ICTP WHEN USED FOR CONSECUTIVE MEASUREMENTS IN PATIENTS WITH MULTIPLE MYELOMA

T. Lund1, J.M. Delaissé,[1] N. Abildgaard,[2] K. Kupisiewicz,[1] T. Plesner[1]

[1]Vejle Hospital, VEJLE; [2]Odense University Hospital, ODENSE, Denmark

Background. In multiple myeloma (MM) there is an uncoupling of bone resorption and formation as the myeloma cell induces factors stimulating osteoclast activity e.g. RANKL and inhibiting osteoblasts activity e.g. DKK-1. In turn these changes in osteoclast and osteoblast activity promote survival and proliferation of the myeloma cells, thereby creating a vicious cycle. Various markers of bone degradation have been tested for prognostic value in MM. A correlation has been found between elevated values and overall survival, disease stage and bone involvement. *Aims*. We have investigated if consecutive measurements of bone resorption markers applied to an unselected group of patients with MM could detect progressive disease, development of osteolysis and response to treatment in the individual patient. *Methods*. In 106 patients the bone resorption markers ICTP (CTX-MMP), NTX-I and CTX-I, as well as serum M-component and serum free light kappa/lambda chains (FLC), were measured every fourth week for up to 22 months. Disease progression and response to treatment were defined according to the International Uniform Response Criteria for Multiple Myeloma 2006. When analysing disease progression, bone marker values at plateau phase were compared to values when progressive disease was first detected. Development of osteolysis was evaluated either with conventional x-ray or computed tomography (CT). Patients who received new treatment were divided into responders and non-responders. Response was defined as partial response or better. Bone marker values prior to treatment start were compared to values when treatment was stopped or when best response during treatment was achieved. *Results*. Progressive disease was observed in 40 cases. Bone status was evaluated in 26 patients at the time of disease progression. A statistically significant increase in CTX-1 of 44 percent was found at the time of disease progression. Changes in serum creatinine were analysed to rule out that the increase in CTX-I was due to deterioration in renal function. No significant changes were observed in the levels of ICTP or NTX-I. Patients who developed osteolysis visible by X-ray or CT-scan had a statistically significant higher rise in CTX-I of 344 percent compared to patients who had no new osteolytic lesions. A new treatment was initiated in 50 cases, partial response or better was achieved in 33 cases. A significant decline in CTX-I of 52 percent was observed in responding patients. A sub-analysis was performed excluding patients for whom bisphosphonate treatment was changed during or up to one month prior to the analysed time period. A significant decline in CTX-I values of 36 percent was observed even in this sub-population. No significant changes in CTX-I were observed in the non-responding group. NTX-I and ICTP showed no significant changes, neither in the responding patients nor in the non-responding patients. *Summary*. Our data indicate that CTX-I may be a useful marker of disease progression, development of osteolysis and response to treatment in multiple myeloma. CTX-I appears to be a better marker, when using consecutive measurements to follow development in multiple myeloma, than the more widely studied markers NTX-I and ICTP.

0661

A SINGLE-CENTRE LONGITUDINAL STUDY ON 1003 MULTIPLE MYELOMA PATIENTS TO EVALUATE THE INCIDENCE, PRESENTING FEATURES AND PROGNOSIS OF EXTRAMEDULLARY MYELOMA

M. Varettoni, A. Corso, G. Pica, S. Mangiacavalli, C. Pascutto, M. Lazzarino

Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy

Background. Multiple myeloma (MM) is usually confined to bone marrow, but extramedullary (EM) spread may occur either at diagnosis or during the course of disease. In the last years, EM relapses have been increasingly reported following high-dose therapy (HDT) or treatment with thalidomide. However, no clinical studies have evaluated whether the incidence of EM myeloma has changed with the expanding use of transplant or biological agents. *Aims*. Aims of this study were: 1) to evaluate the overall incidence of EM involvement at diagnosis and during the follow-up in a large cohort of patients (pts); 2) to assess whether the incidence of EM myeloma has changed over time; 3) to compare the presenting features and prognosis of pts with and without EM disease. *Methods*. The clinical records of 1003 consecutive MM pts seen at our Institution over a 38-year period were reviewed. Pts were grouped into three periods of time: 1969-1993, period of conventional chemotherapy; 1994-1999, corresponding to the introduction of HDT; 2000-2007, the era of biological agents. *Results*. Overall, 132 out of 1003 pts (13%) had EM disease at diagnosis or later. The proportion of pts with EM involvement at diagnosis remained unchanged in the first two periods, while increased significantly after 1999 (8.9%, 8.4%, 20.1% respectively, $p<0.0001$).

Table 1. Presenting features of MM patients according to the presence of EM disease.

Characteristics at diagnosis	EM disease at diagnosis (132 pts)	No EM disease at diagnosis (871 pts)	P-value
Age (years), median (range)	58 (31-80)	60 (26-87)	0.03
Sex, M/F ratio	1.9	1.1	0.007
Prior MGUS, % of patients	18	38	<0.0001
Type of myeloma, % of patients			
IgG	60	65	NS
IgA	19	21	NS
IgM	1	<1	NS
IgD	1	<1	NS
Light chain	11	12	NS
Non secretory	8	1	0.0001
Type of light chain, k/λ ratio	1.1	1.8	0.02
Stage (Durie-Salmon), % of patients			
I	21	37	
II	9	15	<0.0001
III	70	48	
International Staging System (ISS), % of patients			
I	-	-	
II	89	77	NS
III	11	23	
Hemoglobin (g/dL), median (range)	12.7 (4-15.8)	10.6 (7-14.8)	NS
Serum M-protein (g/dL), median (range)	3.36 (0.5-11.4)	1.4 (0.1-12.3)	NS
Urine M-protein (g/L), median (range)	0.3 (0-4.3)	0.4 (0-30.8)	NS
Bone marrow plasma cells (BMPC)	35 (5-100)	40 (1-100)	NS
Number of lytic bone lesions			
none	25	52	
<3	14	14	<0.0001
≥3	61	34	
β_2-microglobulin (mcg/L)	2780 (1064-11700)	3560 (445-23600)	0.005
LDH (U/L)	314 (113-1188)	425 (169-7960)	NS

Table 1 shows the presenting features of pts with and without EM involvement. The median progression-free survival (PFS) of pts with EM disease at diagnosis was shorter as compared with the others (17.8 *vs* 51.6 months, $p<0.0001$), whereas the median overall survival (OS) was not statistically different (39.6 and 52.8 months respectively, $p=0.28$). During the follow-up, 57 out of 1003 pts (5.6%) developed one or more EM relapses (122 events). The incidence of EM relapses increased from 6.5x1000 person-year before 1994 to 15.4 between 1994 and 1999 ($p=0.055$), up to 48.4 after 1999 ($p=0.03$). The mean time from diagnosis of MM to EM relapse was significantly longer in the last period than in the previous two (34.6 *vs* 18.9 months, $p=0.03$). The incidence of EM relapses was not influenced by prior exposure to HDT, bortezomib or thalidomide/lenalidomide in uni- and multivariate analysis. Pts with EM disease were at risk to develop subsequent EM localizations (HR: 12). By time-dependent analysis, the presence of EM disease at any time in the course of the disease was associated with a significantly shorter OS (HR 3.6) and PFS (HR 3.2) ($p<0.0001$). *Conclusions*. The incidence of EM myeloma has significantly increased after 1999. The wider use of sensitive imaging techniques might explain the higher prevalence of EM involvement at diagnosis. The increase of EM relapses during follow-up might just reflect the prolongation of survival, as suggested by the longer time from diagnosis of MM to the appearance of EM disease observed in the last years. In this study, prior exposure to HDT and to novel agents seems not to increase the risk of EM progression. The analysis of presenting features of pts with EM myeloma shows a significant correlation with male gender, younger age, non secretory MM, advanced stage. The presence of EM disease confers a poor prognosis.

0662

ANALYSIS OF THE INVOLVED IGG? / IGG? RATIOS MAY GIVE A MORE SENSITIVE MEASURE OF RESPONSE TO TREATMENT IN MULTIPLE MYELOMA

J. Harding,[1] M. Drayson,[2] J. Hobbs,[3] G. Mead,[2] A. Bradwell[2]

[1]The Binding Site, BIRMINGHAM; [2]University of Birmingham, BIRMINGHAM; [3]The Binding Site Ltd, BIRMINGHAM, UK

Background. Measurement of monoclonal (M) protein by densitometry has been the preferred method of response assessment in patients with multiple myeloma (MM). The production of specific polyclonal antibodies which recognise epitopes spanning the junction of the heavy and light chains of the immunoglobulin has allowed us to develop sensitive nephelometric immunoassays on the Dade Behring BN™II analyser which can determine the serum ratios IgGκ / IgGλ. *Methods.* IgGκ / IgGλ ratios were measured in 109 normal (blood donor) sera to generate a normal range. Total IgG (Dade Behring) was measured on all normal and clinical samples. The MM sera analysed were archived samples collected in the VIIth UK Medical Research Council myeloma trial and sera collected from Heartlands Hospital (Birmingham, UK). Presentation samples were analysed from 20 patients (10 IgGκ /10 IgGλ), with serial sample analysis being completed on 9(4 IgGκ /5 IgGλ) patients through the course of their disease. *Results.* The sum of the IgGκ + IgGλ measurements correlated well with total IgG in normal (Pearsons Correlation 0.8 $p<0.01$) and monoclonal disease sera (Pearsons Correlation 0.74 $p<0.01$). In the multiple myeloma patients all 25 presentation sera that had a positive immunofixation (IFE) had elevated concentrations of the relevant immunoglobulin and an abnormal IgGκ / IgGÏ ratio. For the 9 patients followed through the course of their disease, in all cases the changes in IgGκ / IgGλ ratio reflected the clinical assessments. Four of the 9 did not achieve complete response (CR) and the ratio remained abnormal throughout. In 3 out of the remaining 5 patients relapse from CR was indicated by a change in ratio earlier than by serum protein electrophoresis or IFE. *Conclusions.* This preliminary data indicates it is possible to type monoclonal immunoglobulins using IgGκ /I gGλ ratios. Furthermore, the agreement of the summation indicates it is possible to accurately measure IgGκ and IgGλ in normal and disease-state sera. Analysing the IgGκ / IgGλ ratio gives a more sensitive indication of relapse in some patients.

0663

HYPOADIPONECTINEMIA IS ASSOCIATED WITH MULTIPLE MYELOMA RISK

M. Dalamaga,[1] A. Lekka,[2] M. Triantafilli,[2] K. Karmaniolas,[2] A. Hsi,[3] A. Panagiotou,[4] C. Dimas,[4] C. Mantzoros[5]

[1]Athens University Medical School, ATHENS, Greece; [2]NIMTS General Hospital, ATHENS, Greece; [3]Beth Israel Medical center, BOSTON, USA; [4]Attikon General University Hospital, ATHENS, Greece; [5]Beth Israel Deaconess Medical Center, BOSTON, USA

Background and Aims. Accumulating evidence supports a role for obesity in the etiology of multiple myeloma (MM). The distinct possibility exists that obesity may be linked to MM through altered adipokine secretion and circulating levels, one of which, adiponectin, has a protective role in several malignancies, including leukemia. In this case-control study we investigated the role of serum adiponectin in the etiopathogenesis of MM and we explored its association with several established prognostic factors. *Methods.* Seventy three patients with incident, histo-

logically confirmed MM and 73 hospital controls admitted for non-neoplastic and non-infectious conditions and matched on gender and age were studied between 2001 and 2007, and blood samples were collected. Serum adiponectin and leptin concentrations were measured using ELISA (Avibion Human Elisa, Orgenium Laboratories, Helsinki, Finland) as well as MM prognostic parameters such as C-Reactive Protein (CRP), LDH, calcium, β-2 microglobulin and erythrocyte sedimentation rate were determined. Statistical analysis of the data was performed using univariate and multivariate analyses with SAS 9.1 for Windows XP. *Results.* Cases presented significantly higher height, weight and BMI as well as higher levels of serologic prognostic parameters of MM than control subjects. Cases had significantly lower serum levels of adiponectin and high molecular weight adiponectin than controls ($p<0.001$). Hypoadiponectinemia was associated with higher risk of MM by bivariate analysis and after adjusting for age, gender, BMI and serum levels of leptin ($p<0.0001$). Among cases, only leptin tended to be positively associated with CRP, LDH and calcium, and negatively with adiponectin. No significantly different adiponectin levels were found among different paraprotein classes and among different MM stages. *Conclusions.* Adiponectin, an endogenous insulin sensitizer, may have a protective role in MM, whereas leptin is not associated with risk for MM. Further studies are needed to confirm these associations and to explore the mechanisms underlying adiponectin's role in MM and plasma cell dyscrasias.

0664

THYROID AUTOIMMUNITY AND THE OCCURRENCE OF MULTIPLE MYELOMA

M. Dalamaga,[1] M. Triantafilli,[2] K. Karmaniolas,[2] N. Pelekanos,[3] G. Sotiropoulos,[2] A. Lekka[2]

[1]Athens University Medical School, ATHENS; [2]NIMTS General Hospital, ATHENS; [3]Attikon General University Hospital, ATHENS, Greece

Background and Aims. Thyroid disease has been associated with lymphoma and leukemia. No previous study using clinical and laboratory data has explored whether thyroid disease and in particular autoimmune thyroid disease (ATD) is associated with multiple myeloma (MM) risk. In this case-control study design, we investigated the association of ATD with the occurrence of MM. *Methods.* Seventy three patients with incident, histologically confirmed MM and 73 hospital controls admitted for non-neoplastic and non-infectious conditions and matched on gender and age were studied between 2001 and 2007, and blood samples were collected. All subjects were submitted to clinical, ultrasound thyroid evaluation and serum thyroglobulin and thyroperoxidase antibodies determination using electro-chemiluminescence immunoassay intended for use on Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, USA). MM prognostic parameters such as C-Reactive Protein (CRP), LDH, calcium, β-2 microglobulin and erythrocyte sedimentation rate were also determined. Statistical analysis of the data was performed using univariate and multivariate analyses with SAS 9.1 for Windows XP. *Results.* In univariate analysis, the prevalence of clinical thyroid disease in MM patients was significantly higher than that in controls ($p=0.002$). Specifically, the prevalence of ATD (Hashimoto's thyroiditis and Graves' disease) was significantly higher in MM cases than in controls ($p=0.021$). There was statistically significant evidence that diagnosis of ATD was associated with increased risk of MM, adjusting for age, gender, body mass index and familial history of lymphohematopoietic cancer (OR=5.68, 95%C.I.: 1.69-19.13). Interestingly, controlling for the above variables, an individual suffering from ATD more than ten years has about 2.41 times more likely the risk to develop MM than an individual without any ATD (OR=2.41, 95% C.I. 1.35-4.29). Finally, controlling for the aforementioned variables, presence of serum thyroid antibodies was associated with increased risk of MM. *Conclusions.* Biological plausibility and empirical evidence highlights the importance of ATD in the occurrence of MM. Further studies are needed to explore underlying mechanisms associating thyroid autoimmunity with plasma cell dyscrasias.

0665

THE ROLE OF PARAPROTEIN ON PLATELET DISFUNCTION

D.I. Djunic,[1] E.I. Elezovic,[1] M.N. Milosevic-Jovcic,[2] I.V. Ilic,[2] S.N. Suvajdzic-Vukovic,[1] B.J. Bila,[1] V.A. Vidovic,[1] A.D. Antic,[1] T.D. Tomin[1]

[1]Institute of Haematology, Clinical Center of Serbia, BELGRADE; [2]Institute for Medical research, BELGRADE, Serbia

Background. Some patients with monoclonal gammopathies (MG) have haemostatic disorders at presentation, and arising mechanism is not completely explained. It is more frequently belived to be in connection with paraprotein. *Aims.* The aim of this study was proved by investigation with coagulation tests the incidence of haemostatic disorders at patients with MG, and to estimated with platelet aggregation tests, the role of paraprotein on disturbance of platelet function at healthy donors, *in vitro*. *Methods.* In this study where included 48 patients with MG. Initially coagulation tests and investigation of platelets adhesion on glass pearls, platelets aggregation induced with ADP, collagen (COL), ristocetin (RIS) and epinephrine (EPI) where done. Paraprotein has been separated by Rivanol method from serum of 10 patients (9 with myeloma multiplex and 1 with MGUS), who had decreased platelet aggregation on used inducers: ADP, COL, RIS and EPI, at presentation. Platelet aggregation in platelet rich plasma (PRP) was measured at 10 healthy donors before and after addition of paraprotein isolated from the patients with MG, induced by same inducers. The indentical test was repeted with addition of human immunoglobulins for intravenous used in PRP at 10 haelthy donors. We measured latent time in seconds and maximal platelet aggregation in percent for all inducers. *Results.* Platelets adhesion was disturbed at one half of patients, and platelets aggregation at one third. Platelets aggregation was normalized together with disappearing of paraprotein during a treatment. When patients attained remission, their platelets aggregation was normalized. Paraprotein isolated from serum of these patients inhibited platelets aggregation of healthy donors. Average of maximal levels of platelet aggregation has been significantly decreased in PRP of healthy donors after addition of paraprotein when used inducers: ADP ($p=0.007$), COL ($p=0.008$), RIS ($p=0.001$), and EPI ($p=0.002$). Average of latent time of platelet aggregation was significantly prolonged in healthy donors after addition of paraprotein with inducers: COL ($p=0.008$), RIS ($p=0.008$) and EPI ($p=0.006$). Average of latent time of platelet aggregation was not significantly prolonged after induction by ADP ($p=0.168$). In comparison, when human immunoglobulins added in PRP of healthy donors, maximal platelet aggregation and latent time were not significantly changed. *Conclusions.* These invastigations have proved that paraprotein leads to haemostatics disorder at patients with MG. Paraprotein isolated from patients with MG, who had decrease platelet aggregation at presentation, significantly decreased platelet aggregation when was added in PRP of healthy donors, *in vitro*. This is confirmed with addition of human immunoglobulins in PRP of haelthy donors, thus, platelet aggregation was not significantly changed. Further investigation is necessary.

0666

SUCCESFUL PREVENTION OF OSTEONECROSIS OF THE JAW DURING ZOLENDRONAT USE IN THE PATIENTS WITH MULTIPLE MYELOMA

R. Hajek, L. Pour, Z. Adam, M. Krejci, A. Krivanova, L. Zahradova, V. Sandecka

Masaryk university Brno, Faculty hospital, BRNO, Czech Republic

Background and Aims. Bisphosphonates are non-metabolized pyrophosphate analogues which inhibit osteoclastic activity. Bisphosphonates containing nitrogen have been recently associated with osteonecrosis of jaw (ONJ). It is defined as three month non healing defect in a jaw, usually in mandibule. In multiple myeloma patients the incidence of ONJ is the highest in all cancer type.. Most cases of ONJ is associated with zolendronate and pamidronate use. ONJ develops usually after some stomatological procedure, mostly teeth extraction. We made precautions which was aimed to decrease incidence of ONJ in our routine daily praxis. *Methods.* Total of 43 MM patients were treated with zolendronate with 480 infusions in 2006. Similar number of patients (41) was treated in 2007 with total of 465 applied infusion of zolendronate. Zolendronate was administrated in common dosage schedule 4mg intravenously every month. We retrospectively analysed ONJ incidence during zolendronate use in myeloma patients in 2006. In 2007, an ONJ Preventive Program (ONJ PP) was activated in our department and we reevalauted effectivity of this ONJ PP after one year. The ONJ PP consists from 5. measurments: 1. Stomatological examination before zolendronate treatment, including X-Ray examination; 2. Interruption of zolendrante use two months before planned teeth extraction or other stomatosurgery, and resumption of this treatment two month after jaw is completly healed; 3. Antibiotic prophylaxis with amoxicillin/clavulanate 1g p.o. 2 times per day for 14 days if teeth extraction is made; 4. Regular chlorhexidine use during period after teeth exctraction. *Results.* Together four cases (4/43; 9,3%) of ONJ we monitored in our patients during 2006. All of these patients have used zolendronate for more than one year (median 13 months; range12-36 month). ONJ developed after forgoing teeth exctraction in all cases. None case of ONJ was reported

in 2007(0/41) after precautions was established. Five teeth extraction were planned in patients treated with zolendronate with median of 14 months. These patients had stopped zolendronate treatment and they used ONJ PP as recommended. Neither any case of ONJ was not developed in this patients. Incidenence of ONJ after precautionts establishing statisticaly significantly decrease compare to period when these precautions was not used (p=0,003). *Conclusions*. ONJ is common and dangerous complication in multiple myeloma patiens treated with zolendronate. Its incidence rapidly increasing during time of therapy. Our data confirm that ONJ developing usually after one year of the treatment. Any procedure attacking bone seems to be key risk factor for ONJ formation. If zolendronate use is stopped before teeth extraction and antibiotic prophylaxis is used incidence of ONJ is rapidly decreased and the risk becomes acceptable for patients.

0667

BISPHOSPHONATES (BP) RELATED OSTEONECROSIS OF THE JAW (ONJ): A LONG TERM FOLLOW UP (FU) OF A SERIES OF 35 CASES OBSERVED BY GISL

S. Pozzi,[1] R. Marcheselli,[2] S. Sacchi,[2] L. Baldini,[2] F. Angrilli,[2] S. Falorio,[2] G. Quarta,[2] C. Stelitano,[2] G. Caparotti,[2] S. Luminari,[2] A. Falcone,[2] D. Natale,[2] Ch. Broglia,[2] A. Cuoghi,[3] D. Dini,[3] P. Ditonno,[2] G. Leonardi,[3] G. Pianezze,[2] V. Pitini,[2] G. Polimeno,[2] L. Ponchio,[2] L. Masini,[2] M. Maurizio,[2] M. Spriano,[2] P. Musto[4]

[1]Università di Modena e Reggio Emilia, MODENA; [2]GISL, MODENA; [3]Dipartimento di Oncologia ed Ematologia, MODENA; [4]Ematologia e Trapianto Cellule Staminali, CROB Centro Riferimento Oncol Basicata, RIONERO IN VULTURE (POTENZA), Italy

Background. In 2007 we published a review of 35 cases of BP-associated ONJ observed in cancer patients during a multicenter study performed by the GISL (Gruppo Italiano Studio Linfomi e Mielomi) in the period 2002-2005. Our study strongly suggested an association between the use of BP and the occurrence of ONJ, although we were unable to identify any definitive risk factors with a retrospective study. The most frequently ONJ-associated clinical characteristics were chemotherapy treatment, advanced age, female sex, anemia, parodonthopaties/dental procedures and thalidomide (in the case of MM patients). *Aims.* To update the FU of these 35 patients, evaluating ONJ evolution and the interference with the quality of life. *Methods.* We asked to the 14 centers that participated in the previous study, and that reported cases of ONJ, to up-date the status of the primary disease, the evolution of ONJ, and the quality of life of their pts. *Results.* Five patients were lost to FU. Among the remaining 30 pts, 25 were affected by multiple myeloma, and 5 by other type of neoplasia. Nineteen pts are alive (63%) and 11 patients (37%) died for progression of the primary disease. In the deceased pts the follow-up referred to the status of the ONJ just before the decease. Twenty-two are females, 8 are males with a median FU of 30 months since the diagnosis of ONJ for all patients and a median FU of 34 months for alive patients. In one patient (3%) ONJ resolved, in 11 patients (37%) the lesion is stable, and in 13 cases (43%) the lesion improved, as a result of one or more procedures. Five patients (17%) showed progression of the lesion: in 4 cases due to a fistula and in 1 case of local infection. No recurrence of the event has been reported. ONJ interfered with the ability of eating in 13 pt (43%) determining an impairment of quality of life. In 29 out of 30 (97%) BP has been suspended indefinitely, and only in 1 case the pt went on with the treatment after the diagnosis of ONJ. *Conclusions.* In our population ONJ showed a various range of evolution: in the majority of the cases it was stable or even improved or healed (37%,43% and 3% respectively), but even if rarely it evolved in a even worst complication like fistula and local infection. No cases of recurrence has been reported. The complication doesn't seem to interfere with the survival of the pts, and all patients deceased for progression of the primary disease. ONJ interfere with the quality of life in particular because the lesion reduce the ability of eating. The large majority of treating physicians preferred to indefinitely discontinue BP administration regardless of the bone involvement and this could explaining why we did not observe any recurrence.

0668

NOVEL NEPHELOMETRIC IMMUNOASSAYS FOR THE SENSITIVE DETECTION OF IGA MONOCLONAL GAMMOPATHIES IN MULTIPLE MYELOMA AND AL AMYLOIDOSIS

J. Harding,[1] M. Drayson,[2] H. Lachmann,[3] P. Hawkins,[3] J. Hobbs,[4] G. Mead,[2] A. Bradwell[2]

[1]The Binding Site, BIRMINGHAM; [2]University of Birmingham, BIRMINGHAM; [3]The Royal Free Hospital, LONDON; [4]The Binding Site Ltd, BIRMINGHAM, UK

Background. Specific polyclonal antibodies have been produced which recognise conformational epitopes spanning the junction of the heavy and light chains of the immunoglobulin molecule. Here we describe automated, nephelometric immunoassays on the Dade-Behring BNTMII analyser, which can determine the serum IgAκ / IgAλ ratios and evaluate the utility of measuring these ratios in multiple myeloma (MM) and AL amyloidosis. *Methods.* IgAκ / IgAλ ratios were measured in 118 normal (blood donor) sera to generate a normal range. Total IgA (Dade Behring) was measured on all normal and clinical samples. The MM sera analysed were archived samples collected in the VIIth UK Medical Research Council myeloma trial. 20 IgA (10 IgAκ / 10 IgAλ) patient sera were analysed at presentation, with serial sample analysis being completed on 5 patients (4 IgAκ /1IgAλ). In addition, 17 IgA immunofixation (IFE) positive presentation sera from patients attending the National Amyloidosis Centre (Royal Free and University College Medical School, London) were analysed. *Results.* IgAκ+IgAλ summation correlated well with total IgA in normal (Pearsons Correlation 0.9 p<0.01) and monoclonal disease sera (Pearsons Correlation 0.96 p<0.01). In the multiple myeloma patients, all 26 presentation sera had elevated concentrations of the relevant IgA immunoglobulins and abnormal IgAκ / IgAλ ratios. In all 5 patients with serial samples, the change in IgAκ / IgAλ ratio reflected the course of the disease. In 2 of the 5 patients the ratios indicated residual disease when IFE results were negative. For 1 patient, the monoclonal immunoglobulin was obscured by other proteins after serum protein electrophoresis (SPE) but the disease course could be monitored throughout by changes in the IgAκ / IgAλ ratio. In a second patient, the IgAκ / IgAλ ratio indicated progression 596 days earlier than SPE or IFE. In the 17 IgA IFE positive amyloid patients it was possible to quantify monoclonal bands in 8/17 by SPE. All of the 8 patients with quantifiable M protein and 7/9 with non quantifiable M protein had abnormal IgAκ / IgAλ ratios. Normal serum free light chain measurements were recorded for 3 out of the 17 patients, but in all 3 cases the IgAκ / IgAλ ratios were abnormal. *Conclusions.* The agreement of the summation indicates it is possible to accurately measure IgAκ and IgAλ in normal and diseased sera. Measurement of IgAκ / IgAλ ratios allowed accurate quantification of monoclonal IgA immunoglobulins even when the immunoglobulin was obscured by other proteins on SPE. The assays provided data concerning the monoclonal protein concentration when the SPE gels were negative and in some instances, a more sensitive indication of residual disease than IFE. Finally, in AL amyloidosis patients with normal FLC ratios, measurement of involved and uninvolved intact immunoglobulins might offer a sensitive, quantitative alternative for disease monitoring.

0669

FAMILIAL B-CELL LYMPHOPROLIFERATIVE DISEASES: HYPER-RESPONSIVE B-CELLS IN UNAFFECTED FAMILY MEMBERS

H. Steingrimsdottir,[1] H.K. Einarsdottir,[2] V. Haraldsdottir,[1] H.M. Ogmundsdottir[2]

[1]Landspitali University Hospital, REYKJAVIK; [2]University of Iceland, REYKJAVIK, Iceland

Background. In Iceland eight families have previously been identified with multiple cases of monoclonal gammopathies (MG) and other lymphoproliferative diseases (Õgmundsdottir *et al.* 2005). One of these families with several cases of Monoclonal Gammopathy of Undetermined Significance (MGUS), Multiple Myeloma (MM) and Waldenströms Macroglobulinemia (WM) was first reported in 1978. Subsequent studies revealed that one third of disease-free family members showed a phenotype described as hyper-responsive B-cells on the basis of significantly increased production of immunoglobulins (IgA, IgG and IgM) following *in vitro* stimulation with poke weed mitogen. These eight families have now been traced further, compared with the Icelandic Cancer Registry and family members above 20 years screened for the presence of paraproteins by protein electrophoresis, immunofixation and serum

light chain analysis (Steingrimsdottir *et al.*, abstract IMW in Kos 2006). This revealed a total of 31 new cases of lymphoproliferative disorders. *Aims.* The aim of this study was to screen for the hyper-responder phenotype in these eight families on the basis of increased immunoglobulin production by cultured lymphocytes. *Methods.* Family members were selected on the basis of results of the recently conducted screening for MG. One control was selected for each case matched for age and gender. Peripheral blood samples were collected and mononuclear cells isolated by ficoll/hypaque centrifugation. Peripheral blood mononuclear cell cultures were tested for B-cell hyper-responsiveness by stimulation with poke weed mitogen. Culture supernatants were assayed for IgG and IgM on days 2-4-6-8-10-12-14 by ELISA. The proportion of T cells (total $CD3^+$, and $CD4^+$ and $CD8^+$) and B-cells ($CD19^+$, $CD19^+/CD27^+$, $CD19^+/CD86^+$, $CD19^+/32^+$, $CD19^+/95^+$) was estimated by flow cytometry. Hyper-responders were defined as those with IgG/IgM production more than 2 SD from mean for controls. *Results.* Of the 73 1° and 2° relatives of known cases with lymphoproliferative diseases who were invited to participate, 53 accepted. Of those 39 were from the previously studied family No 8. A total of 11 hyper-responders were idendified. Nine of these hyper-responders were from family no 8, of wich 5 had not previously been detected and 4 were known hyper-responders. No statistically significant differences were detected in the proporations of B-cell sub-populations between hyper-responders and controls or members of family No 8 and controls. In family No 8, cases with lymphoproliferative diseases and hyper-responders form clusters within the pedigree. *Conclusions.* Our results so far have confirmed a functional phenotype (B-cell hyper-responsiveness) in a previously defined family with multiple cases of MGUS/WM/MM and defined further subjects and cases within that family. In two of the seven remaining families the hyper-responsive phenotype was detected in two individuals possibly indicating that this phenotype is related to an underlying cause for familial gammopathies in a subset of such families. This needs to be confirmed by further screening of members of families Nos 1-7 and this work is in progress.

0670

ANALYSIS OF DISTINCT MULTIPLE MYELOMA SUBGROUPS BASED ON BIOLOGICAL CHARACTERISTICS, BONE MARROW MICROENVIRONMENT AND INTERNATIONAL STAGING SYSTEM

J. Bila, I. Elezovic, M. Perunicic, N. Suvajdzic-Vukovic, M. Todorovic, D. Tomin, A. Vidovic, B. Mihaljevic, M. Gotic, D. Boskovic

Institute of Hematology, BELGRADE, Serbia

Background. At present, multiple myeloma (MM) can be considered as a group of disease states based on varying biological behavior. The recently introduced International Staging System (ISS) was developed and validated, providing consistent risk distinction of MM patients (pts). The aim of study was to analyze different MM subgroups in accordance to a distinct laboratory parameters, ISS score and immunohistochemical characteristics of the bone marrow microenvironment, as a surrogate markers of the MM activity. Patients and *Methods.* The study included 60 newly diagnosed MM pts (33 male/27 female, mean age 60yrs, range 35-75). IgG myeloma was diagnosed in 35pts, IgA in 12pts, light chains in 12pts, and non-secretory in 1 pts. According to the clinical stage (CS, Durie&Salmon), patients were distributed as follows: I 8pts; II 24pts; III 30pts. Regarding ISS score, the group included: ISS1 19pts; ISS2 12pts; ISS3 30pts. Renal impairment existed in 16pts. All patients were treated with conventional chemotherapy. Immunohistochemical expression of CD34 as a marker of visualization of microvessel density (MVD); FGFR-3; gp130; VCAM; RANK; and Ki-67 was analyzed on the samples of BM biopsies and graded as weak; moderate; and strong. *Results.* Analysis of biological characteristics of MM pts indicated significant association of renal impairment with III CS; and light chains MM with ISS3 (p<0,004). Most of the pts with ISS3 were in III CS (p<0,002). Although there was no difference regarding the response rate and CS or ISS score (p>0,05), the duration of response was significantly longer in pts with ISS1 *vs* ISS3 (14,4m *vs* 10,1m; p<0,05). Likewise to a shorter overall survival of pts in III CS *vs* I CS (21m *vs* 47m; log rank 6,45; p<0,039), the overall survival of the pts with ISS3 was significantly shorter in comparison to the pts with ISS1 (18m *vs* 39m; log rank 18,31; p<0,0001). Regarding the MM type, pts with IgA MM had a significantly shorter duration of response (11,2m; F 2,910; p<0,05), followed by a shorter survival as well (18,5m; F 3,666; p<0,016). BM microenvironment of pts with ISS3 was distinguished by the strong expression of MVD (p<0,039); FGFR-3 (p<0,025); VCAM (p<0,05); RANK (p<0,028); and Ki-67 (p<0,05). There was no correlation between the ISS score and level of gp130 expression (p>0,05). A strong expression of FGFR-3 (p<0,05); gp130 (p<0,05); RANK (p<0,05); and Ki-67 (p<0,028) existed in III CS. *Conclusions*. A complete assessment of biological characteristics of disease, including ISS score as a marker of MM activity in a correlation to the functional interplay of BM microenvironment, distinguish MM subgroups of different predictive value on course of disease and possible therapeutic targets.

0671

THE INCIDENCE OF JAW OSTEONECROSIS IN MULTIPLE MYELOMA PATIENTS TREATED WITH BISPHOSPHONATES

M. Kraj, R. Poglod, M. Maj, K. Owczarska

Institute of Hematology and Transfusion Medicine, WARSAW, Poland

Background. Osteonecrosis of the jaw (ONJ) has recently been identified as complication in bisphosphonate-treated patients. In a published series the incidence of ONJ in MM patients treated with aminobisphosphonates ranged from 3% to 11% (Badros *et al.* J. Clin Oncol 2006; 24: 945-952; Bamias *et al.* J. Clin Oncol 2005; 23: 8580 - 8587; Zervas *et al.* Br J Haematol 2006; 134: 620 - 623). The aim of the study was assessment of occurrence of jaw osteonecrosis in 113 multiple myeloma (MM) patients treated with different bisphosphonates which have been evaluated in prospective study performed at our institution. *Methods.* Sixty one MM patients received clodronate per os 2,4g/24 hrs. Median treatment duration amounted to 17 months and in 14 patients treatment duration exceeded 24 months. Forty six MM patients received pamidronate intravenously 60 mg monthly. All 46 patients included in pamidronate study were followed up until death or at least for 6 years. Six MM patients received zoledronic acid intravenously either 4 or 8 mg every 3 to 4 weeks for 13 months. *Results.* No patient treated with clodronate or pamidronate experienced jaw osteonecrosis. Osteonecrosis of the mandible developed in 2 of 6 patients treated with zoledronic acid. In both cases treatment with zoledronic acid lasted 13 months and cumulative zoledronic acid dose was 72 mg in one and 144 mg in the other case. The lesions were refractory to conservative debridement, surgery and antibiotic therapy. These 2 cases were subject of separate publication (1. Kraj *et al.* Acta Haematologica Polonica 2004; 35: 227-241, 2. Kraj *et al.* Nowotwory Journal of Oncology 2006; 56: 140-143). *Conclusions.* Clinicians should be aware of the potential serious complication of bone necrosis in MM patients receiving long-term treatment with potent bisphosphonates. It is recommended a dental examination to identify and correct predisposing conditions before bisphosphonate treatment is started. On the basis of our findings and literature review (Kraj Acta Haematologica Polonica 2007; 38 suppl 1: 26-39), including Luigi *et al.* guidelines we suggest that 1) the most common clinical presentation of ONJ is pain and exposed bone of the mandible or maxilla; 2) for patients who develop ONJ, conservative, non-surgical treatment is strongly recommended; 3) clinical dental exmination and panoramic jaw radiograph should be performed before the onset of bisphosphonate therapy; 4) dental treatment and other oral procedures should be completed before initiating bisphosphonate therapy; 5) patients should be informed and instructed on the importance of maintaining good oral hygiene and undergoing regular dental assessment; 6) the medical community needs to be aware of the association between bisphosphonate usage and ONJ so that unnecessary and harmful surgical procedures could be avoided; and 7) we suggest discontinuation of aminobisphosphonate therapy whenever it is possible.

0672

INCIDENCE OF LIGHT CHAIN ESCAPE IN UK MRC MYELOMA VII TRIAL

A.R. Hobbs,[1] K. Sharp,[1] S. Harding,[1] M. Drayson,[2] A.R. Bradwell,[2] G.P. Mead[2]

[1]The Binding Site, BIRMINGHAM; [2]Division of Immunity and Infection, University of Birmingham, BIRMINGHAM, UK

Background. For some intact immunoglobulin Multiple Myeloma (MM) patients in remission, relapse is accompanied by a marked rise in monoclonal free light chains (FLCs) with no associated increase in intact immunoglobulin concentrations - a phenomenon termed *light chain escape (LCE)*. Recent case reports have suggested LCE might be more prevalent with modern chemotherapy and detected earlier by serum FLC analyses. *Aims.* To compare the frequency of LCE in MM patients with IgG *vs* IgA paraproteins and treated on intensive *vs* non-intensive chemotherapy regimens. *Methods.* Stored sera from the first 60 IgA and 58 IgG patients recruited to the Myeloma VII trial (randomised between intensive and non-intensive chemotherapy) were utilised. There were sufficient frozen sera and complete data for 36/60 IgA and 30/58 IgG MM patients. Representative sera from presentation, maximum response and relapse were utilised for sFLC measurement and results compared with recorded urine FLC (urine FLC/creatinine ratio) and serum intact immunoglobulin measurements. Results were classified as *true* LCE (rising sFLC concentrations with stable or falling intact immunoglobulin concentrations) or *partial* LCE (the increase in the involved serum FLC concentration was at least 40% greater than the increase in monoclonal intact immunoglobulin concentration). *Results.* IgA patients showed 8% (3/36) with true LCE and 11% (4/36) with partial LCE. For IgG patients the figures were 3% (1/30) and 10% (3/30) respectively. Of patients showing true LCE, 4/4 had received non-intensive treatment and of patients with partial LCE, 3/7 had been treated non-intensively. For all 11 patients showing some form of LCE, this was corroborated by the urine results in 5/11. For 6/11 the amounts of FLC in the urine were insufficient for consistent analysis. *Summary and Conclusions.* The results from this study support the use of the serum FLC assay for monitoring intact immunoglobulin MM patients to detect LCE. True LCE was seen in 3/36 IgA MM patients and 1/30 IgG MM patients. These preliminary findings do not indicate any greater frequency of LCE with intensive chemotherapy but suggest that it might be more apparent with serum FLC analysis compared with UBJP analysis.

0673

A RANDOMISED CONTROLLED TRIAL OF AN EDUCATIONAL BOOKLET FOR MULTIPLE MYELOMA PATIENTS WITH PERIPHERAL NEUROPATHY

H.L. Clarke, R. Gillibrand

University of the West of England, BRISTOL, UK

Background. As overall survival improves in multiple myeloma (MM) patients, quality of life and symptom management are becoming paramount. Peripheral neuropathy (PN) is a debilitating side effect of MM and its treatment but can be managed by monitoring symptoms and changing medication. *Aims.* To improve patient understanding and participation in the management of PN in MM patients using an education booklet. *Methods.* MM patients diagnosed for at least 1-year were randomised (1:1:1) to three treatment groups (recruitment target of 60 patients per group); Group 1 (control) received no information on PN. Group 2 and 3 received an educational booklet designed specifically for this research project providing in-depth information on PN. Questionnaires were completed at 0, 4, 8 and 12-months to assess impact of educational booklet. Groups 1 and 2 completed the questionnaires themselves and group 3 had the questionnaire completed as part of a telephone interview. *Results.* To date, 58 patients have consented to participate and baseline results for the first 40 patients are presented (15 group 1, 15 group 2, 10 group 3). Baseline characteristics were not significantly different between treatment groups. Patients were male (65%) and age >60years (70%). Year of diagnosis ranged from 1991-2006 and 58% received >3 prior lines of therapy, with 35% actively receiving MM treatment. Three quarters of the patients raise the issue of side effects with 42% discussing this at every hospital visit whilst 20% wait for the doctor to discuss the subject. The subject of side effects is primarily discussed with the doctor but 76% also discuss the subject with a nurse. 96% of patients are still experiencing symptoms of PN after they have completed their treatment (56% >1 year ago). Twice as many patients reported that PN affected their feet and legs compared to their hands and arm, however 53% experienced PN symptoms in all limbs. PN affects patients' walking distances (71%), lifting things (56%), gardening (54%), getting exercise (50%), and climbing stairs (43%). Almost half of the patients (47%) found it a lot more or somewhat more difficult to be optimistic or hopeful about their progress and survival when they have PN. Treatment recommendations included vitamin B12/folates (20%), painkillers (25%), and gabapentin (15%) but 57% of patients were given no treatment for their PN. The majority of patients correctly identify symptoms associated with PN but a proportion of patients wrongly believe that shortness of breath, vomiting, headache and dizziness are symptoms of PN. 10% believe that PN is reversible and a further 53% reported that the reversibility of PN depends on the MM treatment that caused the PN. Consequently, 37% of patients stated that PN is not reversible and once you have the symptoms that they will not disappear. *Summary and Conclusions.* Baseline results show that MM patients have a relatively high level of knowledge of the symptoms but not the management of PN. PN is impacting on the patients lives and it is not being managed effectively either through modification of the patients MM therapy or additional treatment recommendations.

0674

PLASMA HOMOCYSTEINE AND METHYLMALONIC ACID CORRELATE TO RENAL FUNCTION AND NOT TO PLASMA COBALAMIN (VITAMIN B12) IN MULTIPLE MYELOMA PATIENTS

N.E.U. Hermansen,[1] U. Malm,[2] P. Gimsing[3]

[1]Rigshospitalet, COPENHAGEN, Denmark; [2]Dept. Paediatrics, Kristianstad Central Hospital, KRISTIANSTAD, Sweden; [3]Dept. Haematology, Copenhagen University Hospital Rigshospitalet, COPENHAGEN, Denmark

Background. The classical signs of cobalamin (vitamin B12) deficiency - anaemia and neuropathy - are also serious complications in multiple myeloma, occurring frequently at diagnosis and during treatment. Screening and monitoring for cobalamin deficiency is therefore an essential part of work-up and follow-up in multiple myeloma. Cobalamin deficiency can be difficult to diagnose. Serum or plasma cobalamin levels can remain normal despite clinical signs of deficiency or may be low without cobalamin deficiency due to decreased binding protein (low haptocorrin). A more sensitive estimate of cobalamin status can be made by measuring the two metabolites that accumulate in the event of deficiency: plasma total homocysteine (tHcy) and plasma methylmalonic acid (MMA; methylmalonate). However, the validity of these two analyses are debatable on the grounds of low specificities: both increase with reduced renal function, which is frequently seen in multiple myeloma.

Besides, elevated tHcy is associated with such diverse factors as life style (smoking, alcohol, coffee), deficiencies in folate and pyridoxin (vitamin B6), and individual genetic traits. Additional analyses regarding cobalamin status exist, but reduced availability, high price and/or lack of thorough validation prevent general usage. Accordingly, the diagnosis of cobalamin deficiency can sometimes only be ensured ex juvantibus through the administration of cobalamin. (Solomon LR. Blood Rev 2007 May;21(3):113-30.)

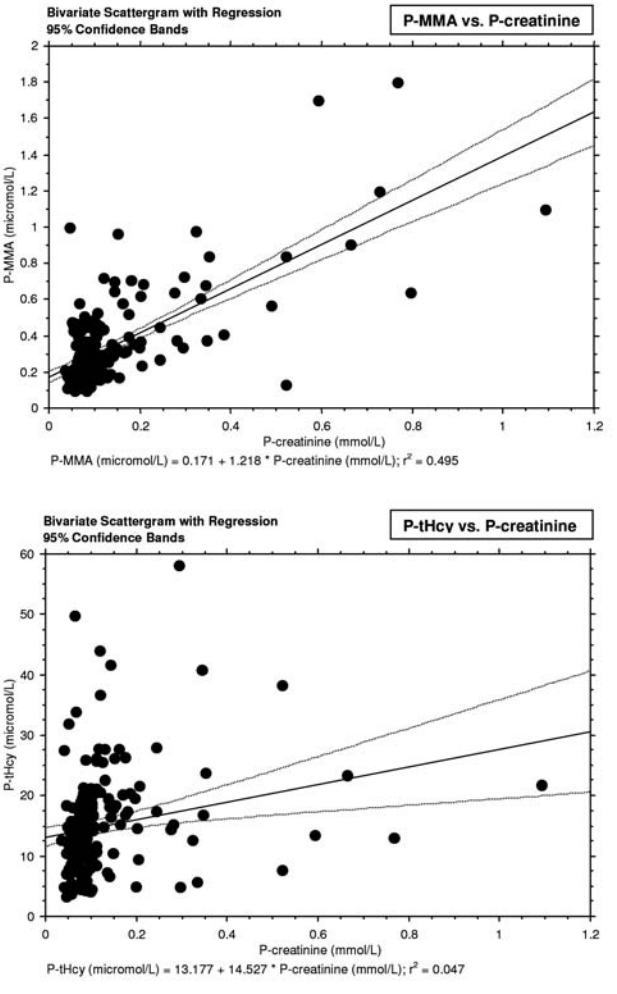

Figure 1. P-MMA and P-tHcy (resp.) vs P-creatinine

Aims. To investigate the value of tHcy and MMA for diagnosing cobalamin deficiency at the time of diagnosis in multiple myeloma. *Methods*. Using unpaired comparisons, contingency and regression analyses, we analyzed retrospective, biochemical data from 217 newly diagnosed myeloma patients at Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark. Data did not include information about neurological status. All statistical analyses were conducted using StatView software, version 5 (SAS Institute, Inc., Cary, NC, USA). *Results*. We found no significant relationships between cobalamin, MMA, tHcy, haemoglobin, and mean cell volume; not even when analyzed by renal status. But we found MMA and tHcy highly correlated to creatinine levels. By linear regression the correlation of creatinine to MMA was r2=0.495 corresponding to $p<0.0001$, and to tHcy r2=0.047 corresponding to $p=0.002$ (Figure 1). *Summary/conclusions*. MMA and tHcy were related only to renal function in this study on 217 newly diagnosed multiple myeloma patients. We therefore suggest that multiple myeloma patients should not be screened for cobalamin deficiency with MMA and tHcy, not even when renal function is unaffected. We would like to stress the need for prospective clinical intervention studies to evaluate the incidence and clinical significance of cobalamin deficiency in multiple myeloma.

Myelodysplastic syndromes - Biology

0675

A NOVEL APPROACH FOR STUDYING CLONAL GRANULOPOIESES IN MDS - MONITORING THE INFLUENCE OF IMIDS

K. Püllmann, A. Ganser

Hannover Medical School, HANNOVER, Germany

Myelodysplastic syndrome (MDS) is a clonal disorder characterized by ineffective hematopoiesis. Available evidence suggests that it belongs to a group of diseases, which manifest as autoimmune processes or as repression of normal hematopoiesis by an expanding malignant cell clone. We recently reported the existence of a TCR based variable immunoreceptor in a subpopulation of neutrophils, which provides an as yet unknown molecular basis for variable and adaptive immunorecognition in neutrophils (Puellmann *et al.* PNAS 2006, 103: 14441-6; N Engl J Med 2006, 355: 2592-3) This study aimed at the analysis of the neutrophil immunoreceptor repertoire in myelodysplasia. We characterized in detail the TCR Vαβ repertoires in neutrophils from MDS patients and monitored the repertoire profiles during the course of MDS using RT-PCR based CDR3 spectratyping. Neutrophil TCR V-chain repertoires were cloned and their CDR3 cDNA sequences were determined. All results were related to clinical data including disease classification and karyotype. Our results demonstrate that human peripheral blood $CD15^+$ neutrophils from MDS patients express highly restricted TCR variable chain repertoires indicative of monoclonality and oligoclonality. Therapy of MDS patients with a 5q- syndrome with lenalidomide revealed a restoration of normal polyclonal granulopoiesis after treatment. TCR Vβ chain profiling of purified neutrophils is a promising new marker for monitoring clonal granulopoiesis and therapeutic effects in MDS.

0676

AML1/RUNX1 MUTATIONS COOPERATE WITH CLASS I MUTATIONS IN THE PROGRESSION OF MDS TO S-AML FOLLOWING MDS IN NORMAL KARYOTYPE

S. Dicker, C. Haferlach, W. Kern, T. Haferlach, S. Schnittger

MLL Munich Leukemia Laboratory, MUNICH, Germany

Background. The mechanism of progression of myelodysplastic syndrom (MDS) to acute myeloid leukemia (AML) is genetically poorly defined and several different scenarios might account for this phenomenon. RUNX1 mutations have been implicated in this process, but also cytogenetic aberrations and mutations of the RAS signal transduction pathway. *Aims.* We wanted to characterize a cohort of MDS (n = 78) and s-AML following MDS (n = 39) patients for RUNX1 mutations, by chromosome banding analysis and for mutations of several molecular markers (FLT3, NRAS, MLL and NPM1) to analyze the results for cooperating mutation patterns. *Methods.* After informed consent the entire coding region of all samples was screened for RUNX1 mutations from cDNA by denaturing high performance liquid chromatography (DHPLC) and mutations were called by direct DNA sequencing. Furthermore, FLT3 was screened for length mutations (FLT3-LM, FLT3-ITD), NRAS for mutations in codons 12/13 and 61, MLL for partial tandem duplications (MLL-PTD) and NPM1 for exon-12 gene mutations. Cytogenetic analysis was done by chromosome banding and FISH analysis. *Results.* RUNX1 mutations were detected in the cohort of MDS patients in 13 of 78 cases (17%). This cohort consisted of MDS patients with RARS (n=1), RCMD (n=6), CMML-1/-2 (n=8), RAEB-1 (n=18), RAEB-2 (n=21) and MDS not further classified (n=24). The incidence of RUNX1 mutations in the MDS cohort was not significantly different from the incidence of RUNX1 mutations in s-AML following MDS with 10 out of 39 cases beeing mutated (26%, $p=0.323$, Fisher's exact test). Next, we further categorized the samples as being cytogenetically aberrant and as being mutated in one of the following genes: FLT3, NRAS, MLL and NPM1. The number of RUNX1 mutations in MDS with normal karyotype (NK) (4/31, 13%) was not significantly different from MDS with aberrant karyotype (9/42, 21%) ($p=0.537$). Likewise, the incidence of RUNX1 mutations in s-AML following MDS with NK or aberrant karyotype was almost identical with 25% (5/20) and 26% (5/19), respectively, and no difference was noted between NK of MDS and s-AML following MDS or aberrant karyotype of MDS and s-AML following MDS ($p=0.289$ and $p=0.747$, respectively). However, when mutations in additional genes (FLT3, NRAS, MLL and NPM1) were included into this analysis, all s-AML following MDS with NK and RUNX1 mutation (n=5) had additional mutation events (FLT3-LM (n=2), NRAS (n=1), MLL-PTD (n=1) and

MLL-PTD/NRAS (n=1)). This was in contrast to MDS with NK and RUNX1 mutation (n=4) and to s-AML following MDS with aberrant karyotype and RUNX1 mutation (n=5), where no or only one additional mutation (NRAS) were detected, respectively, and also in contrast to s-AML following MDS with NK without AML1 mutation (n=15) with four additional mutations (FLT3-LM (n=1), MLL-PTD (n=1), NPM1 (n=2)) (p=0.008). *Summary and Conlusions*. These data implicate that RUNX1 mutations in MDS with normal karyotype cooperate with mutations in receptor tyrosine kinases and the RAS signaling pathways, but also with MLL-PTD in the progression to AML.

0677

THE ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND MATRIX METALLOPROTEINASE 2 AND 9 OVEREXPRESSION IN BONE MARROW CELLS FROM PATIENTS WITH MYELODYSPLASTIC SYNDROME

R. Invernizzi,[1] E. Travaglino,[2] V. Matti,[2] M.G. Della Porta,[2]
L. Malcovati,[2] A. Gallì,[2] E. Bonetti,[2] E. Boveri,[2] V. Rosti,[2] M. Cazzola[2]

[1]Fondazione IRCCS Policlinico S. Matteo, PAVIA; [2]Policlinico S. Matteo, PAVIA, Italy

Angiogenic factors influence the growth and differentiation of hematopoietic cells in normal conditions and in hematologic malignancies. Most angiogenic factors appear to be secreted by hematopoietic cells, and they may have autocrine and paracrine regulatory effects on the hematopoietic system. The expression of various angiogenesis mediators has been found to be altered in myelodysplastic syndrome (MDS) bone marrow and abnormal angiogenesis has been implicated in the pathogenesis of the disorder. Vascular endothelial growth factor (VEGF) is one of the most important agents to stimulate angiogenesis. Also matrix metalloproteinases (MMP), which are able to degrade all the protein components of the extracellular matrix, especially MMP-2 and MMP-9, play a role in angiogenesis. We analyzed by immunocytochemistry VEGF, MMP-2 and MMP-9 expression in bone marrow cells from 117 patients with MDS (55 RA, 26 RARS, 15 RAEB, 4 RAEB-t, 17 CMML) and 55 non hemopathic subjects. We also measured the release of VEGF by ELISA and that of active MMPs by a colorimetric assay in the supernatants of cell cultures from representative cases. Our aims were to evaluate whether abnormalities in the expression of these factors were associated with relevant laboratory or clinical findings and to investigate a possible correlation between VEGF or MMP positivity and altered apoptosis or proliferation. In normal samples MMP-2 was detected in rare myeloid cells, MMP-9 and VEGF in most maturing myeloid cells. In MDS VEGF and MMP-2 myeloid levels were higher than in controls (p<0.0001), and also many erythroblasts expressed VEGF and MMP-2. A few MDS CD34$^+$ stem cells expressed VEGF and/or MMPs, whereas normal CD34$^+$ cells did not express any of these factors. The release of VEGF and active MMP-9 was demonstrated in all samples, while the release of active MMP-2 was observed only in the media conditioned by MDS mononuclear cells. No significant relationship was detected between VEGF or MMP expression and circulating endothelial cell levels or marrow microvessel density. In MDS there was a positive correlation between MMP-2 erythroblast expression and erythroid dysplasia (p=0.002) and, in early MDS, a tendential positive correlation between MMP-2 erythroid expression and erythroid apoptosis levels, as evaluated by TUNEL technique. An inverse correlation between MMP-2 or MMP-9 myeloid expression and blast cell percentage (p=0.05 and p=0.04 respectively) and a positive correlation between VEGF myeloid levels and apoptotic rate (p=0.02) were observed. High VEGF or MMP levels in myeloid cells were associated with longer overall survival (p=0.03) and evolution-free survival (p=0.04). In conclusion, we have demonstrated an abnormal MMP and VEGF expression profile in MDS bone marrow cells. The production and release of these proteins may influence hematopoietic cell behaviour, possibly by a paracrine induction of inflammatory pro-apoptotic cytokines from endothelial cells and macrophages, with a potential prognostic significance for disease progression.

0678

EPIGENETIC REGULATION OF FAS EXPRESSION ALONG PROGRESSION OF MYELODYSPLASTIC SYNDROMES

B. Fontenay,[1] B. Benet,[1] M. Gentil,[1] E. Gyan,[1] E. Frisan,[1]
C. Pierre-Eugene,[1] C. Humbrecht,[1] F. Picard,[1] M. Guesnu,[1] V. Bardet,[1]
O. Beyne-Rauzy,[2] M. Hunault-Berger,[3] B. Quesnel,[4] E. Solary,[5]
C. Lacombe,[1] P. Mayeux,[1] F. Dreyfus[1]

[1]Cochin Institute, PARIS; [2]CHU Purpan, TOULOUSE; [3]CHU, ANGERS; [4]CHRU, LILLE; [5]Faculty of medicine, University of Burgundy, Inserm U866, IFR100, DIJON, France

Background. Myelodysplastic syndromes (MDS) are heterogeneous diseases of the hematopoietic stem cell whose pathophysiology remains largely unknown. We have demonstrated a role for Fas-mediated apoptosis in the ineffective hematopoiesis that characterizes low risk (LR) MDS. We have also shown that evolution of LR-MDS to high risk (HR)-MDS or acute myeloid leukaemia (AML) was associated with Fas down-regulation at the surface of hematopoietic cells. In cancer cells, promoter hypermethylation can account for the repression of genes encoding tumor suppressors and pro-apoptotic molecules including Fas. Drugs such as 5-azacytidine or 5-azadeoxycytidine, which inhibit DNA methyltransferases, can restore Fas expression and sensitivity to Fas agonists in various carcinomas. *Aims.* We investigated whether Fas expression was epigenetically regulated in a cohort of 125 patients at different stages of their MDS natural history. *Methods and Results.* Comparison of LR-MDS to normal bone marrows demonstrated an increase in the expression of Fas at the surface of CD45int/CD34$^+$ cells and in the fas mRNA level quantified by qRT-PCR in mononuclear cells. In contrast, both Fas expression at the cell surface and fas mRNA level were decreased in HR-MDS compared to LR-MDS cells. In a series of 20 patients, we identified a decrease in membrane Fas expression when comparing bone marrow cells before (median RFI 2.3) and after (median RFI 1.7) transformation into AML. The half-life of fas mRNA (30 min) and Fas protein (1 h) was similar in normal and LR-MDS bone-marrow cells. Our results suggested an increased transcription of fas gene in LR-MDS. The -1119 to +8 region of human fas gene promoter, which contains a putative target (CpG-rich islet) for DNA methylation was amplified by PCR. Methylation of 36 CpG dinucleotides was analyzed after treatment of CD34$^+$ cell DNA with sodium bisulphite in 10 LR-MDS, 10 HR-MDS and 5 control samples by cloning and sequencing. fas promoter methylation was decreased in LR-MDS compared to control cells and increased in HR-MDS compared to LR-MDS. The percentage of methylated CpG inversely correlated with fas mRNA level. *Ex vivo* treatment with 5-azadeoxycytidine of bone marrow mononuclear cells from 6 patients with HR-MDS induced a significant increase in fas mRNA level and membrane Fas expression. We also studied 9 patients before and after 4 cycles of 5-azacytidine treatment (75mg/m^2/d, 7 days, every 4 weeks). In all cases, Fas expression increased at the cell surface of CD45int/CD34$^+$ cells. Of these 9 patients, 1 demonstrated a complete response and 8 showed a stabilized blast cell count, including 2 with improved red cell and platelet counts. Flow cytometry analysis identified an increased apoptosis of bone marrow cells in stabilized patients. *Conclusions.* Altogether, our data demonstrate that Fas expression at the surface of bone marrow cells is epigenetically regulated along the progression of MDS and can be modulated by hypomethylating agents, both *ex vivo* and in patients.

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DEFECTIVE SUPPRESSOR ACTIVITY AND BONE MARROW TRAFFICKING OF TREGS IN EARLY-STAGE MDS

I. Kotsianidis,[1] I. Bouchliou,[2] E. Spanoudakis,[2] E. Nakou,[2]
D. Margaritis,[2] A.V. Christophoridou,[2] A. Anastasiades,[2] C. Tsigalou,[3]
G. Kambouromiti,[3] G. Bouricas,[2] C. Tsatalas[2]

[1]Democritus University of Thrace, ALEXANDROUPOLIS; [2]Democritus University of Thrace, Medical School, ALEXANDROUPOLIS; [3]Department of Microbiology, University hospital of Alexandroupois, ALEXANDROUPOLIS, Greece

Background and Aims. CD4$^+$CD25$^+$Foxp3$^+$ T regulatory cells (Tregs) protect against autoimmune diseases, but they also convey excessive suppression of antitumor immunity. Appropriate compartmentalization of Tregs is crucial for the effective regulation of immune responses. Interestingly, human bone marrow (BM) attracts Tregs by CXCL12/CXCR4 signals. As MDS is frequently associated with immunologic abnormalities and immune-mediated BM failure, we

explored the frequency, function and BM trafficking of Tregs in MDS. *Patients and Methods*. Peripheral blood (PB), BM and BM fluid (BMF) samples were obtained from 52 treatment-naïve MDS patients with a median age of 70 (42-85) years. Based on IPSS, patients were subdivided in early-stage MDS (E-MDS, low/intermediate-1 risk, n=36) and late-stage MDS (L-MDS, intermediate-2/high risk, n=16) patients. Fourteen normal age-matched individuals undergoing orthopaedic surgery were used as controls. According to WHO classification, 8 patients had RA, 22 RCMD, 12 RAEB-I, 7 RAEB-II and 3 RAS. Treg frequency and CXCR4 expression was measured by flow cytometry. Treg suppressor activity was assessed by CFSE-based proliferation assays of MACS-isolated $CD4^+CD25^+$orcells and by the inhibition of IFNγ and TNF-α secretion from effector cells. PB $CD4^+$ cells from patients and controls were induced to migrate towards CXCL12 or BMF. CXCL12 was detected in BMF by ELISA. Significance of differences was assessed by one way ANOVA or paired Student's t test as appropriate. *Results*. PB (17.8±3.3×10^6/L, p=0.01) and BM (2.28±0.4%) Tregs were expanded in L-MDS patients compared to controls (PB: 7.5±0.9×10^6/L, p=0.01, BM: (1.1±0.33%, p=0.01) and E-MDS patients (PB: 6.5±0.6×10^6/L, p=0.01, BM: (0.79±0.14%, p=0.01). In E-MDS patients, the suppressive capacity of Tregs from both cellular compartments was significantly compromised compared to controls (p<0.001) and L-MDS patients (p=0.001 in PB and p<0.001 in BM). Similarly, PB and BM Tregs from E-MDS patients failed to suppress IFN-γ and TNF-α production from autologous $CD4^+CD25^-$ cells compared to controls (IFN-γ: p=0.01 and p=0.088, TNF-α: p=0.015 and p=0.233, for PB and BM, respectively) and L-MDS patients (IFN-γ: p=0.049 and p=0.036, TNF-α: p=0.1 and p=0.4, for PB and BM, respectively). In contrast to L-MDS patients and controls, E-MDS patients displayed significantly decreased Tregs in their BM (1.4±0.22%) compared to PB (0.79%±0.14%, p>0.001). Additionally, PB Tregs from E-MDS patients exhibited decreased chemotaxis towards CXCL12 compared to controls (p=0.036) and L-MDS patients (p=0.086), whereas CXCR4 was underexpressed on Tregs from E-MDS patients (15.8±1%) compared to controls (24.9±1.3%, p=0.002) and L-MDS patients (25.9%±3.3%, p=0.001). On the contrary, normal Tregs migrated equally towards BMF from all groups and BM CXCL12 levels were comparable among all groups (E-MDS: 4.98±1.57 ng/mL, L-MDS: 5.33±1.9 ng/mL, controls: 4.1±1.2 ng/mL). No differences in Treg suppressor function, cytokine inhibition, migration towards CXCL12 and CXCR4 expression were observed between L-MDS patients and controls. *Conclusions*.Treg function and BM trafficking is compromised in E-MDS, potentially promoting immune derangement and bone marrow insufficiency. Conversely, in L-MDS, Treg expansion and restoration of their function and migratory capacity may allow the immune escape of the malignant clone. Our findings indicate Treg involvement in the pathophysiology of MDS, rendering them candidate targets for immunotherapy of the disease.

0680

REDIFINING MONOSOMY 20 BY MOLECULAR CYTOGENETICS IN 10 MYELODYSPLASTIC SYNDROMES/ACUTE MYELOID LEUKEMIA (MDS/AML) PATIENTS

P. Bernasconi,[1] I. Dambruoso,[2] M. Boni,[2] R. Zappatore,[2]
P.M. Cavigliano,[2] I. Giardini,[2] S. Calatroni,[2] B. Rocca,[2] M. Caresana,[2]
P. Tarantino,[2] M. Lazzarino[2]

[1]Foundation IRCCS Policlinico San Matteo, PAVIA; [2]Division of Hematology, Foundation IRCCS Policlinico S. Matteo, PAVIA, Italy

Deletions of the long arm of chromosome 20 (20q-) are recurrent karyotype defects in patients with MDS and AML. In these patients conventional cytogenetic, fluorescence *in situ* hybridisation (FISH) and molecular analyses have revealed a common deleted region (CDR) 2.6 Mb long. Further studies have pointed to L3MBTL, a tumor suppressor gene (TSG), as the critical gene residing within this CDR. However, the involvement of L3MBTL in the pathogenesis of 20q- MDS/AML has not yet been confirmed. In addition, MacKinnon *et al.*, (2007) have revealed that the most proximal segment of 20q is consistently preserved in deletions and unbalanced rearrangements of this chromosomal segment and have suggested the presence of a gene/sequence crucial for MDS/AML pathogenesis. The present study was aimed at verifying this hypothesis in 10 MDS/AML patients with a complex karyotype, defined by the presence of ≥3 chromosome defects and presenting either a 20q deletion or a monosomy 20. According to FAB classification 2 patients were classified as refractory anemia (RA), 5 as refractory anemia with excess of blasts (RAEB), 2 as RAEB in transformation (RAEB-t) and one as AML. According to WHO classification 2 patients were classified as refractory cytopenia with multilineage dysplasia (RCMD), 2 as RAEB-1, 3 as RAEB-2 and 3 as AML. CC and FISH studies were performed as already reported (Bernasconi *et al.*, 2007). FISH analyses were carried out on mitotic figures with the D20S108, mapped in 20q12, and WCP20SO probes from Vysis (Abbott Molecular/Vysis, North Chicago, IL, USA) and with an arm chromosome painting (ACP) 20q probe from QBiogene (Qbiogene Inc., Carslbad, CA, USA)). The D20S108 probe revealed a 20q deletion (one signal only) in 7 patients and an amplification (≥4 signals) in 3 patients. The WCP20 probe revealed a normal chromosome 20 and various fragments of number 20 on other chromosomes in 8 patients, a pentasomy 20 in one patient and a true monosomy 20 in the remaining one. In 7 of the former 8 patients the ACP 20q probe revealed a normal long arm of chromosome 20 and showed that all fragments, which the WCP20 probe had identified as material derived from number 20, belonged to 20q. In 3 of these 7 patients the ACP probe confirmed 20q amplification and revealed that the DS20108 probe was contained within the amplified region. In one of these 8 patients FISH was unsuccessful. Finally, the ACP probe confirmed pentasomy 20q in one patient and true monosomy 20 in the last one. In conclusion, our data confirm that i) true monosomy 20 is a very uncommon event in MDS/AML, ii) in most patients an apparent monosomy does occur since material from chromosome 20q is always retained, iii) the common feature in all our patients is 20q amplification (observed in 7 patients) rather than band 20q12 deletion (observed in 4 patients), a datum which strengthens the suggestion of a still undetected oncogene mapped within the amplified area. Our future goal will be to define the extent of the amplified area searching for a possible oncogene.

0681

GENETIC IMBALANCES IN MYELODYSPLASTIC SYNDROMES(MDS) AND ACUTE MYELOID LEUKEMIA (AML) PATIENTS WITH CHROMOSOME 11Q13 ABNORMALITIES

P. Bernasconi,[1] I. Dambruoso,[2] M. Boni,[2] R. Zappatore,[2]
P.M. Cavigliano,[2] I. Giardini,[2] M. Caresana,[2] P. Tarantino,[2]
R. Invernizzi,[3] M. Lazzarino[2]

[1]Foundation IRCCS Policlinico San Matteo, PAVIA; [2]Division of Hematology, Foundation IRCCS Policlinico S. Matteo, PAVIA; [3]1 Department of Internal Medicine, Foundation IRCCS Policlinico S. Matteo, PAVIA, Italy

In MDS and AML patients a deletion of band q13 on the long arm of chromosome 11 is a recurrent, yet rare chromosomal defect. In fact, in large MDS patients series the incidence of this chromosomal lesion is about 1.0% (Solè *et al.*, 2005; Bernasconi *et al.*, 2007). Based on FAB and WHO classifications most del(11)(q13) MDS patients are classified as RA and RARS. The present study, which included 9 patients, was aimed at excluding that del(11)(q13) was due to cryptic chromosomal translocations, at better defining the extent of the deleted region and eventually at revealing any possible genetic imbalance leading to the amplification of some chromosome 11 fragments, possibly suggesting the involvement of an oncogene. Four patients were classified as MDS (2 RA and 2 RARS) and 5 as AML (one M1, 2 M2 and 2 M4). Conventional cytogenetic (CC) and FISH studies were carried out as already reported (Bernasconi *et al.*, 2007). CC revealed del(11)(q13) in 6 patients (3 MDS and 3 AML) and 11q abnormalities in a complex karyotype in the other 3, one MDS and 2 AML. FISH was applied to all the 9 patients with commercial probes from Vysis (Abbott Molecular/Vysis, North Chicago, IL, USA) and BAC probes provided by the Sanger Institute (Wellcome Trust Sanger Institute, Cambridge, UK). In order to define the proximal and distal breakpoints of the deleted chromosomal area, all patients were at first analysed with two BAC probes RP11-93M11 and RP11-5N5, mapping at bands 11q13.4 and 11q22.3 respectively. Subsequently, a set of twenty-one BAC probes was used to further restrict the breakpoint region. In 5 patients, 2 low-risk MDS and 3 AML, the deleted region was comprised between the BAC probes RP11-93M11 and RP11-23O14 (between bands 11q13.4 and 11q14.2). In 3 of these patients we succeeded in further reducing the breakpoint area to a region comprised between the BAC probes RP11-19P3 and RP11-23O14 (between bands 11q14.1 and 11q14.2). Another MDS patient presented an interstitial deletion involving a more telomeric region than that of the other 5 patients. In fact, she showed the loss of a chromosomal segment comprised between the BAC probes RP11-179B7 and the LSI MLL from Vysis (between bands 11q22.3 and 11q23). In the remaining 3 patients, one MDS and 2 AML, the BAC probes applied did not detect any deletion, instead they revealed the amplification of a region beginning from the cyclin D1 gene (LSI Cyclin D1 probe from Vysis) mapped at 11q13. So, the extent of the deleted area was variable while that of the amplified area was relatively constant. In addition, the two regions differed for the chromosomal segment comprised between the LSI cyclin D1 and the BAC probe RP11-93M11 (between bands 11q13 and 11q13.4). In conclusion, our study

excludes cryptic translocations of the investigated regions and suggests the existence of either a possible oncogene residing between bands 11q13 and 11q13.4 and activated by the amplification, or of a cyclin D1 regulatory mechanism disrupted by the chromosomal deletion.

0682

DYNAMICS OF TELOMERE LENGTH, THE STATUS OF CDKN2B GENE METHYLATION AND TELOMERASE ACTIVITY AS A POTENTIAL UNSPECIFIC PROGNOSTIC MARKERS IN MDS

S. Vcelikova,[1] H. Cechova,[1] H. Zizkova,[1] P. Baresova,[1] M. Skalova,[1] Z. Zemanová*,[2] K. Michalová*,[2] J. Cermak[1]

[1]Institution of Hematology and Blood Transfusion, PRAGUE; [2]1st Medical Faculty, Charles University, PRAGUE, Czech Republic

Background. Telomeres have an important role in chromosome function and the maintenance of genome integrity. Erosion of telomeres associated with the activation of telomerase synthesizing telomeric sequences and methylation of CpG islands within gene promoters in patients with myelodysplastic syndromes (MDS) might represent one of the critical factors for leukemic transformation. *Aims.* We investigated variability of telomere length, activity of telomerase and status of CDKN2B (p15INK4b) gene methylation in patients with MDS to compare dynamic changes of the parameters during leukemogenesis and to evaluate their usefulness as prognostic markers. *Methods.* Molecular analyses were performed in bone marrow (BM) samples obtained from 26 patients with MDS subdivided according to WHO classification: 6 x RA, 1 x RARS, 8 x RCMD, 3 x 5q-syndrome, 1 x RAEB1, 5 x RAEB2 and 2 x MDS/MPS. BM cells of 10 age matched healthy donors served as controls, 5 leukemic cell lines were examined as positive controls. Telomere length was determined as terminal restriction fragment (TRF)index in kbp on the base of Southern analysis of genomic DNA. Activity of telomerase was assessed by Quantitative TRAP assay based on photometric enzyme immunoassay on solid phase. TRF shorter than 7.5 kbp was postulated as reduced telomere length and values of TA higher than 0.0263 as increased telomerase activity. Level of methylation DNA was quantified by methylation specific PCR after sodium bisulphate modification of DNA. Methylation status was dedicated as Methylation Indices (MI) in the range 0.0-1.0. *Results.* Aberrant methylation of CDKN2B gene was present in 77% (20/26,MI=0.26±0.17) of patients with MDS, telomere reduction was observed in 65% (17/26TRF= 5.8±1.02) of patients and 38% of patients (10/26, TA=0.1335±0.1065) had increased TA. A rapid erosion of telomeres was connected with high level of methylation (13/26,TFR=6.04±1.02; MI=0.31±0.15) in a half of MDS patients (50%) and with positive activity of telomerase in 38% of patients (TA=0.1279±0.1316). A high level of methylation CDKN2B gene and the presence of shortened telomeres was observed in advanced forms of MDS with excess of blasts (TRF=5.79±0.95; MI=0.25±0.14) when compared to early forms of MDS (TRF=7.26±2.1;MI=0.21±0.17). The difference in TA between early and advanced forms was not significant because of a high incidence of increased TA level in early MDS (TA=0.078±0.1031). *Summary and Conclusions.* The presence of methylation in CpG islands of gene promoters, reduction of telomere length and telomerase activity in patients with MDS was connected with disease progression towards advanced forms. Our data suggest that telomere length as well as methylation status of CpG islands might serve as an unspecific prognostic marker and contribute to optimalization of treatment strategy.

0683

LENALIDOMID INFLUENCES THE T-CELL RECEPTOR REPERTOIRE IN PATIENTS WITH 5Q- MYELODYSPLASTIC SYNDROME

T. Momot, C. Dobbelstein, J. König, O. Schulz, J.-E. Pautsch, A. Ganser, E.M. Weissinger

Medical School Hannover, HANNOVER, Germany

Background and aims. MDS is a complex disease of the stem cells, ranging from a pre-malignant (low risk) to highly malignant (high risk) phenotype. A T-cell involvement has been shown for low risk MDS. A relatively newly described subtype of low risk MDS by WHO classification is the 5q- syndrome, which is characterized by a loss of the part of the long arm of chromosome 5. A novel immunomodulatory agent lenalidomide shows encouraging results in 5q- syndrome with durable transfusion independence. To investigate a T-cell involvement in the pathophysiology of MDS 5q- and the mechanisms of action of lenalidomide we studied the T-cell receptor repertoire of patients with 5q- syndrome before and after lenalidomide therapy (n=14, 5 mg/day for 60 days). *Methods and results.* $CD3^+$, $CD4^+$ and $CD8^+$ T-cells were isolated from the bone marrow and peripheral blood samples. RNA from these T-cells was isolated and spectratyping performed. The data were compared to the results obtained from age-matched controls (n=12). Thirteen of 14 patients with MDS 5q- showed atypical expansion in particular Vβ subfamilies before therapy with lenalidomide in both bone marrow and peripheral blood T-cells, the TCR repertoire in controls was normal. The most frequent skewing of TCR Vβ fragments before lenalidomide treatment occurs in Vβ 1, Vβ 3, Vβ 13, Vβ 22 and Vβ 24. Interestingly, Vβ 1 and Vβ 3 are also often over expressed in patients with RAEB subtype of MDS. Three of the patients are now far enough after lenalidomide therapy to evaluate the changes of the spectratyping pattern. Normalization of 8 initially skewed Vβ profiles after lenalidomide therapy occurred in all three treated patients. Eight MDS 5q- patients with lenalidomide and evaluable to date achieved a complete hematologic remission (CR), 3 a partially remission (PR), while 2 showed no response (NR). Karyotypic abnormalities in 2 patients had completely resolved to show a normal female karyotype after lenalidomide treatment, 5 patients showed partially cytogenetic remission. *Summary*. Our results show interesting new insights in the mechanism of lenalidomide action. We see changes in the TCR repertoire in MDS 5q- patients that need further evaluation, but point towards an involvement of T-cells in the pathogenesis of MDS 5q.

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PI3K, FLT3, JAK2 MUTATIONS AND MDM2 POLYMORPHISM IN BRAZILIAN PATIENTS WITH MYELODYSPLASTIC SYNDROME

F. Traina, P. Campos, M. Andreoli, J.A. Machado Neto, K.B.B. Pagnano, I. Lorand-Metze, F.F. Costa, S.T.O. Saad

State University of Campinas, CAMPINAS, Brazil

Background. Myelodysplastic syndrome (MDS) encompasses a heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis, refractory cytopenia and a tendency to progress to acute myeloid leukemia (AML). Accumulation of genetic alterations is closely associated with the progression of MDS to AML and efforts are being made to determine the significance of various genetic aberrations in adult patients with MDS. *Aims.* We investigated, in MDS patients, the incidence of mutations of genes that control proliferation, survival and motility, which are involved in leukemia and other cancers: Phosphatidylinositol 3-kinases (PI3Ks), fms-like tyrosine kinase 3 (FLT3), Janus kinase 2 (JAK2). In addition, we studied the MDM2 SNP309 polymorphism. MDM2 directly binds to and inhibits p53 by regulating its location, stability and activity as a transcriptional activator. This polymorphism results in higher levels of MDM2, attenuates the p53 pathway and correlates with poor survival in cancer. SNP309 is shown to associate with accelerated tumour formation in both hereditary and sporadic cancers. A model is proposed whereby SNP309 serves as a rate-limiting event in carcinogenesis. Patients and *Methods.* We studied 65 patients with diagnosis of MDS. According to FAB, patients were distributed as follows: 41 RA, 11 RARS, 7 RAEB, 3 RAEBt and 2 CMML. Samples were obtained from peripheral blood (PB) (n= 45) or bone marrow (BM) (n = 49) and were screened for the presence of the PI3K E542K, E545K, and H1047R mutations (41 BM), the FLT3/ITD mutation (44 BM), the JAK2 V617F mutation (50 BM; 16 PB) and the MDM2 SNP309 (46 PB). PI3K mutations were screened through PCR analysis and sequencing using specific primers. The FLT3/ITD mutation was screened by PCR analysis with specific primers. JAK2 and MDM2 mutations were screened through PCR analysis with specific primers and appropriate restriction enzyme. *Results.* PI3K and FLT3 mutations were not found in the patients studied. One patient was heterozygote for JAK2 mutation. Interestingly, JAK2 mutation was only identified in this patient after disease progression. Initially, the patient was classified as CMML with less than 5% of blasts in the bone marrow and without JAK2 mutation. His disease progressed with increased blasts in the bone marrow and leukocytosis and was found to have the JAK2 mutation at the time of progression. Among the 46 patients tested for the MDM2 mutation, 17 were found to have heterozygous mutations and one was homozygous. MDM2 SNP309 allele frequency was found to be 20% in the patients studied and 29% in a control Brazilian population (142 subjects) (unpublished data). There were no differences between the MDM2 mutation group and the other groups regarding age of diagnosis, bone marrow blasts, transfusion requirement and low risk *vs* high-risk disease according to FAB. *Conclusions.* PI3K, FLT3, JAK2 mutations are rare in MDS patients. JAK2 mutation may be present in MDS, especially in CMML with myeloproliferative disease. In MDS, MDM2 SNP309 did not correlate with clinical features, but it should be pointed out that this is the first study of this polymorphism in hematological malignancy and merits further study.

0685

T CELL MEDIATED AUTOLOGOUS CYTOTOXICITY AGAINST HEMATOPOIETIC PRECURSOR CELLS IN LOW AND INTERMEDIATE-1 RISK MYELODYSPLASTIC SYNDROME

M.E.D. Chamuleau, T.M. Westers, J. Groenland, L. Dreunen, A. Zevenbergen, G.J. Ossenkoppele, A.A. van de Loosdrecht

VU University Medical Center, AMSTERDAM, Netherlands

Background and Aims. It remains unclear whether the altered T cell function in MDS reflects an autoimmune reaction against normal haematopoiesis or represents immunesurveillance against dysplastic and pre-leukemic hematopoietic cells. To address this question we have studied the T cell function of 41 low/int-I risk MDS patients. *Methods*. We analyzed peripheral blood lymphocytes of 41 MDS patients (IPSS score 0 in 54% of patients, IPSS 0.5 in 36% and IPSS 1 in 10% of patients) and from 20 healthy donors. All analyses were done by flow-cytometry. Autologous cytotoxic capacity was assessed by co-culturing purified peripheral $CD8^+$ T lymphocytes (CTL) with purified bone-marrow derived $CD34^+$ hematopoietic precursor cells in different E:T ratios. Apoptosis was measured by 7AAD/syto16 staining. *Results*. We could confirm high apoptotic rate of hematopoietic precursor cells in MDS patients. As compared to healthy control samples, a significant lower percentage of naïve $CD4^+$ and $CD8^+$ T lymphocytes and an increased percentage of activated effector $CD8^+$ T lymphocytes were measured in MDS ($p<0.0001$ for all analysis). $CD8^+$ cells in MDS revealed a cytotoxic phenotype (high granzyme B and perforin levels) and were clonal of origin when analysing the Vbeta repertoire of the T cell receptor. In addition, a significant lower level of FoxP3 expression was detected in $CD4^+$/CD25high cells (regulatory, suppressing function, $p<0.0001$). A significant increased percentage of WT1 specific lymphocytes could be detected in the peripheral blood of MDS patients ($p=0.025$). Cytotoxicity of these activated T cells was demonstrated by co-culturing $CD8^+$ T cells and blasts in different E:T ratios of 8 MDS patients. Patients with high levels of activated T cells revealed high autologous cytotoxicity activity (up to 75% apoptosis). In 2 healthy controls no autologous cytotoxicity against hematopoietic precursor cells could be demonstrated. Loss of granzyme B and perforin expression during disease progression could be demonstrated, providing evidence that immunesurveillance could be of critical importance in controlling the disease. *Summary*. In low/int-I risk MDS patients, the high cytotoxic phenotype of leukemic specific $CD8^+$ T cells and the low frequency of FoxP3high regulatory T cells may reflect an active controlling immune system. This provides evidence for carefully monitoring and ultimately critical reconsideration of the treatment of low/int-I risk MDS patients with immune-modulating agents.

0686

EXPRESSION ANALYSIS OF DNA REPAIR ENZYMES OF THE NON HOMOLOGOUS END JOINING MECHANISM IN THE BONE MARROW OF ADULT DE NOVO MYELODYSPLASTIC

P. Economopoulou,[1] F. Kontsioti,[2] V. Pappa,[2] E. Ioannidou,[2] S. Chondropoulos,[2] S. Papageorgiou,[2] K. Girkas,[2] V. Giannopoulou,[2] E. Papageorgiou,[2] J. Dervenoulas,[2] T. Economopoulos[2]

[1]Attikon University General Hospital, ATHENS; [2]Second Department of Internal Medicine, Attikon University General Hospital, ATHENS, Greece

Background. Myelodysplastic syndromes (mds) are an heterogenous group of clonal malignant hemopoietic disorders characterized by different degree of ineffective hemopoiesis resulting in cytopenias and a frequent evolution to acute leukemia. Numerical and structural clonal chromosomal abnormalities are frequently observed, suggesting genetic instability which at the molecular level is strongly associated with abnormal DNA repair mechanisms. The most lethal type of DNA damage are double strand DNA breaks (DSB) which can cause accumulation of genomic rearrangements and promote tumorigenesis. Their repair is by one of two pathways: the homologous recombination and the Non Homologous End Joining (NHEJ) mechanisms. The latter is thought to play a more dominant role in higher eukaryotes. The NHEJ mechanism at the molecular level is dependent on the activity of the Ku70/Ku80 heterodimer that binds to DNA ends at a DSB, DNA -PK cs important in phosphorylation of proteins involved in the pathway and XRCC4 stimulating DNA ligase IV to join the broken ends. *Aims.* The aim of the present study was the analysis of the expression of proteins involved in the NHEJ nechanism in bone marrow cells of adult *de novo* myelodysplastic syndromes and their association with clinical characteristics and prognosis. *Methods*. Our analysis included 25 cases of newly diagnosed adult *de novo* myelodysplastic syndromes before the initiation of any treatment . There were 18 males and 7 females with a median age 71classified according to FAB classification as follows: 8 RA, 5 RARS, 11 RAEB, 1 CMML. Cytogenetic analysis was performed in all cases and the IPSS was defined as follows: 0 n=10, 0,5 n=5, 1 n=5, 1,5 n=1, 2 n=3, 3 n=1. Bone marrow mononuclear cells from 17 cases of lymphoma patients without bone marrow involvement were used as normal controls. The expression of the enzymes Ku70, Ku80, XRCC4, DNA-PKs, ligase IV was determined on bone marrow mononuclear cell extracts by Western Blotting. Samples of 20 μg of protein were heated to 95°C resolved on 12.5% polyacrylamide Trig/glycine gels and blotted onto Hybond ECL nitrocellulose membrane (Pharmacia) using Tris/Glycine transfer buffer under the recommended conditions. Blots were blocked in Odyssey blocking buffer for 1h and incubated overnight in antibody buffer containing primary antibody: DNA PKcs, ab230, 1:2000 (Abcam, Cambridge, UK). Actin clone AC-15, 1:1000 (Sigma, Gillingham, UK), XRCC4 1:4000 (Serotec, Oxford, UK), Ku-80, Ab-7, 1:1000 (Biocarta, Oxford, UK), ligase IV, ab6145, 1:2000 (Abcam). After three 15 min washes in PBS-T blots were incubated with ssecondary antivody (1:5000) (Alexafluor 680-conjugated goat anti-rabbit or goat anti-mouse, Molecular Probes, Leiden, the Netherlands). Following three washes in PBS-T labeled proteins were detected and quantified using the Vision Works, Image Analysis Software System (UVP). *Results.* XRCC4 was expressed in 20/21, Ku70 in 17/18, Ku80 in 21/21, ligase IV in 11/13, DNA PKcs in 16/20 cases examined. All the enzymes were expressed in all cases of normal controls. The median DNA PKcs expression value was significantly lower in MDS compared to normal controls (0,35 *vs* 0,82, $p=0.02$). All other enzymes expression values were not significantly different between patients and controls. Moreover the mean and median expression values were not significantly different among FAB , IPSS and different cytogenetics subgroups. No significant differences were observed in hematological parameters, FAB, IPSS and cytogenetics subgroups between cases expressing or not all the enzymes. A trend towards a negative correlation was observed between the karyotype risk group and ligaseIV median values suggesting that as the cytogenetic risk group increases the median ligase IV values tend to decrease although statistically not significant ($p=0.07$). Moreover all cases with ligase IV values <median control value had IPSS >0 compared to 3/7 cases with lower values although the difference was not statistically significant ($p=0.07$). *Conclusions*. Enzymes involved in the NHEJ mechanism are expressed in the majority of cases with MDS. DNA PKcs expression is significantly lower in MDS compared to controls. Although the number of cases screened is relatively small no significant differences were observed in the expression of all other enzymes and no correlation was found with hematological parameters, FAB, IPSS and cytogenetic subgroups. The analysis of the integrity of the mechanism as well as the expression of all these enzymes following exposure to DNA damaging signal is under way to help to clarify the pathogenetic significance of this key DNA repair mechanism in myelodysplastic syndromes.

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CYTOGENETIC AND IMMUNOREGULATORY PROPERTIES OF BONE MARROW MESENCHYMAL STEM CELLS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

M. Klaus, P. Fragioudaki, K. Giannikou, M. Ximeri, M. Psyllaki, C. Kastrinaki, H. Papadaki

University of Crete School of Medicine, HERAKLION, CRETE, Greece

Background. The application of autologous bone marrow (BM) mesenchymal stem cells (MSCs) in haemopoietic stem cell transplantation procedure of patients with haematologic malignancies including myelodysplastic syndromes (MDS) is under investigation. We have previously shown normal immunophenotypic characteristics and differentiation potential of BM MSCs in MDS patients. However, the cytogenetic and immunoregulatory properties of BM MSCs in these patients have not been extensively studied. *Aims.* To characterize BM MSCs in MDS patients by investigating their cytogenetic characteristics in comparison to haemopoietic cells, their cytogenetic stability during passages and their immunosuppressive properties *in vitro*. *Methods.* BM MSCs were expanded from MDS patients (n=13) and healthy controls (n=5) using posterior iliac crest aspirates after informed consent. MSC identification was based on the morphologic and immunophenotypic ($CD45^-$, $CD14^-$, $CD34^-$, $CD90^+$, $CD73^+$, $CD44^+$, $CD29^+$, $CD105^+$, $CD146^+$) characteristics and their potential to differentiate towards adipocytes (Oil red-O stain and aP2/PPAR-γ expression by RT-PCR), osteoblasts (ALP/Von Kossa stain and ALP/CBFA1 expression by RT-PCR) and chondrocytes (Alcian blue stain and Collagen II/Aggrecan expression by RT-PCR). Classical cytogenetics (GAG-banding) and fluorescence *in situ* hybridization (FISH) analysis were performed in both BM haemopoietic cells and MSCs at passage-2 and in serial passages. MSC immunosuppressive properties were evaluated by a 3^H-thymidine (3^H-TdR)-based mixed lymphocyte reaction (MLR) using normal purified CD3+ cells stimulated with phytohemagglutinin (PHA; 2microg/mL) or interleukin-2 (IL-2; 500IU/mL) in the presence or absence of allogeneic normal or patient MSCs from passage-2. *Results.* Eight of the 13 MDS patients studied, displayed chromosomal abnormalities in the BM haemopoietic cells: five patients with del(5q), two patients with +8, and one patient with -Y. Chromosomal analysis of the corresponding MSCs didn't show any of these abnormalities. The del(5q) and +8 presence in BM haemopoietic cells and their absence in MSCs was verified by FISH. Overall, three MDS patients and one healthy control displayed trisomy 5 in the chromosomal analysis of MSCs that was also verified by FISH and one additional MDS patient displayed trisomy 7 in MSCs that was further confirmed by FISH. These numerical changes remained stable through passages. In five MDS cases with chromosomal abnormalities in BM haemopoetic cells, serial karyotypic analysis of BM MSCs until late passages (passage 2, 4, 6, 8) showed absence of the karyotypic abnormalities found in BM haemopoietic cells. MLR assay showed that the percentage of inhibition of PHA- or IL-2-activated T-lymphocytes by MSCs did not differ significantly between MDS patients (91.55%±5.86% and 81.54%±15.82%, n=5) and healthy controls (84.08%±12.43% and 80.73%±10.76%, n=5) suggesting normal immunosuppressive properties of BM MSCs in MDS patients. *Conclusions.* BM MSCs from MDS patients do not harbor the cytogenetic abnormalities present in BM haemopoietic cells and accordingly, they do not belong to the abnormal clone. MSCs from MDS patients display also cytogenetic stability through passages and normal immunosuppressive properties. Numerical aberrations such as trisomy 5 and 7 may represent an *in vitro* phenomenon without pathophysiologic significance as they have been described in cultures of normal haemopoietic and non-haemopoietic cells and have also been associated with age progression.

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WT1 AND CXCR4 EXPRESSION IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

I.S. Improta,[1] M.R. Villa,[1] A. Lucania,[1] M.R. Esposito,[1] M. Sagristani,[1] N. Pepe,[2] P. Rotondo,[2] M. Sansone,[2] M.T. Polistina,[2] L. Mastrullo[1]

[1]P.O. San Gennaro U.O.C. Ematologia, NAPOLI; [2]U.O.C. Biochimica e Genomica Molecolare P.S.I. Loreto Crispi, ASL NA1, NAPOLI, Italy

Background. Myelodysplastic Syndromes (MDS) are clonal hematopoietic stem-cell disorders characterized by ineffective dysplastic hematopoiesis involving one or more cell lineages, by peripheral-blood cytopenias and a high risk of progression to acute myeloid leukemia (AML). In normal peripheral blood (PB) and bone marrow (BM), WT1 expression is reported to be low and sometimes undetectable even by RT-PCR. By contrast, WT1 is highly expressed in most acute leukemias, and its level of expression is associated with the presence, persistence, or reappearance of leukemic hematopoiesis. Stromal cell-derived factor-1 (SDF-1) is a homeostatic chemokine that is constitutively secreted by marrow stromal cells. SDF-1 signals through CXCR4, which plays an important role in hematopoiesis, development and organization of the immune system. Prognostic impact of CXCR4 expression levels on the neoplastic cells has been demonstrated in breast cancer, renal cell cancer and AML. *Aims*. We investigated WT1 gene expression and its association with the expression of the chemokine receptor CXCR4 on bone marrow $CD34^+$ cells of MDS patients. *Methods*. BM samples from 36 MDS patients (according to WHO classification: 16 RA, 7 RAEB I, 4 RAEB II, 4 RARS, 3 deletion of 5q, 2 MDS unclass) were tested for WT1 expression at diagnosis and every 6 months. WT1 gene expression was evaluated by methods of real-time quantitative PCR (RQ-PCR). Surface CXCR4 expression were measured flow cytometrically. *Results*. At diagnosis, 22BM samples (10 RA, 6 RAEB I, 4 RAEB II, 1 RARS, 1 MDS unclass) expressed WT1 transcript amounts greater than the ranges level. The degree of WT1 expression was highly correlated with the type of MDS, was much higher in RAEB I and II compared with RA, and other types, and increased during disease progression. Moreover, a significant correlation was found between WT1 expression levels, blast cell percentage and CXCR4 over-expression on blast cells (as defined by CXCR4 mean fluorescence intensity ratio thresholds of more than 5). The patients received only a supportive therapy if necessary. After 6 months, 9 patients (2 RA, 5 RAEB I, 2 RAEB II) converted to AML. All of these patients showed at diagnosis an high WT1 and CXCR4 expression and a further elevation of WT1 expression level after 6 months. *Conclusions*. WT1 expression has been previously reported to be increased also in myelodysplastic syndromes. In this study, the data obtained show that in most MDS, including a large percentage of RA and almost the total number of RAEB I and II, WT1 is expressed above the range observed in normal controls in BM and that its expression is directly correlated with the type of MDS. A strong association is present between the level of WT1 expression and the blast percentage and the CXCR4 over-expression. The identification of a molecular marker so able to establish the tendency of MDS to progression can be of great help in decision making for MDS patients. Our results justify further investigation into the role of CXCR4 in MDS and suggest that WT1 and CXCR4 should be incorporated into the risk assessment of MDS patients

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EXPRESSION AND REGULATION OF KILLER CELL IMMUNOGLOBULIN (IG)-LIKE RECEPTORS (KIR) IN 5Q-MYELODYSPLASTIC SYNDROMES (MDS)

T. Momot, C. Dobbelstein, J. König, O. Schulz, J.-E. Pautsch, A. Ganser, E.M. Weissinger

Medical School Hannover, HANNOVER, Germany

Introduction. In the pathogenesis of low risk MDS a probably T-cell mediated autoimmune response is dicussed. As a novel immunomodulatory agent lenalidomide showed exiting results in the treatment of the low risk MDS, especially in patients with 5q- syndrome. For other autoimmune disorders like SLE and scleroderma several receptors including killer cell immunoglobulin-like receptors (KIRs), have been identified to probably influence the or as a result of the underlying disease. The repertoire of KIRs that are involved in the activation of T-cells and natural killer (NK) cells is highly variable. Currently, 13 known activating or inhibitory KIR exist. They play an important modulating role in the activation of T- and NK-cells via T-cell receptor and are inherited in a highly variable way. Therefore, the individual KIR repertoire is diverse. So far approximately 50 combinations of KIR have been described. *Meth-*

ods. To further characterize KIR-mediated T-cell involvement in the pathogenesis of MDS we used KIR genotyping and quantitative Real Time PCR. Total DNA and RNA was isolated from whole blood samples of MDS patients (n=17) receiving immunomodulatory treatment with lenalidomide (10 mg every other day for 60 days) and healthy controls (n=100). Quantitative RT-PCR was done to look for the up- or down-regulation of KIR in MDS patients compared with healthy blood donors. *Results*. Eight new combinations of KIR, so called *phenotypes* never described before in the literature, were detected in MDS patients compared with only 2 new KIR phenotypes in normal donors. The new phenotypes were characterized by overrepresentation of activating KIR2DS2 and KIR2DS3. The activating KIR were analysed by quantitative RT-PCR to confirm the genotyping results. *Discussion*. Our results indicate a possible role of KIR expression in patients with MDS. Since mainly activating KIR are expressed in the MDS patients, we speculate that in these cases KIR-phenotypes might influence T-cell activity without involvement of any pathogens. On the other hand, it is possible that KIR expression is changed due to the malignant clone and its progeny. To answer this question we currently compare DNA and RNA isolated from buccal mucosa and compare the KIR expression pattern to that of hematopoietic cells.

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STUDY OF BONE MARROW HAEMOPOIESIS AND PERIPHERAL BLOOD LYMPOCYTE SUBSETS IN PATIENTS WITH MYELOSDYSPLASTIC SYNDROMES (MDS) TREATED WITH LENALIDOMIDE

M. Ximeri,[1] A. Galanopoulos,[2] A. Symeonidis,[3] Z. Kartasis,[4] V. Pappa,[5] D. Liapi,[6] E. Hatzimichael,[7] S. Kokoris,[8] K. Giannikou,[1] M. Psyllaki,[1] M. Klaus,[1] H. Papadaki[1]

[1]University of Crete School of Medicine, HERAKLION, CRETE; [2]G. Gennimatas General Hospital, ATHENS; [3]University of Patras Medical School, PATRAS; [4]General Hospital of Halkida, HALKIDA; [5]Attikon General Hospital, ATHENS; [6]Venizeleion General Hospital, HERAKLION, CRETE; [7]University of Ioannina School of Medicine, IOANNINA; [8]Laiko General Hospital, ATHENS, Greece

Background. Lenalidomide has been approved for the treatment of transfusion-dependent low-/intermediate-1 risk MDS patients with chromosome 5q deletion[del(5q)]. Mechanisms of action of lenalidomide are not entirely known. *Aims.* To study the reserves and adhesion properties of bone marrow (BM) haemopoietic progenitor cells and the haemopoiesis supporting capacity of BM stromal cells in MDS patients under lenalidomide treatment. *Methods.* Twelve MDS patients (Low:n=5; Intermediate-1:n=5, Intermediate-2:n=2) with del(5q) alone(n=10) or with additional chromosome abnormalities(n=2) were treated with 10mg lenalidomide/daily after informed consent. Before(day-0) and after(day-120) treatment we studied: (a) the proportion and apoptotic characteristics of BM CD34$^+$ cells and the percentage of CD34$^+$ cells expressing the CD11a/CD43/CD44/CD48/CD49d/CD49e/CD54/CD62L/CXCR4 adhesion molecules using flow-cytometry; (b) the number of myeloid, erythroid, megakaryocytic progenitor cells using clonogenic assays; (c) the capacity of BM stroma to sustain haemopoiesis using long-term BM cultures (LTBMCs). Peripheral blood (PB) lymphocyte subsets were also studied. Response to treatment was defined according to standard criteria. Data were analyzed by the Wilcoxon's rank test. *Results.* One intermediate-1 risk patient with isolated del(5q) didn't display any response and discontinued lenalidomide three months after treatment initiation. Patient's data are not included in the analysis. The remaining patients displayed major haematologic(n=10) and cytogenetic(n=10) responses including normal FISH for del(5q) (n=8). One intermediate-2 risk patient displayed stable disease. The proportion of BM CD34$^+$ cells decreased significantly post-treatment compared to baseline(p=0.0039). Interestingly, the proportion of apoptotic cells within the CD34$^+$ cell fraction increased significantly post-treatment (p=0.0098). The proportion of GlycophorinA$^+$ cells increased significantly post-therapy(p=0.0137) and this increase was associated with a significant increase in BFU-E numbers obtained by BM mononuclear cells(BMMCs) post-therapy (p=0.0020). A significant increase was also observed in CFU-GM and CFU-Meg numbers obtained by BMMCs post-therapy (p=0.0020 and p=0.0078, respectively). The expression of CD11a/CD43/CD44/CD49d/CD49e/CD54/CD62L/CXCR4 within the CD34$^+$ cells did not change significantly post-therapy. A significant increase was observed in the proportion of CD34$^+$ cells expressing the CD48 molecule post-treatment (48.73%±35.05%) compared to baseline (10.82%±9.07%,p=0.0371). The haemopoiesis supporting capacity of LTBMC adherent layers increased significantly post-treatment compared to pre-therapy, as was demonstrated by the colony-forming cell numbers in standard and irradiated LTBMCs recharged with normal CD34$^+$ cells. A significant increase was observed in the proportion of CD3$^+$ T-cells post-therapy (p=0.0488) but not in the CD19$^+$,CD16$^+$,CD56$^+$ and CD57+ cells. A significant increase was obtained in the proportion of CD3+ cells expressing the CD69, CD38, Fas, and CD71 activation markers post-therapy compared to baseline (p=0.0195, p=0.0273, p=0.0137, p=0.0371, respectively). *Conclusions.* Haematologic and cytogenetic responses in lenalidomide-treated MDS patients are associated with a significant decrease in the proportion of BM CD34$^+$ blasts, improvement of CFU-GM, BFU-E and CFU-Meg numbers and amelioration of haemopoiesis supporting capacity of BM stroma. The adherent properties of BM CD34$^+$ cells remain unchanged. The increased expression of CD48 in CD34$^+$ cells, representing a co-stimulatory molecule for cytotoxic T-cells, in association with the increased apoptosis of CD34$^+$ cells and the increase number and activation status of PB T-cells following treatment, suggest a T-cell mediated apoptotic death of the abnormal cell clone as a possible mechanism of action of lenalidomide.

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INTRONIC RNA EXPRESSION IN STROMAL CELLS OF PATIENTS WITH MYELODYSPLATIC SYNDROME BY RNA MICROARRAY ANALYSIS

M.O. Baratti,[1] Y.B. Moreira,[2] F. Traina,[1] L. Borges,[1] F.F. Costa,[1] S. Verjovski-Almeida,[2] S.T.O. Saad[1]

[1]Hematology and Hematherapy Center / State University of Campinas, CAMPINAS; [2]Institute of Chemistry / University of São Paulo, SÃO PAULO, Brazil

Background. Myelodysplatic syndromes (MDS) are a group of clonal hematological disorders characterized by ineffective hematopoiesis with morphological evidence of marrow cell dysplasia resulting in peripheral blood cytopenia. There is increasing evidence that abnormal function of stromal environment may play a role in the disease pathophysiology. Microarray technology has permitted a refined high-throughput mapping of the transcriptional activity in the human genome. RNAs transcribed from intronic regions of genes are involved in a number of processes related to post-transcriptional control of gene expression, and in the regulation of exon-skipping and intron retention. The characterization of intronic transcripts in progenitor cells of MDS patients could be an important strategic to understand the gene expression regulation in this disease. *Methods.* We conducted a pilot study in stromal cells of 3 MDS-RARS patients and 4 healthy individuals. Gene expression analysis was performed using a 44k intron-exon oligoarray custom-designed by the group of Verjorvski-Almeida and collaborators and printed by Agilent Technologies. This oligoarray includes probes for protein-coding genes, for sense and antisense strands of totally intronic noncoding (TIN) and for partially intronic nonconding (PIN) RNAs. Stromal cells were isolated from bone marrow samples using Ficoll-Paque Plus (Amersham) gradient. The integrity of total extracted RNA was confirmed with the Agilent Bioanalyzer 2100. We amplified 300ng of each total RNA using the Agilent Low RNA Input Fluorescent Linear Amplification Kit PLUS, two-Color and samples were hybridized using the Gene Expression Hybridization Kit (Agilent) and then scanned on a GenePIX 4000B Scanner (Molecular Devices). Data extraction was performed with Agilent Feature Extraction Software 9.5. Each transcript was considered in the analysis only when the intensity was significantly above the average background in all samples. Data were normalized among the samples by quantil using Spotfire DecisionSite® for Microarray Analysis. To identifying genes differentially expressed between MDS-RARS and healthy individuals, we applied leave-one-out cross-validation following the SAM (Significance Analysis of Microarray) approach using as parameters: two-class unpaired response, t-statistic, 500 permutations and FDR <5%. After using SAM, a fold change > 2 filter was applied. *Results.* We identified 45 differentially expressed genes (40 up-regulated and 5 down-regulated), of which 12 were TIN and PIN transcripts (10 up-regulated and 2 down-regulated). These intronic transcripts were grouped according to the main role of the corresponding protein-coding genes transcribed from the same loci: gene transcription (FLII and KIAA1267); receptor (PPP2R1B); cell motility and adhesion (SPINT2, TBCE and TBCD); cell differentiation (GAS7); cell cycle and apoptosis (ARHGAP30, ARHGEF10L and CROCC) and cellular trafficking (GLIPR1). *Conclusions.* These results demonstrated that 26% of the total amount of differentially expressed genes corresponds to TIN and PIN transcripts in stromal cells of MDS-RARS patients, suggesting that intronic transcripts can play an important role during the development of myelodysplastic syndrome.

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EVALUATION OF GENE METHYLATION STATUS IN PATIENTS WITH MYELODYSPLASTIC SYNDROME: NEW PROGNOSTIC MARKERS?

E.N. Cortesao,[1] A.C. Gonçalves,[2] M.I. Sousa,[1] C. Moucho,[1] M.A. Pereira,[3] J. Nascimento Costa,[1] A.B. Sarmento[2]

[1]University Hospital of Coimbra, COIMBRA; [2]Faculty of Medicine and CIMAGO, University of Coimbra, COIMBRA; [3]Distrital Hospital of Figueira da Foz, FIGUEIRA DA FOZ, Portugal

Myelodysplastic syndrome(MDS) is a clonal hematopoietic stem cell disorder characterized by dyshematopoiesis and high susceptibility to acute myeloid leukemia(AML). One mechanism contributing to the constellation of hypercellular marrow and peripheral blood cytopenia is a significant increase in programmed cell death(apoptosis) in hematopoietic cells. The TNF-related-apoptosis-inducing ligand(TRAIL) is a member of the TNF-family and induces apoptosis preferentially in transformed and tumor cells but generally not in normal cells. The exact mechanism by which TRAIL eliminates tumor cells preferentially or selectively is not known. One possibility is differential expression of agonistic receptors 1 and 2(TRAIL-R1 and -R2), also known as death receptors(DR4 and DR5), and antagonistic or modulatory receptors(TRAIL-R3 and -R4), also known as decoy receptors(DcR1 and DcR2). However, it is clear that the regulation of hemopoiesis in MDS is complex and multiple factors are involved that need to be clarified. Deregulated epigenetic mechanisms are likely involved in the pathogenesis of MDS. Gene silencing through aberrant CpG island methylation is the most extensively analyzed epigenetic event in human tumorigenesis and has huge diagnostic and prognostic potential. Aberrant methylation of gene promoter region is responsible for inappropriate gene silencing, and it has been associated to initiation and progression of cancer. Aberrant DNA methylation is frequently observed in adults with MDS, and is recognized as a critical event in the disease's pathogenesis and progression. Which genes are silenced by aberrant promotor methylation during MDS hematopoiesis as well as the prognostic significance of these epigenetic alterations has not been equivalently investigated. We hope to contribute for the identification of molecular markers involved in apoptotic signalling pathways and the role of epigenetic gene modulation that could interfere with prognosis, evolution to AML and therapeutic approach. We have examined the methylation status of several genes namely the cell cycle regulators p15, p16 and p53 and also the apoptotic modulators TRAIL-Rs, R1, R2 R3 and R4, in bone marrow cells collected at diagnosis of 16 patients with *de novo* MDS. For this analysis, we performed sodium bisulfite treatment of genomic DNA, followed by methylation specific PCR(MS-PCR). The median age was 77 years(33-88), M/F=7/9, WHO subtypes: RCMD(n=8), RA(n=3), CMML(n=2), RCMD-RS(n=1), RAEB-2(n=1), 5q- syndrome(n=1) and IPSS: low(n=6) and intermediate-1(n=2), in patients with cytogenetic results. Our preliminary results show that p15 methylation occurred in 8(50%) and p16 methylation occurred in 12(75%) MDS patients. None of the patients had methylation of p53 or TRAIL-Rs. Patients with and without p15 methylation had similar WHO subtypes, however p16 methylation was found in 8 of the 9 patients with RCMD subtype. The two patients with IPSS intermediate-1 have p15 and p16 methylation. None of the patients evolve to acute leukemia, with a median follow-up of 19 months(7-60). This study suggest that in this cohort of patients, p15 and p16 seem to be an event in the MDS development and the use of hypomethilant agents may be considered as a therapeutic approach. However to the identification of prognostic markers we need to increase our data.

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Myelodysplastic syndromes - Clinical II

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IRON OVERLOAD AND CHELATION THERAPY PATTERNS IN LOW RISK MYELODYSPLATIC SYNDROMES WITH TRANSFUSION REQUIREMENTS. A CROSS SECTIONAL OBSERVATIONAL NATIONAL STUDY IN SPAIN (IRON STUDY)

F. Remacha,[1] B. Arrizabalaga,[2] C. Del Cañizo,[3] G. Sanz,[4] A. Villegas[5]

[1]Hospital de Sant Pau, BARCELONA; [2]Hospital de Cruces, BARAKALDO(VIZCAYA); [3]Hospital Universitario Salamanca, SALAMANCA; [4]Hospital La Fe, VALENCIA, Spain; 5Hospital Clínico San Carlos, MADRID, Spain

Background. Most patients with low risk myelodysplastic syndromes (MDS) require blood transfusions and develop iron overload (IO). Iron chelation therapy (ICT) can be used to avoid organ damage secondary to IO. *Aims.* To evaluate the current ICT patterns in low risk MDS with transfusion requirements in Spain *Methods*. Cross sectional observational multicenter study carried out in Spain (Iron Study). *Inclusion criteria*. MDS patients with low or intermediate IPSS risk score or 0-1 Spanish score who had received at least 10 red blood cell units (RBCu). Spanish score was used because cytogenetics were not available in 28 % of the patients. The following variables were collected: serum ferritin (sFt) at diagnosis, sFt before chelation, final sFt, total number of RBCu and amount of transfused iron received (both lifelong and in the last year). ICT administration and changes in sFt following ICT. *Results.* In the present study, 626 patients from 81 Spanish centers were enrolled. Seventy seven patients were excluded for different reasons and 549 cases were available for evaluation. (median age 77 years, median time from diagnosis 3 years). According to WHO classification 34.1% were refractory anemia with ringed sideroblasts, 24% refractory anemia, 12.8% refractory cytopenia with multilineal dysplasia without ringed sideroblasts and 8.2% with ringed sideroblasts (8.2%), 10% were 5q- syndrome, and other subtypes were 11.3%. Median time from the first transfusion to inclusion was 2.7 years. Mean number of RBCu transfused was 56 units per patient lifelong and 25.7 units during the last year. Total iron transfused was 11.2 g and 5.1 g during last year. Mean sFt level at diagnosis was 589 microg/L, (13% of cases with sFt > 1000 microg/L) and 1964 microg/L at the beginning of ICT that was started in 202 patients (36.8%). Moreover, 91.7 % of MDS patients with age <65 years and sFt > 1000 microg/L received ICT. Deferoxamine (DFO) was used in 187 patients (92.6%). However, only 13% of patients received minimally effective dosage of DFO therapy (>25 mg/kg sc over > 8 hours or 1 g/12 h/d sc in bolus, at least 3 d/week). DFO remained in only 11 cases (5.4%) after 12 months. Due to this limited use of DFO there was an overall significant increase in sFt (1986 microg/L before *vs* 2480 microg/L after DFO, p=0.001). However, when only the 27 (13%) patients with minimally effective ICT were considered, sFt did not increase during the study period (sFt 2037 microg/L *vs* 1798 microg/L, p=NS). *Summary and Conclusions.* ICT was started in 36.8% of MDS patients with transfusion requirements, especially in youngest patients. Most patients received DFO, but few of them (5.4%) remained on treatment after one year. For those patients (13%) who received a minimally adequate DFO dosage (see above), DFO was able to maintain IO. Therefore, our data show that chelation with DFO is inadequate to control IO in the majority of MDS patients (87%) with transfusion requirements.

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EFFICIENCY AND SAFETY OF ADMINISTRATION OF ORAL IRON CHELATOR DEFERIPRONE IN PATIENTS WITH EARLY MYELODYSPLASTIC SYNDROME (MDS)

J. Cermak,[1] A. Jonasova,[2] J. Vondrakova,[3] L. Walterova,[4] M. Siskova,[2] I. Hochova,[5] R. Neuwirtova[2]

[1]Institute of Hematology and Blood Transfusion, PRAHA; [2]1st Department of Internal Medicine,Faculty Hospital Prague 2, PRAHA; [3]Department of Hematology, Faculty Hospital, OLOMOUC; [4]Department of Hematology, Regional Hospital, LIBEREC; [5]Department of Hematology, Faculty Hospital Motol, PRAHA, Czech Republic

Background and Aims. Since approximately 80% of patients with early MDS develop transfusion dependency in the course of the disease, an effective iron chelation represents an important treatment approach preventing accumulation of toxic iron within the body. In this study we

have performed a retrospective analysis of efficiency and safety of administration of oral iron chelator deferiprone (Ferriprox®, Apotex, Canada) in patients with MDS treated in the years 2005-2007. *Patients and Methods.* Ferriprox was administered in a daily dose of 40-90 mg/kd/day in 48 patients (28 females, 20 males) with early MDS without excess of blasts (6x RA, 15xRCMD, 11xRARS, 2x RCMD-RS, 14x 5q-syndrome). The median age of patients was 64,5 years (range 29-84 years), the median duration of Ferriprox administration was 9,5 months (range 1-24 months). *Results.* In 4 patients with initial serum ferritin 800-1000 μg/L, a daily dose 40-50 mg/kg of deferiprone was sufficient to maintain iron balance in patients receiving ≤3 TU of red blood cells (RBC) per month. In 18 patients with initial serum ferritin 1000-2000 μg/L, iron balance was maintained with 50-60mg/kg/day of Ferriprox in 5 patients depending on ≤2 TU/month and a negative iron balance was achieved with the dose of 75 mg/kg/day (6 cases). In 12 patients with serum ferritin 2000-3000 μg/L, only 75-90 mg/kg/day of chelator was maintaining iron balance in patients with ≤ 2 TU/month and the same dose was effective in only 2 out of 14 patients with initial serum ferritin > 3000 μg/L and receiving 1-2 TU/month. A concomitant administration of 30-40 kU/week of recombinant human erythropoietin (rHuEPO) in 13 patients led to a significant increase in daily urinary iron excretion and enabled to decrease an effective daily dose of Ferriprox to 50-60 mg/kg in patients with serum ferritin 1500-3000 μg/L. A combination of rHuEPO with Ferriprox (75-80 mg/kg/day) was the only approach maintaining iron balance in 4 heavily iron overloaded patients requiring > 2 TU/month. Gastrointestinal symptoms (abdominal discomfort, pain, nausea, vomiting, diarrhea) occurred in 18 patients (37,5%) and were the most frequent adverse effect of deferiprone administration. These symptoms limited an effective escalation of the daily dose and led to discontinuation of the treatment in 6 patients. Moderate granulocytopenia (NS = 0,5-1,0×10^9/L) occurred in 5 patients (13%), severe granulocytopenia (NS< 0.5×10^9/L) was observed in 2 patients (4%). The cessation of therapy resulted in restoration of NS counts in all but one patient. In general, the chelation was effective in 16 out of 22 patients (73%) with serum ferritin <2000 μg/L in contrast to only 12 out of 26 patients (46%) with serum ferritin >2000 μg/L. *Summary and Conclusions.* Administration of deferiprone may be an alternative approach in treatment of mild and moderate iron overload in MDS patients who cannot be treated with deferasirox. A beneficial effect of concomitant administration of rHuEPO allowed to decrease the dose of chelator and thus the incidence of adverse effects. However, the efficiency of this combination should be proved in larger studies.

0695

EFFICACY OF THE FLAG-IDA REGIMEN AS FIRST LINE TREATMENT FOR MYELODYSPLASTIC SYNDROMES (MDS) AND ACUTE MYELOID LEUKEMIA (AML): RESULTS FROM A SINGLE CENTER

M. Bernardi, M. Tassara, A. Crotta, C. Messina, J. Peccatori, A. Assanelli, D. Clerici, S. Mastaglio, F. Ciceri

San Raffaele Scientific Institute, MILAN, Italy

Background. poor prognosis MDS (IPSS intermediate-2/high risk) have a high probability to progress to AML within 2-14 months. Median survival of patients (pts) with poor prognosis MDS and secondary AML (sAML) is about 3-12 months. Allogeneic stem cells transplantation (SCT) is the therapy of choice for these patients; high dose chemotherapy with autologous SCT (ASCT) is an alternative in patients unfit for allogeneic SCT. Disease Complete Remission (CR) has to be pursued with induction chemotherapy, before autologous stem cells collection or allogeneic SCT; unfortunately, MDS and sAML are often refractory to first-line conventional treatments including cytarabine and an anthracycline, with CR<50%. The FLAG-IDA (fludarabine plus cytarabine and idarubicin) regimen seems to induce a better response rate in these cases, as documented by different reports; it probably exploits the synergistic activity of fludarabine and cytarabine, although a recently published study showed no difference in CR induction with or without fludarabine combined to cytarabine and idarubicine. *Aims.* to retrospectively evaluate the response to first-line FLAG IDA in MDS and AML pts treated at our Center. *Methods.* Between 1/1999 and 1/2008 we treated 74 newly diagnosed MDS/AML pts with the FLAG-IDA regimen (Fludarabine 30 mg/sqm days 1 to 5, cytarabine 2 g/sqm days 1 to 5, idarubicin 10 mg/sqm days 1 to 3, lenograstim 0.3 mg/day days 1 to 5 and from day +12 to hematologic recovery). Median age: 59.4 (22-76), pts ≥60=35. Diagnosis according to WHO: RCMD=2, RAEB1=6, RAEB2=13, MDS/MPD=1, therapy related MDS=3, AML MD=36, *de novo* AML=3, therapy related AML=10. Diagnosis according to FAB: RA=2, RAEB=25, RAEB-T=13, sAML=31, AML=3 Cytogenetics: good=2, normal=32, poor=7, complex=14, intermediate=16, not evaluable=3. IPSS (40 MDS): int-1=3, int-2=16, high=19, not evaluable=2. *Results.* overall CR rate after induction with one single FLAG-IDA was 63.5% (47/74); CR was 68.1% in the 69 pts evaluable around day 30, after hematologic recovery. Treatment related mortality was 8.1% (6/74). CR rate in different age groups: pts <60=69.2% (27/39), pts ≥60=57.1% (20/35), *p*=ns; toxic deaths were 3 for each age group, 7.7% and 8.6%, respectively. CR rate did not statistically differed according to diagnosis, cytogenetics and IPSS. When AML and MDS pts were compared, CR was 59.1% (29/49) and 72% (18/25), respectively (WHO, *p*=0.05), 55.8% (19/34) and 70% (28/40), respectively (FAB, *p*=0.02). *Conclusions.* in our experience the FLAG-IDA regimen proved to be most effective for MDS, before progression to acute leukaemia. Older age, unfavourable cytogenetics/IPSS and diagnosis of MDS/AML secondary to previous chemo-radiotherapy did not affect the response in our pts. Treatment related mortality was low, also in the older group of pts. According to our data, the FLAG IDA regimen confirms to be effective in poor prognosis MDS and sAML; MDS pts with a high risk of progression to AML should be treated promptly, regardless of age and disease-related risk factors, to increase their chance to accede to an appropriate transplant program.

0696

EQUIVALENT EFFICACY OF AZACITIDINE FOR PATIENTS WITH POOR CYTOGENETICS AND HIGH RISK MYELODYSPLASTIC SYNDROME

D.Y. Kim,[1] M.J. Jang,[2] J.H. Won,[3] Y.K. Kim,[4] J.S. Ahn,[4] S.H. Bae,[5] H.J. Shin,[6] I. Kim,[7] H.M. Ryoo,[5] J.H. Lee,[8] S.J. Choi,[8] Y.C. Mun,[9] S.S. Yoon,[7] D. Oh,[2] J.H. Lee,[10] H.K. Lee,[11] K.H. Lee,[8] H.J. Kim,[4] S. Park,[7] Y.H. Min,[12] B.K. Kim,[7] S.M. Bang,[7] J.H. Lee[8]

[1]Asan Medical Center, SEOUL; [2]Pochon CHA University College of Medicine, SUNGNAM; [3]Soonchuhyang University College of Medicine, SEOUL; [4]Chonnam National University College of Medicine, GWANGJU; [5]Daegu Catholic University School of Medicine, DAEGU; [6]Pusan National University School of medicine, BUSAN; [7]Seoul National University College of Medicine, SEOUL; [8]Asan Medical Center, University of Ulsan College of Medicine, SEOUL; [9]Ewha Woman's University College of Medicine, SEOUL; [10]Gachon University of Medicine and Science, INCHON; [11]Konkuk Univerisity College of Medicine, SEOUL; [12]Yonsei University College of Medicine, SEOUL, South-Korea

Background. The methylated portions of the gene, turning on the cell cycle, increase according to the progression of myelodysplastic syndrome (MDS). The methylation rate of p15 gene was 32% in RA and RARS, which increased up to 58% in RAEB, RAEB-t, and CMMoL. It can be assumed that the more methylated the genes were, the more responsive the patients with MDS were to a hypomethylating agent. According to the previous reports, it was suggested that the hypomethylating agent such as azacitidine (AZA) might be as effective in advanced stage of MDS as in early stage. *Aims.* We analyzed the efficacy of AZA on the basis of cytogenetic groups and international prognostic scoring system (IPSS). *Methods.* Patients with MDS were treated with AZA according to the dose adjustment schedules. Chromosome analysis was performed before the initiation of treatment and informed consent for the use of genetic information was obtained from each participant. Patients had received AZA until they had hematologic response, intolerable toxicity, or progression despite of treatments. Responses and toxicities were monitored regularly after the each cycle of AZA. *Hematologic response* (HR) to AZA was defined as hematologic improvement (HI) in at least one lineage of cytopenia. *Results.* From May 2006 till August 2007, 118 patients with MDS had been treated with AZA in 12 centers and 113 patients were included in analysis. The median age of all patients was 59 (20-83). A median time from the diagnosis to treatment was 3.2 months (0-183) and 72.6% of patients received AZA within less than a year after the diagnosis of MDS. The diagnoses of patients just before the initiation of AZA treatment were RA(20.4%), RARS(4.4%), RCMD(23.9%), RCMD-RS(5.3%), RAEB-1(24.8%), and RAEB-2(18.6%). Patients were distributed in LOW (3.5%), INT-1 (64.6%), INT-2 (22.1%), and HIGH (9.7%) risk groups according to the IPSS, and good (62.8%), intermediate (21.2%), and poor (15.9%) cytogenetic groups according to the chromosomal abnormalities. A median number of 4 cycles (1-15) and a median relative dose intensity of 75mg/m^2 of AZA per cycle was administered to each patient. In terms of best response, 49.6% of patients achieved HR (CR:8.0%, PR:1.8%, marrowPR:1.8%). Response rates (RR) were not different significantly between three cytogenetic groups (good: intermediate: poor = 47.9%: 50.0%: 55.6%, *p*=0.573). Difference of IPSS made no significant influence to the response rate (LOW: 25.0%, INT-1: 46.6%, INT-2: 64.0%, HIGH: 45.5%, *p*=0.329). Of the 46 patients evaluable for cytogenetic response, 13 patients achieved cytogenetic CR.

Among the 56 patients who had responded to AZA, patients with *more than PR* were evenly distributed in 3 cytogenetic groups (20.6%: 25%: 20%, p=0.953). Duration of response was not different between the 3 cytogenetic groups (6.7: 8.3: 6.5 months, p=0.507). Overall survival of all patients was 10.4 months (0.4-17.0) and patients who responded to AZA showed more favorable survival than those who did not (1-year survival: 80.9% *vs* 60.6%, p=0.018). No difference of overall survival was observed between 3 cytogenetic groups. *Conclusions*. AZA showed equivalent efficacy even in patients with poor cytogenetics and high risk international prognostic score.

0697

BORTEZOMIB IS ABLE TO INACTIVATE NF-KB AND DOWN-REGULATE WT1 GENE IN P39 CELL LINE: A POSSIBLE USE IN HIGH-RISK MYELODYSPLASTIC SYNDROMES?

S. Galimberti,[1] M. Canestraro,[1] H. Savli,[2] G. A. Palumbo,[3] B. Nagy,[4] F. Di Raimondo,[3] M. Petrini[1]

[1]Hematology, PISA, Italy; [2]Biology department - Kocaeli University, KOCAELI, Turkey; [3]Hematology Univeristy of Catania, CATANIA, Italy; [4]1st Department of Obstetrics and Gynecology, Semmelweis University, Budapest, BUDAPEST, Hungary

Background. NF-kB is one of the fundamental transcription factors, able to control the expression of genes involved in apoptosis (BCL2 and BCLxL), cell cycle progression, inflammation, and angiogenesis (including IL6, IL8, VEGF). NF-kB is normally bound in the cytosol to inhibitor KB-a; degradation of IKBa is required for NF-kB translocation into the nucleus and activation of target genes. Bortezomib, a proteasome inhibitor already adopted in treatment of resistant/relapsed patients affected by multiple myeloma, by impending degradation of IKB, allows NF-kB to remain in the cytoplasm, so blocking activation of its downstream targets. In the 2006 Braun and coworkers unequivocally showed that NF-kB is constitutively activated in P39 cell line and that its activity levels were higher in advanced myelodysplastic syndromes (MDS), with a higher risk of transformation into overt acute leukemia. These Authors also reported that treatment with proteasome inhibitors, including bortezomib, resulted in loss of the mitochondrial transmembrane potential, apoptosis, and loss of viability. Another gene over-expressed in high-risk MDS is the Wilm's tumor (WT1) gene. Its expression levels have been reported to play a relevant prognostic role both in evolution into acute leukemia and in predicting relapse of myeloid leukemia, even after allogeneic transplantation. Interestingly, the presence of two NF-kB binding sites within the WT1 promoter has been reported; thus, we decided to assess if a compound able to inactivate NF-kB would be also able to down-regulate the WT1 expression in the P39 cell line. Also in our model, bortezomib was able to inactivate NF-kB, as shown both by EMSA and immunofluorescent tests; it exerted an anti-proliferative and pro-apoptotic effect, by blocking cell cycle in the G2 phase. Moreover, we found that bortezomib significantly increased the release of reactive oxygen species (ROS), without any differentiating effect. Finally, bortezomib significantly down-regulated the WT1 expression, in a dose- and time-dependent way. *Materials and methods*. Because no information about which genes were de-regulated by bortezomib, we performed gene expression assays, by using both TaqMan® Low Density Array Human Apoptosis Panel (Applied Biosystem), and Agilent platforms. The genes identified as de-regulated by bortezomib were then analyzed for network and gene ontology by Ingenuity Pathway Analysis software.

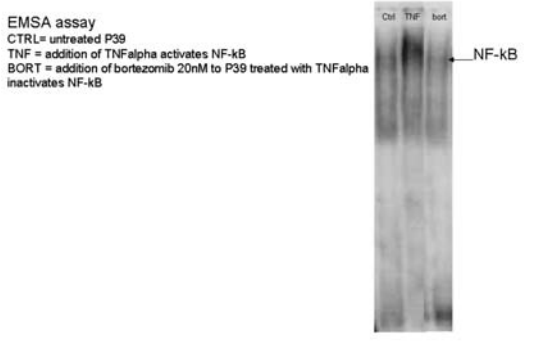

Figure 1.

Results. In the untreated P39, 84 of the 93 genes involved in the apoptosis pathway and represented in the Taqman Low-Density Arrays were expressed. Already after 12h-treatment, bortezomib was able to up-regulate DIABLO and NFKBIB, (a NF-kB inhibitor), and down-regulate NF-kB1, NF-kB2, and BIRC1, an anti-apoptotic gene. These results arising from gene expression studies support the observed NF-kB inactivation and the pro-apoptotic effect exerted by bortezomib. Moreover, microarray assays showed that the PPAR, P53, IL6, IL2, hypoxia, Huntington's disease, TLR and cell cycle were the pathways more significantly modified after exposure to bortezomib. Interestingly, among down-regulated genes, JUN, HSP70 and HSP90 seem to be clinically relevant. Indeed, high levels of HSPs have been reported to negatively condition the overall survival of patients affected by high-risk MDS. Other interesting genes down-regulated by bortezomib were CREBBP, PMAIP1, SPP1, and some adhesion molecules, such as ICAM1. Even reduction of integrins could be a relevant effect exerted by the proteasome inhibitor, because higher serum levels of ICAM1 have been reported to be associated with high-risk MDS and to negatively condition the survival of these patients. *Conclusions*. In summary, biological results and gene expression assays suggest the possible use of proteasome inhibitors in treatment of high-risk MDS. *in vivo* trials will be useful to confirm this hypothesis coming from *in vitro* studies.

0698

THE TCR REPERTOIRE OF PATIENTS WITH MYELODYSPLASIA DISPLAYS A HIGH FREQUENCY OF SELECTIVE EXPANSIONS IN BOTH CD4+ AND CD8+ T-CELLS ALONG WITH A DRAMATIC CONTRACTION CONFINED TO THE CD8+ SUBSET

C. Fozza, S. Contini, A. Galleu, M.P. Simula, M. Longinotti

University of Sassari, SASSARI, Italy

Background. The clinical history of patients with myelodysplastic syndrome (MDS) is often characterized by autoimmune manifestations. Moreover, several studies suggest an immune-mediated origin for the marrow failure observed in this group of disorders. However, the exact degree of perturbation of the immune system as well as its potential role in the pathogenesis of MDS are yet to be elucidated. *Aims.* In this study we deeply analysed the status of the T-cell immune system in patients with MDS, correlating its pattern with the most relevant clinical and laboratory parameters. In particular, we focused on the degree of skewing and, more specifically, on the overall frequency of oligoclonalities in the T-cell receptor (TCR) repertoire of different T-cell subsets. *Methods.* We performed our analysis in 30 patients (7 RA, 4 RARS, 10 RCMD, 8 RAEB and one 5q- syndrome) and 15 age-matched controls. We firstly analysed the profile of the third complementarity-determining-region (CDR3) in separated helper and cytotoxic T-cells by spectratyping. After immunomagnetic $CD4^+$/$CD8^+$ cell separation, RNA extraction and reverse transcriptase PCR, CDR3 fragment analysis was performed through capillary electrophoresis. Spectratyping evaluation was carried out by determining the percentage of skewed and oligoclonal beta-variable (BV) subfamilies. Flow cytometric analysis was based on a panel of 24 BV family-specific antibodies, combined in groups of 3, one antibody being conjugated to FITC, another to PE, and the third to FITC/PE. Costaining was performed with anti-CD4/anti-CD8 PerCp. *Results.* By using a qualitative approach, we first looked at the overall degree of TCR repertoire skewing in the two cell subpopulations, via spectratyping. The repertoire of MDS patients was mostly Gaussian in $CD4^+$ T-cells, whereas $CD8^+$ T-cells were characterized by an extremely high frequency of skewed BVs when compared to normal controls (mean 88 *vs* 74%). We then considered the frequency of oligoclonal BVs in the T-helper and T-cytotoxic repertoires. Once again we detected a very low number of oligoclonal profiles in $CD4^+$ T-cells, whilst the $CD8^+$ subset showed in all the patients an unexpectedly high frequency of oligoclonal BVs (mean 28 *vs* 10% in normal controls). We then determined by flow cytometry the frequency of quantitatively expanded T-cell subpopulations, showing in patients an increased number of oligoclonal lymphocyte subpopulations when compared to normal controls, in both $CD4^+$ (5 *vs*1%) and CD8+ (5 *vs* 2%) T-cells. When we looked at the possible influence of several disease-related factors, such as IPSS score, WHO subtype, degree of cytopenia etc., we could not find any difference between patients with a less or more aggressive disease. *Summary and Conclusions.* The present study shows that the immune system of patients with MDS is overall extremely contracted, especially in the $CD8^+$ T-cell subsets. Moreover, it is characterized by an extremely high frequency of selective proliferations of both cytotoxic and helper T-cells. Our findings further underline the deepness of the immune dysregulation observed in course of MDS. Moreover, they highlight the difficulty of discriminating immun-

odominant clones hypothetically responsible of the functional inhibition of the bone marrow haematopoiesis in the context of a highly skewed TCR repertoire.

0699

USAGE PATTERNS AND TRANSFUSION REQUIREMENTS IN PATIENTS ENROLLED IN AVIDA, A LONGITUDINAL REGISTRY OF PATIENTS WITH HEMATOLOGIC DISORDERS RECEIVING AZACITIDINE

L. Grinblatt,[1] X. Narang,[2] M. Malone,[3] A. Sweet,[4] S. Dunne,[4] A. Sullivan[4]

[1]Evanston Northwestern Healthcare, EVANSTON; [2]White River Diagnostic Center, BATESVILLE, AR; [3]Oncology-Hematology Medical Associates of the Central Coast, SAN LUIS OBISPO, CA; [4]Pharmion Corporation, BOULDER, CO, USA

Background. Myelodysplastic syndromes (MDS) are a heterogeneous group of myeloid neoplasms that are characterized by ineffective hematopoiesis and peripheral cytopenias. Until recently, most patients received supportive care alone and will require a transfusion at some point during the course of their disease. Treatment decisions are often based on International Prognostic Scoring System (IPSS) classification, MDS subtype, transfusion independence, cytopenias, performance status (PS), and age. Azacitidine, a hypomethylating agent, is approved in the US for a dosing schedule of 75 mg/m^2/day x 7 day q 28 days for the treatment of all 5 MDS subtypes. However, the dose and schedule of azacitidine used in clinical practice varies. AVIDA is a unique, longitudinal, multicenter patient registry designed to prospectively collect data from community-based hematology clinics on the natural history and management of patients with MDS and other hematologic disorders, including acute myeloid leukemia, who are treated with azacitidine. *Aims.* To further the understanding of current azacitidine treatment patterns in the community, identify common concomitant care procedures and concomitant treatments, and document transfusion requirements. *Methods.* Baseline demographics and disease characteristics were obtained at enrollment. Azacitidine treatment patterns (dose and dosing schedule), administration methods, transfusion requirements, and onset of red blood cell (RBC) transfusion independence (no transfusions for 56 days and have received 2 or more cycles of azacitidine) were recorded.*Results.* As of February 19, 2008, 136 patients (95 males, 41 females; mean age, 73.7 yrs) have been enrolled in AVIDA. Most patients had low-risk MDS per IPSS (62% had low or Int-1 risk). Median time from first MDS diagnosis until azacitidine treatment was 2.8 months (mean, 13.8 months). Majority (82%) of patients had primary MDS and 77% had a baseline performance status of 0 or 1. Eighty (59%) patients had a history of RBC transfusion and 25 (18%) patients had a history of platelet transfusions; 47 (35%) patients had no history of any transfusion. Treatment data are available for 126 patients. A total of 360 cycles (median, 2; range, 1-14) of azacitidine have been administered (46% via subcutaneous injection). The most common dose and schedule is 75 mg/m^2 (81%) at 5 days on treatment (53%). Seventy patients have received at least 2 cycles of azacitidine and had available prior transfusion data. Of patients with transfusion history, 37% (15/41) achieved RBC transfusion independence; onset occurred within the first 2 cycles for 67% (10/15) and continued to occur during cycles 3 to 6. Of patients without a transfusion history, 79% (23/29) remained RBC transfusion independent; onset occurred within the first 2 cycles for 100% (23/23). *Conclusions.* Based on available data from the first 136 patients from AVIDA, the characterization of azacitidine treatment patterns in the community-based setting is beginning to emerge. Early AVIDA data suggest that alternative dosing regimens may provide benefit in achieving transfusion independence.

0700

REVISITING GVL EFFECT OF AZACITIDINE TREATMENT IN RELAPSED MDS PATIENTS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

S.G. Cho, S.Y. Kim, B.S. Cho, M. Kim, K.S. Eom, Y.J. Kim, H.J. Kim, S. Lee, C.K. Min, D.W. Kim, J.W. Lee, W.S. Min, J.W. Park, C.C. Kim

Catholic University of Korea, SEOUL, South-Korea

The age preponderance and frequent co-morbidity of the patients with of myelodysplastic syndrome (MDS) and acute myeloid leukemia secondary to MDS (AML-MDS) made non-myeloablative stem cell transplantation (NST) being explored widely as an important treatment modality. However, the therapeutic options for patients with relapse after transplantation are limited. Attempts to maximize the GVL effect by donor lymphocytes infusion (DLI) have been generally unsuccessful for restoration of complete remission and in combination with chemotherapy, the response rates can be increased, but remission duration is short and long-term survival is rare. These results come from their lack of effectiveness and treatment related mortality caused by toxicity, life threatening graft-versus-host disease (GVHD) and their associated infections. The hypomethylating agents used in MDS and AML secondary to MDS have an advantage over conventional cytotoxic chemotherapeutic agents in terms of organ toxicity. Therefore, these agents may be safely applied for the patients who relapse after allogeneic stem cell transplantation. We have treated three MDS patients who relapsed post-transplant with 3 to 4 cycles of azacitidine (50-75 mg/m^2) for 7 consecutive days at intervals of 4 weeks. All the patients responded to azacitidine treatment, with two patients achieving complete remission and one patient having partial remission, and achieved a restoration of the previous level of donor chimerism. Times to initial response and best response were after 2-3 cycles and after 3-4 cycles, respectively. The common toxicity of azacitidine was grade III to IV cytopenia and associated neutropenic fever. Toxicity was transient and patients usually recovered in time for the next treatment cycle. Of note, all three patients experienced reemergence or aggravation of chronic GVHD after azacitidine treatment which was transient or easily controlled with immunosuppressive agents, which suggests revisiting GVL effect of azacitidine can strengthen antileukemic effect. All three responders are still surviving in a transfusion-independent state. Follow up durations from azacitidine treatment are 4, 7, and 15 months. The relatively high response rate of these posttransplant patients compared with non-transplanted patients is probably due to its additional GVL effect. The earlier works demonstrated that treatment with DNA- demethylating agents resulted in a rapid and stable induction of transcription and cell surface expression of formerly unexpressed killer Ig-like receptors in NK cells, the increased expression of MHC class I and II molecules on the surface of the recipient leukemic cells, and the expression of tumor specific antigen to levels above the threshold for immune recognition *in vivo* which is recognized by tumor specific antigen-specific cytotoxic T lymphocytes. With the limitation of small number of cases and short duration of follow-up, the effectiveness of the hypomethylating agent seems to be considerable and its toxicity in transplanted patients is acceptable. Hypomethylating treatment can be promising alternative treatment strategy for relapsed posttransplant MDS patients, which can not only have a direct antileukemic effect on recipient leukemic cells but also can enhance GVL effect.

0701

TWICE-WEEKLY HIGH-DOSE RHUEPO FOR THE TREATMENT OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES

R. Latagliata,[1] E.N. Oliva,[2] P. Volpicelli,[1] I. Carmosino,[1] M. Breccia,[1] I. Vincelli,[2] C. Alati,[2] L. Napoleone,[1] F. Vozella,[1] F. Nobile,[2] G. Alimena[1]

[1]Hematology- University "La Sapienza", ROME; [2]Hematology - "Bianchi-Melacrino-Morelli" Hospital, REGGIO CALABRIA, Italy

Background. Recombinant human erythropoietin (rHuEpo) is effective in about 30% of anemic patients with myelodysplastic syndrome (MDS). Recently, it has been suggested that adequate doses may increase response rates. We performed a prospective non-randomized Phase II study to evaluate the efficacy in terms of erythroid response of high dose rHuEpo in 60 adult patients with primary MDS. *Methods.* Patients were included if Hb < 11 g/dL and previously untreated. Patients received rHuEpo 40,000 U s.c. twice weekly until erythroid response evaluation (according to Cheson criteria) at 3 months. Once weekly dosing was considered for patients with Hb increase ≥2 g/dL within the first 2 weeks of therapy or in patients reaching Hb = 12 g/dL. Responders continued to receive treatment at dose adjusted to maintain Hb level not exceeding 12 g/dL until loss of response while non responders at 3 months went off the study. *Results.* Sixty patients (26M/34F, median age 73.1 years. interquartile range 63.2 - 80.4) were included. Of the 38 patients with evaluable cytogenetics, 21 were normal, 6 had del(5q) as a single abnormality, 2 had trisomy 8, 3 had other single abnormalities, 3 had double abnormalities and 3 had complex karyotypes. IPSS score among evaluable patients was low and Int-1 in 92%, while in 3 patients it was Int-2. Median disease duration was 12 months (interquartile range 3-31). Serum Epo levels were < 500 mU/mL in all patients. Mean Hb in 28 transfusion-free (TF) patients was 9.1±SD 0.9 g/dL; 32 transfusion-dependent (TD) patients required a median number of monthly transfusions of 1.5 (interquartile range 1-2). Thirty patients (50%) had an erythroid response in a median time of 7 weeks (interquartile range 5-9). Nineteen out of 28 TF patients (67.8%) had a mean Hb increase of

2.2±SD 0.6 g/dL, 6 reached target Hb 12 g/dL and 15 required dose reduction for increase in Hb above 12 g/dL. Eleven out of 32 TD patients (34.3%) achieved a reduction in transfusion requirement in a median time of 9 weeks (interquartile range 6 - 10) and 8 patients (25%) became transfusion-free. Median duration of response was 12 months (interquartile range 5-19) in TF patients and 6 months (interquartile range 2-21) in TD patients. At univariate analysis, factors associated with response were transfusion independence (p=0.019), serum erythropoietin levels (median 68 in responders, median 152 in non responders, p=0.059), baseline Hb levels (mean 8.8±SD 1.2 in responders, 8.0±SD 0.8 in non-responders, p=0.003), and cytogenetics (p=0.016), and these factors maintained their significance at multivariate analysis. In particular, at logistic regression analysis for each g/dL increase in baseline Hb, the probability of response increased by 98% (p=0.02). *Summary.* Adequate doses of rHuEpo show a good response rate compared to previous results reported with lower dosing schedules. Randomized studies comparing different dosing regimens are thus required to confirm these findings.

0702

AZACITIDINE IN COMBINATION WITH EPO+G-CSF AND VALPROIC ACID RAPIDLY DETERMINES HEMATOLOGICAL IMPROVEMENT IN PRETREATED NON RESPONSIVE IPSS INT-1 MDS PATIENTS

V. Santini,[1] A. Gozzini,[2] T. Lunghi,[2] A. Bosi[2]

[1]University of Florence, FIRENZE; [2]AOU Careggi, University of Florence, FLORENCE, Italy

Background. The DNMTinhibitor azacitidine (AZA) has been approved by FDA for treatment of patients with myelodysplastic syndromes (MDS) of all IPSS risk scores. In Europe AZA is under approval on the basis of excellent survival results obtained in the AZA-001 international phase III study, involving only INT-2 and high risk MDS patients. In fact, a large number of MDS patients with lower IPSS score could be advantageously treated with azacitidine alone or combined with other agents, but data focused on this specific subset of patients are lacking. *Aims.* We evaluated whether azacitidine, in combination with growth factors and the histone deacetylase (HDAC) inhibitor valproic acid (VPA) could determine haematological response in pretreated, refractory MDS patients with IPSS score INT-1. *Methods.* We treated 11 patients with azacitidine 50 mg/m^2/day for five days, plus erythropoietin (hrEPO) 40.000 U twice weekly, granulocyte colony stimulating factor (hrG-CSF) 300mg, once weekly and VPA 600-1200mg/day. These patients were not eligible for treatment with AZA in any of the ongoing trials and all of them had undergone previous treatment with EPO plus G-CSF for more than 24 weeks without response. One patient had been treated also with thalidomide 100mg/day, but no response was observed, due to intolerance to treatment and subsequent early drop out. All patients were RBC transfusion dependent, 4/11 both for RBC and platelets. Mean age was 62.8 (34-80). None of the patients had more than 6% bone marrow blasts and only 1/9 had a complex karyotype. Patients received a mean of 6 courses, which were very well tolerated, with only nausea grade 1-2. *Results.* Myelosuppression was extremely mild and cycles were administered at regular interval of 21-28 days without delay. In particular, the slow escalation in VPA doses prevented CNS side effect. VPA blood concentrations were kept within neurological therapeutic range. At present, 11/11 patients showed haematological improvement (1/11 CR). In particular, RBC and platelet transfusion independence or significant reduction in transfusion requirement was achieved for 9/11 patients, with also general reversal of neutropenia. In one patient, starting with platelets counts below 10x109/L and regularly transfused weekly, platelet number was within normal range after only 2 cycles of therapy. AZA was used at lower doses than in CALGB and Phase III trials, according to recent evidence of efficacy of the drug, even at 50 mg/m^2/die. *Conclusions.* Combination treatment with low dose-azacitidine, growth factors and the HDACi VPA was safe and well tolerated, and was extremely effective in inducing rapid hematological improvement in this limited cohort of pretreated, resistant INT-1 risk MDS patients.

0703

ERYTHROPOIETIN (EPO) THERAPY AND MYELODYSPLASTIC SYNDROME (MDS): SEARCHING FOR A COMMON IMMUNOPHENOTYPE IN RESPONDERS

P. Font,[1] D. Subirá,[2] J. Loscertales,[3] L. Villalón,[4] S. Ramiro,[5] C. Aláez,[1] C. Serrano,[2] C. Martínez-Chamorro,[3] A. Escudero,[3] J.M. Fernández-Rañada[3]

[1]Clinica Moncloa, MADRID; [2]Fundación Jiménez Díaz, MADRID; [3]Hospital Quirón, MADRID; [4]Fundación Hospital Alcorcón, MADRID; [5]Gemolab Laboratory, MADRID, Spain

Background. Response to EPO in MDS patients has been associated with low serum EPO (sEPO) levels, low previous transfusion requirements, low-risk IPSS categories, and limited marrow blasts. Flow cytometry immunophenotyping (FCI) has been recently introduced for studying MDS, showing a good correlation with FAB and WHO categories. However, predictive value of FCI in current therapies has not been addressed. *Aims.* To establish whether there is a correlation between response to EPO and FCI features. *Patients*. 30 MDS patients (17 male/13 female) classified as: 1 refractory anaemia (RA), 9 refractory anaemia with ringed sideroblasts (RARS), 9 refractory cytopenia with multilineage dysplasia (RCMD), 3 refractory anaemia with excess of blasts (RAEB), and 1 5q-. Seven patients did not fulfil WHO criteria, and were labelled anemia and multilineage dysplasia (A-MD), two of them, had ringed sideroblasts. All patients belonged to low or intermediate-1 IPSS. Twenty-nine patients were treated with EPO and 1 with EPO+G-CSF. Pretreatment sEPO levels were <200 IU/L in 18/20 patients studied. *Methods.* Bone marrow samples were evaluated by FCI. Data studied were: 1 - In myeloid lineage: abnormal granularity, CD45 distribution, phenotypic maturation pattern (CD16/CD11b/CD13), and absence of CD10 expression on mature granulocytes. 2 - In monocytic lineage, decreased (<2%) or increased (>10%) percentage of monocytes; and aberrant antigenic expression. 3 - In myeloblasts, identification of >5% CD34+ cells, and evaluation of CD7 and TdT expression (positive expression was described when >10% of CD34$^+$ cells were positive for any of these antigens). 4 - In B-cells, detection of a low percentage of CD10$^+$/TdT$^+$ B-cells (<1% of BM B-cells). 5- In red cells, proportion of nucleated erythroid cells. *Results.* According to the International Working Group 2006 consensus criteria for treatment response, 16 patients (1 RA, 7 RARS, 2 RCMD, 1 RAEB, 5 A-MD) showed response to EPO at week 12, and the remaining 14 patients did not. Responders to EPO needed few, or none previous transfusions, and sEPO was <200 IU/L in 14/16 patients responders. Non-responders had high requirements of transfusion and the two patients with high sEPO levels were in this group. Comparison of FCI data between the 2 groups showed that percentage of CD34$^+$ cells was <2% in 16/16 responders, and in 5/14 non-responders (p<0.05). Early stages of B-cell maturation were preserved in 14/16 responders, and in 2/13 non-responders (p<0.05). No statistically significant differences were detected in the remaining data: aberrant CD7 expression was similar in both groups (9/16 in responders, and 8/14 in non-responders, p>0.05). Proportion of erythroid precursors ranged from 7 to 48%, in responders and 3 to 32% in non-responders. All cases with isolated erythroid dysplasia (9 RARS, 1 RA) showed granulocytic dysplasia by FCI. *Conclusions.* With the number of cases studied, low percentage of CD34$^+$ cells, and normal B-cell precursors were associated with response to EPO. These data might be an indirect evidence of a better preserved medullar compartment. Neither abnormal expression of CD7 in CD34$^+$ cells, proportion of nucleated red-cells, nor myeloid FCI dysplasia in RA/RARS, showed any impact in response to EPO.

0704

DEFERASIROX IS THE ONLY IRON CHELATOR ACTING AS A POTENT NF-KB INHIBITOR IN MDS CELLS AND LEUKEMIC CELL LINES

E. Messa,[1] R. Catalano,[1] V. Rosso,[1] F. Messa,[1] F. Arruga,[1] I. Defilippi,[1] S. Carturan,[1] A. Rotolo,[1] D. Gioia,[2] E. Bracco,[1] A. Roetto,[1] A. Levis,[2] C. Camaschella,[3] G. Saglio,[1] D. Cilloni[1]

[1]University of Turin, TURIN; [2]Hematology Institute, ALESSANDRIA; [3]Vita Salute S.Raffaele, UNIVERSITY AND IRCC OF MILAN, Italy

Background. Patients affected by MDS undergo iron overload due to a large amount of blood transfusions. Recently oral chelation is available to reduce iron induced organ damage. It was also been reported that iron chelation therapy can reduce red cells and platelets transfusion requirement. This finding has been confirmed by our group. Iron activates NF-

kB through TNFα release. It was demonstrated that NF-kB is abnormally activated not only in AML but also in MDS patients. *Aims.* to evaluate the effects of the iron chelators commercially available on NK-kB activity and to identify a possible mechanism responsible for the observed reduced transfusion requirements during chelation therapy. *Methods.* 40 PB samples were collected from MDS patients: 18 were RA, 14 RAEB, and 8 AML secondary to MDS (s-AML). 30 of them presented iron overload (by SQUID biomagnetic liver susceptometry) and high serum ferritin levels. The remaining 10 patients were collected before starting transfusion therapy and were not affected by iron overload. MNC cells were incubated with 50 μm Deferasirox for 18 hrs. K562 and HL60 cells were analyzed as control and were incubated with Deferasirox, Deferiprone and Deferioxamine. Incubated and control cells were evaluated for NF-kB activity using both EMSA and ELISA method. Results We detected an increased activation of NF-kB as compared to healthy subjects in 6 out of 18 RA, 12 out of 14 RAEB, in all the cases of s-AML and in cell lines. No significant difference was detected in NF-kB activity comparing patients with or without iron overload (p=0,5). The levels of NF-kB activity increase during disease progression being higher in RAEB and s-AML as compared to RA (p=0,003). Among patients with increased NF-kB (n=14) the incubation with Deferasirox induced a significant reduction of NF-kB activity (p=0,0002). No difference was detected in NF-kB inhibition comparing patients with or without iron overload. In addition, we incubated HL60 and K562 cells with all the 3 iron chelators. Only Deferasirox was able to reduce NF-kB activity *in vitro*, while both Deferioxamine and Deferiprone trigger NF-kB activation. *Conclusions.* NF-kB is abnormally activated in MDS patients and this is not apparently related to iron overload being present in many patients with normal serum ferritin levels and in cell lines as well. Deferasirox acts as a potent NF-kB inhibitor so explaining the improvement of Hb levels and the observed reduction of transfusion requirement occurring in a percentage of patients. Moreover, this effect looks independent from the reduction of iron strorage induced by oral chelation and is peculiar of Deferasirox since this effect is not shared by the other iron chelators acting *in vitro* in the opposite way.

0705

POLYMORPHISMS OF GSTP1, GSTM1, GSTT1 AND NQO1 GENES IN PATIENTS WITH TREATMENT - RELATED MYELODYSPLASTIC SYNDROME OR ACUTE LEUKEMIA

E. Georgiou,[1] M. Papaioannou,[2] F. Samarah,[1] J. Christakis,[2] N. Vavatsi -Christaki[1]

[1]Dept. of Biochemistry, School of Medicine, Aristotle University of Thessaloniki, THESSALONIKI; [2]Dept. of Hematology, "Theagenion" Cancer Center, THESSALONIKI, Greece

Glutathione S-transferases (GSTs) constitute a multigene family of enzymes that deactivate many carcinogenic substrates by catalyzing their conjugation to glutathione. NAD(P)H quinone oxidoreductase (NQO1), is an enzyme that depending on the substrate can either bioactivate or detoxify quinones. Their genetic polymorphisms have been associated with susceptibility to solid tumors as well as primary and therapy-related leukemias and myelodysplastic syndromes. The aim of this study was to evaluate genetic susceptibility to therapy-related leukemia and myelodysplastic syndrome (TRL/MDS) in Greek patients by analyzing the gene polymorphism of the enzymes; NQO1, GSTP1, GSTM1, and GSTT1. *Patients and Methods.* DNA was isolated from the peripheral blood leucocytes of 12 (4 male and 8 female) patients with TRL/MDS and 36 age- and sex- matched healthy individuals. Among the patients, 7 had previously received both chemotherapy (CT) and radiotherapy (RT) for a previous neoplasm, while 3 had received only CT and 2 only RT. The primary neoplasms were solid tumors in 7 of the patients and hematological malignancies in the remaining 5 (2 Hodgkin's and 1 non- Hodgkin's lymphoma, 1 multiple myeloma and 1 B-acute lymphocytic leukemia). PCR- RFLP technique was used to detect the GSTP1 A1578G and the NQO1 C609T polymorphisms while PCR alone was used to detect the GSTM1 and GSTT1 deletion polymorphisms. Beta-globin gene amplification was used as an internal control. Fischer's exact test for small samples and odds ratio (OR) were used to evaluate possible susceptibility to TRL/MDS in polymorphism carriers. *Results.* Homozygous NQO1 C609T polymorphism was the only one that showed statistically significant higher frequency among patients compared to controls (25% *vs* 2.8%, p=0.04 OR = 11.3, 95% CI=1.0-122.3). The difference in frequency of the other three gene polymorphisms, although higher in the group of patients, was not statistically significant (p=0.44, p= 0.08, p=0.07 and OR = 1.0, OR = 3.0 and OR = 3.0 for GSTP1, GSTM1, GSTT1 respectively). Besides, patients were more likely to carry two different gene polymorphisms than controls (OR=2.5, 95% CI 0.5-11.0). Three of the patients and none of the controls had three gene polymorphisms (p=0.012). *Conclusions.* Homozygous NQO1 C609T transition and combination of multiple gene homozygous polymorphisms appear to increase the risk of developing TRL/MDS in Greek patients receiving cytotoxic therapy, although some of these polymorphisms are frequent in the general population. Extension of this survey to a higher number of patients is necessary to confirm these results.

0706

GENOME-WIDE COPY NUMBER ANALYSIS IN CHILDHOOD MDS USING ARRAY-CGH

I. Praulich,[1] C. Kratz,[2] C. Flotho,[2] G. Göhring,[3] P. Lichter,[4] C. Niemeyer,[2] B. Schlegelberger,[3] D. Steinemann[3]

[1]Hannover Medical School, HANNOVER; [2]Pediatric Hematology and Oncology, University of Freiburg, FREIBURG; [3]Institute of Cell and Molecular Pathology, Hannover Medical School, HANNOVER; [4]Deutsches Krebsforschungszentrum (DKFZ), HEIDELBERG, Germany

Background. Although characteristic chromosomal aberrations like monosomy 7, trisomy 8 and trisomy 21 have long been known in childhood MDS, about 50% of patients show normal karyotypes. In adult MDS, novel copy number alterations (CNAs) have been identified in patients with normal karyotypes and in patients with known cytogenetic aberrations using molecular karyotyping. *Aims.* We aimed to systematically evaluate CNAs by means of array-CGH in MDS with monosomy 7 (25 cases) in comparison to JMML (10 cases), advanced MDS (5 cases) and RC (5 cases) to identify molecular targets that may play a role in the pathobiology of MDS. *Methods.* A microarray containing around 8000 BAC/PAC clones leading to a genome-wide resolution of at least 1 Mb was used. In 18 cases with monosomy 7, high resolution oligo-CGH-arrays with a 7 kb probe spacing were additionally used. To validate the identified CNAs, quantitative PCR was performed. *Results.* The number of CNAs observed in individual cases ranged from 0 to 36. Using BAC arrays, the following CNAs with a size of 1 MB to 100 MB were found: loss of 3p12-3p14 containing the TSG FHIT (1 advanced MDS), a loss of 3q22-3q29 (1 JMML), a loss of 6q27 (2 JMML), a loss of 9q34 (1 JMML, 1 MDS-7), a loss of 12p12-13 (1 MDS-7), a gain of 12q13 (1 JMML), a microdeletion in 17p11.2 containing GRAP, a novel SH3-SH2-SH3 adaptor protein that couples tyrosine kinases to the RAS pathway (1 RC), a loss of 17p13 (1 JMML, 2 MDS-7), a loss of 21q21 with coexistent gain of 21q22 (1 JMML) and other CNA. Chromosomal deletions were more often observed than gains or amplifications. 18 cases with monosomy 7 were analysed with high resolution arrays to search for minute alterations in the submegabase range. Reucurrent focal deletions targeting GSTT1 (22q11.23) in 8/18, NOTCH1 (9q34) in 8/18, NRAS (1p13) in 12/18, and API5 (11p12) as well as gain of IFITM1 (11p15.5) in 5/18 cases each were identified and confirmed by quantitative PCR. *Conclusions*. The application of high resolution array-CGH to study karyotypic abnormalities leads to the identification of recurrent regions of allelic imbalances containing candidate tumor suppressor genes or oncogenes and may help to identify deregulated cellular pathways or signal cascades in childhood MDS.

Myelodysplastic syndromes - Diagnostic

0707

EUROPEAN LEUKEMIANET (ELN) PROJECT DIAGNOSTIC PLATFORM (WP10): PRELIMINARY RESULTS OF THE EUROPEAN MORPHOLOGY CONSENSUS PANEL

G. Zini,[1] B. Bain,[2] G. Castoldi,[3] J. Cortez,[4] J. Csomor,[5] E. Faber,[6] A. Giagounidis,[7] T. Haferlach,[8] P. Kacirkova,[9] K. Lewandowski,[10] V. Liso,[11] E. Matutes,[12] M. Maynadie,[13] J. Meletis,[14] A. Porwit,[15] M.L. Ribeiro,[16] L. Sréter,[17] A. Tichelli,[18] T. Vallespì,[19] M.B. Van't Veer,[20] S. Woessner Casas,[21] M.C. Béné[22]

[1]Catholic University Sacred Heart, ROME, Italy; [2]St. Mary Hospital, LONDON, United Kingdom; [3]S. Anna Hospital,University of Ferrara, FERRARA, Italy; [4]Faculty Medica Science, New University Lisboa, LISBON, Portugal; [5]Haemopathology Laboratory - Semmelweis University, BUDAPEST, Hungary; [6]University Hospital, OLOMUC, Czech Republic; [7]St.Johannes Hospital, DUISBURD, Germany; [8]MLL Münchner Leukämielabor GmbH, MUNCHEN, Germany; [9]Institute of Hematology and Blood Transfusion U Nemocnice, PRAGUE, Czech Republic; [10]Medical University, GDANSK, Poland; [11]Hematology, University of Bari, BARI, Italy; [12]Haemato-oncology Institute of Cancer Research, LONDON, United Kingdom; [13]Service d'Hématologie Biologique CHU, DIJON, France; [14]University of Athens School of Medicine, ATHEN, Greece; [15]Department of Pathology, Karolinska University Hospital, STOCKHOLM, Sweden; [16]Departamento de Hematologia,Centro Hospitalar, COIMBRA, Portugal; [17]Semmelweis University Ii Med, BUDAPEST, Hungary; [18]Hematology Laboratory, University Hospital, BASEL, Switzerland; [19]Department of Hematology,Vall d'Hebron Hospital, BARCELONA, Spain; [20]Department of Hematology,Erasmus Medical Center, ROTTERDAM, Netherlands

Background. The European LeukemiaNet (ELN) Network of excellence, an EU project funded by the 6th FP, is coordinated by R. Hehlmann and includes 237 scientists from 22 countries. Its major goal is the construction of a cooperative network for improvement of leukemia diagnosis, care and research. The Diagnostic Platform (WP10) is focused on Flow cytometric and Morphological panels, chaired by MC Benè and G Zini. Morphological cell identification results from an expert consensus agreement: in this era the ICT allows to exchange information and images via internet in real time, and scientists can collaborate from their workplace, sparing time and resources. We used this opportunity to try and get a consensus on haematopoietic cells morphology. In April 2007, 21 expert morphologists from 13 different European Countries formed a Committee, the European Morphology Consensus Panel (EMCP). *Aims*. The main goals of the EMCP are to: harmonize the identification of haematological cells for a common European morphological diagnostic pathway ; take into account national specific skills, competences and methods; provide patients with comparable morphological diagnosis all over Europe. The first step was to create a consensus-based cell library of meaningful images of haematopoietic cells identified and named by top level European morphologists for lineage, maturation stage and terminology and agreed by the EMCP, as a valuable tool to train and test European morphologists. *Methods*. From May to July 2007, 165 images containing 438 labelled hematopoietic cells from peripheral blood and bone marrow were collected from all the EMCF members. Each member has submitted her/his own cell images providing each submitted and labelled cell with her/his proposal in terms of lineage, maturation stage and terminology. All images were uploaded in a restricted web page together with an excel file containing the author's proposal for each cell. Faculty Members were asked to indicate in this file their agreement or personal alternative definition for each labelled cell. *Full consensus* was stated for all cells with an agreement of at least 17 out of 21 investigators (>80%). For all cells where less than 17 people agreed and for which at least 3 Faculty members proposed the same denomination, a second round of examination was performed. *Results*. The distribution of cell types is as follows: Erythroid series # 78 (18%); Granulocytic series # 143 (32%); Monocytic series # 40 (9%); Lymphoid series # 113 (26%); Megakaryocytic series # 26 (6%); Histiocytes-Macrophages # 12 (3%); Other # 27 (6%). Full consensus was obtained for: 259 cells (59%), with the following partition : Erythroid series 20%, Granulocytic series 30%, Monocytic series 4%, Lymphoid series 26%, Megakaryocytic series 5% , Histiocytes-Macrophages 8%, Other 7%. The lineage distribution for the 179 cells (41%) cells with disagreement (agreement < 17/21) was as follows: Erythroid series 14%, Granulocytic series 35%, Monocytic series 1%, Lymphoid series 25%, Megakaryocytic series 16%, Histiocytes-Macrophages 3%, Other 6% *Conclusions*. This first project showed that agreement was obtained homogenously among the different cell lineages, indicating that no specific type of cell presents major difficulties. The project is still ongoing and cells with disagreement are now going to be submitted to a Delphi consensus scoring system. Most of the discrepancies are currently of terminology, not lineage After the final results are obtained, all the cells will be uploaded into the Leukemianet web site as a valuable tool to train and test European morphologists. A second phase of the project is planned using a restricted number of cells with equivocal morphological identification and will be submitted to a more extended Faculty without providing any suggestion. An European Morphology Expert Meeting will be organized to find a face to face consensus on the cells not fully agreed upon.

0708

CLINICAL AND PROGNOSTIC CHARACTERISTICS OF 484 PATIENTS WITH THERAPY-RELATED MYELODYSPLASTIC SYNDROME (T-MDS) OR ACUTE MYELOID LEUKEMIA (T-AML)

A.K. Kündgen,[1] D. Haase,[2] A. Giagounidis,[3] S. Blum,[4] S. Fischer,[2] U. Platzbecker,[5] R. Schlenk,[6] B. Hildebrandt,[7] P. Valent,[8] M. Stadler,[9] R. Stauder,[10] O. Krieger,[11] K. Götze,[12] W.K. Hofmann,[13] A. Ganser,[9] C. Mende,[1] R. Haas,[1] N. Gattermann,[1] U. Germing[1]

[1]Heinrich-Heine University, DÜSSELDORF, Germany; [2]Department of Hematology/Oncology, University of Göttingen, GÖTTINGEN, Germany; [3]Hematology/Oncology , St Johannes Hospital, DUISBURG, Germany; [4]Centre Hospitalier Universitaire Vaudois, LAUSANNE, Switzerland; [5]Department of Internal Medicine I, University Hospital Dresden, DRESDEN, Germany; [6]Department of Internal Medicine III, University of Ulm, ULM, Germany; [7]Institute of Human Genetics, DÜSSELDORF, Germany; [8]Division of Haematology & Haemostaseology, Medical University of Vienna, VIENNA, Austria; [9]Department of Haematology and Oncology, Medizinische Hochschule, Hannover, HANNOVER, Germany; [10]Department of Internal Medicine, University of Innsbruck, INNSBRUCK, Austria; [11]1st Department of Internal Medicine, Elisabethinen Hospital, LINZ, Austria; [12]3rd Department of Medicine, Klinikum Rechts der Isar, Technical University, MUNICH, Germany; [13]Department of Hematology, University Hospital Benjamin Franklin, BERLIN, Germany

Based on the assumption that all patients with treatment-related disease share a particularly poor prognosis, in the current WHO classification, patients with t-MDS and t-AML are included into one combined subgroup, termed AML and MDS, therapy-related, regardless of dysplastic features, medullary blast count or cytogenetics. To validate the appropriateness of this classification, we combined the MDS/AML data sets of the German-Austrian MDS and AML study groups. We identified 484 patients with t-MDS or t-AML, 205 males and 279 females. Median age was 59 (16-89) years. 49% of patients underwent chemotherapy, 18% radiotherapy, 3% radioiodine-therapy and 33% combined radio-chemotherapy prior to t-MDS/AML diagnosis. When classified according to the WHO-proposals for primary MDS (pMDS) 8% of the patients had RAEB-II, 9% RAEB-I, 1% CMML-II, 3% CMML-I, 4% RA, 1% RARS, 16% RCMD, 7% RCMD-RS, 1% 5q-Syndrome and 50% had AML. Cytogenetics were available in 357 patients (74%). 7% of the AML patients had a favorable, 61% an intermediate (36% normal) and 32% an unfavorable karyotype (AML-criteria, CALGB). Of the MDS patients 42% showed good-risk (35% normal), 11% intermediate-risk, and 47% poor-risk cytogenetics according to MDS criteria (IPSS). Median survival of the entire group was 13 months (treated (intensive chemotherapy or allogeneic transplantation): 16 months, n=181; untreated: 10 months, n=257). Important prognostic parameters for p-MDS/AML, like age, medullary blast count, peripheral cell counts, as well as classification and scoring-systems (FAB, WHO, IPSS) had no prognostic influence on outcome in t-MDS/AML. However, in the subgroup of untreated MDS patients we observed a significant impact on survival for a marrow blast count </>5% (15 *vs* 6%, p=0,0008) and </> 20% (11 *vs* 5, p=0,001). In a next step we compared survival of 178 untreated t-MDS to 2405 p-MDS patients and found, as expected, a considerable difference in survival (10 *vs* 26 months). Interestingly, in subgroup analyses of cytogenetic risk groups there was no statistically significant survival difference between p- and t-MDS, although survival was slightly lower for t-MDS in all subgroups (favorable: 55 *vs* 41 months; intermediate: 31 *vs* 25 months; poor: 11 *vs* 8 months). The survival differences between p- *vs* t-MDS appeared to be largely due to a higher proportion of high-risk karyotypes in the latter group (19 *vs* 47%). Earlier studies on t-MDS/AML have mostly combined untreated and intensively treated patients, while established prognostic scoring sys-

tems were derived from untreated p-MDS patients only. Possibly, the alterations caused by prior cytotoxic therapy represent the main reason for an inferior outcome observed for t-MDS/AML patients receiving chemotherapy. *Conclusions.* 1. The inferior outcome of tMDS/AML patients might not be related to a special disease biology. 2. Normal karyotypes appear to occur in t-MDS/AML with a higher frequency than previously described. 3. t-MDS patients with a normal karyotype have a relatively long median survival of 41 months. 4. In untreated t-MDS patients an influence of medullary blast count can be observed, challenging the current WHO-approach of combining t-MDS and AML.

0709

INFLUENCE OF HISTOLOGICAL BONE MARROW FINDINGS ON OUTCOME IN 1175 PATIENTS SUFFERING FROM MYELODYSPLASTIC SYNDROME

S. Blum,[1] S. Baghikar,[2] S. Braunstein,[2] P. Reinecke,[2] A. Schmidt-Gräff,[3] M. Schapira,[1] U. Germing[2]

[1]Centre Hospitalier Universitaire Vaudois, LAUSANNE, Switzerland; [2]Heinrich-Heine-University Düsseldorf, DÜSSELDORF, Germany; [3]University of Freiburg, FREIBURG, Germany

Background. Myelodysplastic Syndromes (MDS) are heterogeneous disorders with outcomes depending on subtypes according to different classifications and risk factors. No classification takes histological findings into consideration. Incidence of fibrosis in bone marrow (BM) histology is only reported on small series of patients. A shorter survival and increase of transformation to acute myeloid leukaemia (AML) is suggested, but reached statistical significance only occasionally. *Aims*. We performed this study in order to obtain data on BM cellularity and presence of fibrosis in patients with primary and secondary MDS and on survival and transformation to AML depending on histological findings. *Methods*. Between 1975 and 2007, 1175 patients with MDS from the registries in Düsseldorf and Lausanne with available histological examination results were diagnosed with cytogenetics available in 535 patients. The product limit method (Kaplan-Meier), Mantel-Cox test, stepwise multivariate regression of Cox, c2 and Wilcoxon rank sum test were used. *Results*. Mean age was 69 years (range 14-96), 664 were male, 511 female. BM was hypocellular in 150 patients (13%), normocellular in 402 (34%) and hypercellular in 623 (53%). Hypocellular MDS showed significantly lower white blood cell and neutrophil counts (2.8 and 1.6 $\times 10^9$/L *vs* 3.6 and 1.7$\times 10^9$/L) and lower platelet counts (70 *vs* 133$\times 10^9$/L). There was no difference in hemoglobin level, BM blasts, risk of transformation to AML or distribution to FAB and WHO classification, IPSS or WPSS. Fibrosis was more frequent in hypercellular BM (22%) as compared to hypocellular (9%) and normocellular BM (6%) (p=0.003). Survival was best in the group with normocellular (41ms) or hypocellular (34ms) and was worst in hypercellular BM (19 ms) (Figure 1, p=0.005).

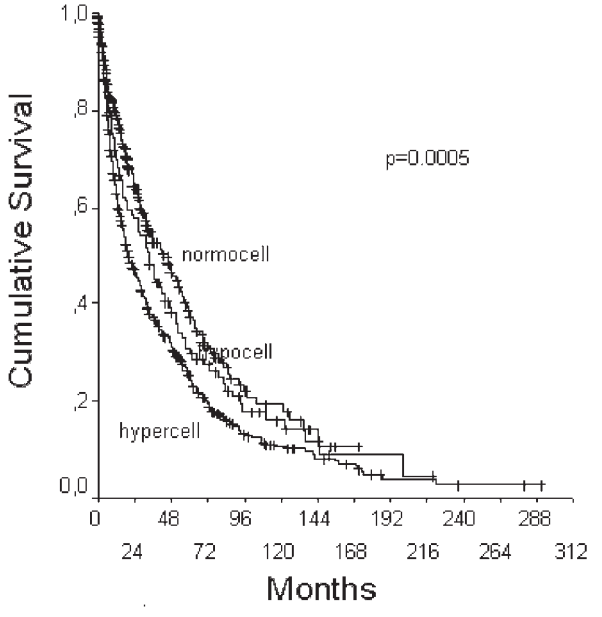

Figure 1.

This remained significant when analysed for FAB, WHO, IPSS and WPSS, and also if CMML was withdrawn. Of interest within the high risk group, hypocellular BM was associated with the best prognosis, whereas in the low risk groups, there was no difference between normo- and hypocellular BM. 150 initial BM biopsies (14.5%) showed presence of fibrosis, 886 (85.5%) did not. Median survival in the fibrosis group was 14 months as compared to 28 months without presence of fibrosis (p<0.000005). This survival difference was not related to AML-transformation, as there was no significant difference. Cytogenetic aberrations were found more often when fibrosis was present with an abnormal non complex karyotype in 60 *vs* 45% (p=0.03) and complex anomalies in 27 *vs* 13% (p=0.002). Survival difference remained significantly different in IPSS cytogenetic low and intermediate risk groups. Within the cytogenetic high risk group there was no difference in survival between patients with or without fibrosis. In a multivariate testing, chromosomal risk group, medullary blast count as well as cellularity were independent risk factors for survival. *Conclusions*. 1) MDS patients with normocellular and hypocellular bone marrow had a better prognosis as compared to hypercellular BM. 2) About 15% of the patients presented with fibrosis in MDS, which was correlated to presence of cytogenetic anomalies. 3) Histology findings at diagnosis give important information on additional risk factors in MDS that are not considered in existing classifications.

0710

DYSPLASTIC FEATURES DETECTED BY MULTIPARAMETER FLOW CYTOMETRY AND THEIR RELATION TO CYTOMORPHOLOGIC AND CYTOGENETIC FINDINGS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

W. Kern, C. Haferlach, S. Schnittger, T. Haferlach

MLL Munich Leukemia Laboratory, MUNICH, Germany

Background. Multiparameter flow cytometry (MFC) can identify dysplastic features in different cell lineages in myelodysplastic syndromes (MDS). *Aims and Methods.* We correlated dysplastic features detected by MFC with findings in cytomorphology (CM) and cytogenetics (CG) in 307 bone marrow samples from patients with suspected/proven (n=130/177) MDS. *Results.* In cases rated MDS or no MDS by CM there was a strong concordance with MFC: both methods MDS, 75.8%; both methods no MDS, 17.9%; CM MDS, MFC no MDS, 2.2%, CM no MDS, FC MDS, 4.0%, p<0.001. Blast counts by CM and MFC ranged from 0-21% (median, 3.5%) and from 0-23% (median, 3%; r=0.271, p<0.0001). Median number of aberrant features detected by MFC were 0 for blasts (range 0-4), 2 for granulocytes (0-5), 1 for monocytes (0-5), and 0 for erythroid cells (0-2). Most frequent dysplastic features observed in blasts included aberrant coexpression of CD11b (13.7%), CD15 (10.4%) and CD64 (10.4%). Most frequent dysplastic features observed in granulocytes included reduced side-scatter signal (67.1%), aberrant coexpression of CD56 (32.9%), aberrant pattern of CD13/CD16 expression (31.6%), aberrant pattern of CD11b/CD16 expression (24.1%), and reduced expression of CD33 (13.4%). Most frequent dysplastic features observed in monocytes included aberrant coexpression of CD56 (42.7%) and of CD16 (18.9%). Most frequent dysplastic features observed in erythroid cells included lack of CD71 expression (15.0%) and an aberrantly homogeneous expression of CD71 (9.1%). As compared to cases with no indication of MDS by CM (=non-MDS) cases with MDS according to CM were significantly associated with a reduced side-scatter signal in granulocytes (ratio granulocytes:lymphocytes 6.53±1.27 *vs* 7.44±1.17, p<0.0001) as well as a higher number of dysplastic features in granulocytes (1.98±1.09 *vs* 1.00±1.31, p<0.0001), monocytes (0.81±0.84 *vs* 0.35±0.63, p<0.0001), and erythroid cells (0.33±0.47 *vs* 0.20±0.40, p=0.061). Particularly, an aberrant expression of CD56 in monocytes occurred more frequently in 33 cases with CMML as compared to non-MDS cases (84.8% *vs* 15.7%, p<0.0001). In cases with possible MDS according to CM the differences to non-MDS cases were less pronounced (reduced side-scatter signal in granulocytes 7.77±1.47 *vs* 7.44±1.17, n.s., dysplastic features in granulocytes 1.90±1.21 *vs* 1.00±1.31, p=0.003, monocytes 0.50±0.63 *vs* 0.35±0.63, n.s., and erythroid cells 0.30±0.47 *vs* 0.20±0.40, n.s.). In cases with aberrant cytogenetics (n=80, excluding loss of Y as sole aberration) dysplastic features by MFC occurred more frequently as compared to cases with normal karyotypes (reduced side-scatter signal in granulocytes 6.35±1.18 *vs* 7.09±1.38, p<0.0001, dysplastic features in granulocytes 2.05±0.95 *vs* 1.68±1.31, p=0.021, monocytes 0.80±0.89 *vs* 0.71±0.81, n.s., and erythroid cells 0.38±0.49 *vs* 0.31±0.48, n.s.). >2 dysplastic features in blasts, granulocytes, and monocytes and/or blasts >5% occurred in 68.4%, 63.3%, and 35.3% of cases with MDS, possible MDS, and non-MDS

according to CM (p=0.0005). In some cases aberrant antigen expression was observed in cell lineages not rated dysplastic by CM. *Conclusions.* This evaluation suggests that MFC may be used to identify dysplastic features in patients with suspected MDS. Sensitivity and specificity varies between MDS subtypes and should be clearly defined in future studies.

0711

MAJOR DIFFERENCES WITH SCORING KARYOTYPE RESULTS FOR PATIENTS WITH MYELODYSPLASTIC SYNDROMES USING IPSS CRITERIA: A CALL FOR INTERNATIONAL STANDARDIZATION

A.M.M.J. Hagemeijer,[1] K. Chun,[2] A. Iqbal,[3] G. Dewald,[4] M.L. Slovak[5]

[1]University of Leuven, LEUVEN, Belgium; [2]Cytogenetics and Molecular Genetics North York General Hospital Genetics Program, TORONTO, Canada; [3]Department of Pathology, University of Rochester Medical Center, ROCHESTER, NY, USA; [4]Laboratory Medicine and Medical Genetics, Mayo Clinic, ROCHESTER, MN, USA; [5]Department of Cytogenetics, City of Hope National Medical Center, DUARTE, CA, USA

Background. The International Prognosis Scoring System (IPSS) is widely used for myelodysplastic syndromes (MDS) as a clinical gauge to estimate overall survival and time of progression to acute myeloid leukemia. An important component of the IPSS is karyotype status and complexity. AIM/*Methods.* To investigate the degree of consistency of scoring karyotype results for IPSS, the International Working Group on MDS Cytogenetics invited 22 survey participants, composed of both clinical cytogeneticists and clinicians, to evaluate cytogenetic results for 32 challenges. The number of cytogenetic aberrations and the IPSS score[Good(G),Intermediate(INT) or Poor(P)] were to be given for each example karyotype. Useful responses were provided by 20 participants by the due date. *Results.* Excellent concordance was observed among participants in evaluation of simple deletion, balanced translocation and highly complex hyperdiploid or hypodiploid karyotypes. However, scoring tetraploidy was inconsistent with karyotype scores ranging over all three risk groups G, INT, and P. Scoring loss of the Y chromosome was problematic when additional clonal aberrations were present, separating survey participants between INT and P scores. In cases of multiple clones, every single aberration was counted by most participants, while others considered only the largest clone. Scoring inconsistencies were most frequently associated with differences in scoring derivatives, dicentrics, isochromosomes, and adds; these aberrations were interpreted by some participants as one chromosome rearrangement and as two abnormal chromosomal events by others. For example, der(1;7)(q10;p10) was counted as one abnormality by 11 participants and two aberrations by 9 participants due to the resulting net imbalance of del(1p) and del(7q). Confusion in scoring was also prevalent when karyotypes had 1) a single chromosome showing clonal evolution [e.g., del(5q), del(5q)x2, or del(5q) with -5]; 2) ill-defined and rare clonal aberrations like mar, ring and jumping translocations; and 3) chromosome 7 balanced translocations (simple and complex), with participants divided between one aberration (INT) *vs* poor because a chromosome 7 abnormality was present. Specific examples and proposals for standardization will be provided. *Conclusions*. Based on these survey results, confusion in scoring karyotype complexity and IPSS values is widespread and argues for the immediate need of 1) international standardized complexity scoring criteria; 2) corresponding IPSS cytogenetic scoring revisions for clinical practice; and 3) future studies designed to reveal the natural history of the karyotype changes observed in MDS.

This work is presented on behalf of the International Working Group on MDS Cytogenetics (MDS Foundation)

0712

DO PROGNOSTIC INDICES IN MDS LOSE THEIR PREDICTIVE POWER OVER TIME?

H. Tuechler,[1] T. Noesslinger,[2] A. Makrai,[2] E. Pittermann,[2] M. Pfeilstoecker[2]

[1]Ludwig Boltzmann Institute for Leukemia Research, VIENNA; [2]Hanusch-Hospital, Dept of Hematology, VIENNA, Austria

Background. Usually prognostic indices such as the IPSS were developed and later validated assuming their predictive values to be unchanged over time. This is not necessarily plausible, because it is easily imaginable that, with a growing interval from the date of diagnosis, initially extremely favourable or unfavourable prognoses tend to an average expectation, but it follows from using the Cox-proportional-hazards-model as framework, a central assumption of which, the proportionality of hazards, translates to constant predictive values. *Aims.* The aim of this study was to examine well known prognostic indices for a possible loss in their predictive power over time. *Methods.* The study is based on data of a series of 243 primary MDS patients treated with supportive care only. Median survival was 31 months and the median follow up 34 months. The median age was 72 years. 124 patients (51%, median survival 29,5 months) were male and 119 (49%, survival 31 months) female. According to the FAB classification the distribution and median survival were as follows: RA 74 patients (30,5%),68 mo; RARS 27 patients (11,1%),65 mo; RAEB 55 patients (22,6%)14 mo; CMML 62 patients (25,5%),25 mo; RAEBT 25 patients (10,3%),9 mo. For these patients the values of the following scores were available: IPSS, c-IPSS, PI-Score, Lille, Duesseldorf, Sanz, Bournemouth, Lausanne-Bournemouth. The assumption of proportional hazards was tested according to Grambsch, Therneau (1994). Changes in the predictive importance were quantified by the correlation of the scaled Schoenfeld residuals with Kaplan-Meier-transformed time. *Results.* IPSS (r=0.36, p<0.001), Lausanne-Bournemouth-score (r=0.34, p<0.001), Bournemouth-score (r=0.28, p=0.005), Lille-score (r=0.25, p=0.013) and Duesseldorf-score (r=0.20, p=0.037) show strong to moderate loss of predictive power over time. The loss of the Sanz-score (r=0.17, p=0.099) is moderate. On the other hand the c-IPSS (r=0.02, p=0.81) and the PI-score (r=0.05, p=0.61) maintain their predictive power over time. All scores are strongest in predicting the first 9 months circa. The IPSS, the Lausanne-Bournemouth-score and the Bournemouth-score showed no correct risk group discrimination for the survival times above three years. *Summary and Conclusions.* These results show surprisingly clear that predictive systems mainly based on clinical characteristics are relevant in the initial period after diagnosis, roughly in the first 18 months. Purely cytogenetically defined risk groups instead remain discriminatory even after a long observation time. Consequently the development and comparison of prognostic systems have to take into account their stability *vs* the possibility or need for re-evaluation of a specific scoring system as this is being attempted in so called dynamic scores (eg WPSS). Possibly not only reevaluation after time is of importance, but also different weighting of score constituting items.

0713

SEQUENTIAL FISH-ANALYSES OF CIRCULATING CD34+-CELLS IN MDS - A SUITABLE METHOD FOR CYTOGENETIC MONITORING UNDER 5-AZACYTIDINE OR LENALIDOMIDE

F. Braulke, R. Steffens, C. Schuetze, K. Plischke, B. Chapuy, D. Haase

Georg August University, Goettingen, GOETTINGEN, Germany

Background. Chromosomal aberrations occur in about 50% of patients (pts) with de novo-myelodysplastic syndrome (MDS) and in up to 80% of pts with secondary MDS. Most of these anomalies are detectable by fluorescence *in situ* hybridisation (FISH) and they are also provable in circulating $CD34^+$-progenitor cells. *Aims and Methods.* We present a novel method for cytogenetic monitoring of MDS pts under different therapeutic regimens: circulating $CD34^+$-cells from peripheral blood were enriched by immunomagnetic cell sorting and then analysed by FISH. By that chromosomal aberrations were followed in peripheral blood of MDS pts under therapy (off-label use) with 5-azacytidine (5-aza) or lenalidomide (len). *Results.* For every aberrant karyotype there was an informative FISH probe available detecting the same chromosomal anomalies in circulating $CD34^+$-cells as in bone marrow cells. In all cases a sufficient quantity of circulating $CD34^+$-cells for FISH analyses could be enriched by immunomagnetic cell sorting (85-42×10^3 $CD34^+$-cells/20mL blood). Microscopic analyses of these sorted cells demonstrate morphologically homogeneous cell populations. As yet 20 pts were treated with 5-aza at our institution. Sixteen (2 female, 14 male) received at least 4 cycles and can be analysed now: 1 RA, 3 RCMD, 1 RAEB-1, 4 RAEB-2, 7 AML. The median number of 5-aza cycles was 5.2 (4-9), the median follow-up time was 34 weeks (17-60). The karyotypes were: 1 normal, 10 pts with 1-2 anomalies, 5 pts with complex abnormal karyotype. Out of 16 pts 11 (69%) responded to therapy: 5 complete remission (CR), 1 partial remission (PR), 2 marrow-PR, 2 hematologic improvement (HI), and 4 partial cytogenetic remission (cyPR) - there is an overlap of pts reaching e.g. HI and cyPR but not fulfilling modified IWG-criteria for PR. Furthermore, 5 pts stayed in stable disease (SD) during therapy. Among 16 pts, 15 had chromosomal aberrations suitable for FISH-analyses. According to modified IWG criteria for classical cytogenetics cyPR means a reduction of >50% of circulating CD34+-cells showing the respective anomaly and cyCR is defined as a reduction of <10% at all. In our study cytogenetic response was detectable in 9 pts

(60%), 2 showed cyCR. Interestingly cyPR preceded HI by 4-8 weeks. The duration of cyPR was 12 weeks (4-42) as yet; transfusion independence lasted for 19 weeks (8-43). In 8 pts with -7/7q- (2 as part of complex anomalies) 2 reached CR, 1 cyPR and 5 stayed in SD. We used the same method to monitor MDS pts with 5q-syndrome receiving lenalidomide. So far, 3 pts (2 male, 1 female) were observed under treatment with len: The median number of cycles was 4 (3-6), the median follow-up time was 32 weeks (31-33). All pts reached CR. Cytogenetic and haematological response correlate with each other. Cytogenetic remission lasted for 15 weeks (13-16) as yet; the median duration of HI was 21 weeks (19-22). *Conclusions.* Analysing circulating CD34$^+$-cells by FISH is a less invasive and practicable method for a frequent cytogenetic monitoring in MDS pts under different treatment regimens and implies obviously some predictive value.

0714

RISK FACTORS FOR AML TRANSFORMATION AND MORTALITY IN TRANSFUSION-DEPENDENT DELETION 5Q MDS

A.F. List,[1] A. Giagounidis,[2] N. Brandenburg,[3] K. Wride,[3] A. Glasmacher,[4] U. Germing[5]

[1]*University of South Florida, H. Lee Moffitt Cancer Center and Research Institute, TAMPA, FLORIDA, USA;* [2]*St. Johannes Hospital, DUISBURG, Germany;* [3]*Celgene Corporation, SUMMIT, NJ, USA;* [4]*Celgene Germany GmbH, MUNICH, Germany;* [5]*Heinrich-Heine-Universität, DÜSSELDORF, Germany*

Background. Patients with myelodysplastic syndromes (MDS) are at risk for early mortality and transformation to acute myeloid leukemia (AML). MDS patients with transfusion-dependent anaemia have higher rates of AML transformation and mortality. Lenalidomide reduces transfusion needs and suppresses the disease clone in transfusion dependent, low/intermediate-1 IPSS risk MDS with a chromosome 5q31 deletion (del(5q)). We have analyzed outcomes in Celgene study MDS-003 (the largest experience to date with lenalidomide treatment of these patients) and in matched patients in the Düsseldorf MDS Registry (the largest current prospective MDS Registry), to identify covariates linked to AML transformation or death and to examine the impact of lenalidomide treatment. *Aims.* Objectives were to (1) identify key risk factors for AML transformation or death in this patient population, and (2) compare event rates in MDS-003 patients *vs* rates in similar Registry patients who had not received lenalidomide. *Methods.* MDS-003 enrolled 148 pts from July 2003 - May 2004. A protocol amendment allowed extended follow-up for long-term outcomes through 2007. Cox proportional hazard models were used to identify risk factors for AML transformation and mortality. In each model, a *Treatment* variable compared MDS-003 pts with transfusion dependent, centrally-confirmed low/intermediate-1 IPSS risk, del(5q) MDS, *vs* similar Registry patients with a defined transfusion requirement at baseline who were not treated with lenalidomide. Other variables included cytogenetic complexity (isolated del(5q) *vs* del(5q) and ≥1 additional abnormality), age, gender, FAB category (RA/RARS *vs* RAEB/RAEBT/other), number of cytopenias, platelet count, neutrophil count, and number of RBC units transfused and minimum haemoglobin level in the 8 week baseline period.

Table 1.

Risk Factors for AML Transformation and Mortality: Final Cox Models

Variable	Hazard Ratio (HR)	HR 95% Confidence Limits		P Value
		Lower Bound	Upper Bound	
AML Transformation				
RBC Units (per 8 weeks)	1.14*	1.01	1.28	0.03
Hb minimum (g/dL)	0.81	0.62	1.04	0.10
Mortality				
Age	1.04	1.02	1.07	<0.001
Gender	0.69	0.43	1.12	0.14
RBC Units (per 8 weeks)	1.10*	1.01	1.18	0.02
Hb minimum (g/dL)	0.76	0.63	0.92	0.004
FAB	1.55	0.88	2.70	0.13
Cytogenetic Complexity	1.49	0.91	2.45	0.12

*Reflects 14% increased AML transformation rate and 10% increased mortality rate, with each additional unit of blood transfused per 8 weeks at baseline.

Results. Analyses included 116 MDS-003 patients (median follow-up 37 months, max=47) and 34 Registry patients (median follow-up 37 months, max=235) who met the above criteria and had complete data on all variables. Four-year cumulative incidence of AML was 21% (24/116) in MDS-003 *vs* 24% (8/34) in Registry patients; four-year cumulative mortality was 51% (59/116) *vs* 50% (17/34), respectively. Risk factors identified in the final backward Cox model for AML transformation (p<0.15) were baseline transfusion burden (Hazard Ratio (HR) 1.14; p=0.03) and haemoglobin (HR 0.81; p=0.10). Risk factors identified in the final Cox model for survival were age (HR 1.04; p<0.001), gender (HR 0.69; p=0.14), baseline transfusion burden (HR 1.10; p=0.02), haemoglobin (HR 0.76; p=0.004), FAB (HR 1.55; p= 0.13), and cytogenetic complexity (HR 1.49; p=0.12). Table 1 summarizes the final Cox models for these analyses. No differences in AML transformation (p=0.88) or survival (p=0.42) were observed between MDS-003 and Registry patients. *Conclusions.* In this population of transfusion-dependent del(5q) MDS patients, the magnitude of maturation impairment and bone marrow failure as measured by baseline transfusion burden and haemoglobin were determinants of both AML risk and survival; age was a determinant of survival, but not for AML risk. There was no apparent effect of lenalidomide on these outcomes, but the small comparison cohort provided limited power to discern treatment effect. Prospectively randomized studies are needed to determine whether lenalidomide treatment impacts the risk of AML transformation and/or mortality in this MDS population.

0715

A STANDARDIZED APPROACH FOR THE ASSESSMENT OF BONE MARROW DYSPLASIA IMPROVES ON THE ACCURACY OF WHO CLASSIFICATION IN MYELODYSPLASTIC SYNDROMES

R. Invernizzi,[1] E. Travaglino,[2] C. Benatti,[1] L. Malcovati,[2] M.G. Della Porta,[2] M. Cazzola[2]

[1]*Fondazione IRCCS Policlinico S. Matteo, PAVIA, Italy;* [2]*Policlinico S. Matteo, PAVIA, Italy*

In 1999, the World Health Organization (WHO) updated the FAB classification introducing multilineage dysplasia as parameter to increase prognostic accuracy in myelodysplastic syndrome (MDS) classification. Nevertheless, a structured and reproducible approach for the precise definition and quantification of bone marrow (BM) dysplasia is still lacking. Moreover, the precise relationship between cytopenia and dysplasia needs to be clarified. In particular it is unclear how anemia associated with multilineage dysplasia, and bi- or pancytopenia associated with unilineage dysplasia should be classified. In order to identify a panel of reproducible morphological criteria associated with MDS useful for a correct application of WHO classification, we retrospectively examined the cytological features of May-Gruenwald-Giemsa and Prussian blue stained BM smears from 362 MDS patients previously classified according to FAB criteria (181 RA, 66 RARS, 69 RAEB, 12 RAEB-t, 34 CMML),143 patients with hyporegenerative anemia and 52 healthy subjects. By counting 100 nucleated cells for the erythroid and granulocytic lineages and at least 20 megakaryocytes and classifying them for their dysplastic changes, a panel of dysplastic features showing a better sensitivity and specifity for MDS identification was developed. In addition, to evaluate the degree of concordance for recognition of dysplastic features, BM aspirates from 54 MDS patients and 43 controls were evaluated by three independent morphologists who analyzed each sample two times with a between-investigators and within-investigator agreement of 92% and 95% respectively. Some of the single morphological abnormalities were associated with poor outcome; moreover, total granulocytic or megakaryocytic dysplasia showed a statistically significant independent unfavorable prognostic value (p=0.01). Also the degree of granulocytic or megakaryocytic dysplasia was of prognostic relevance. The morphological panel was employed in association with the evaluation of blast and sideroblast percentages, besides blood count and cytogenetic analysis, to reclassify MDS patients by WHO proposal using the 10% threshold to record dysplasia in each lineage: 260 MDS cases were correctly reclassified (39 RA, 14 RARS, 81 RCMD, 39 RCMD-RS, 38 RAEB-1, 35 RAEB-2, 14 MDS del(5q)), 42 were unclassifiable for inadequate BM smears and 61 belonged to other hematopoietic neoplasms. On univariate analysis, percentage of BM blasts, multilineage dysplasia and two or more cytopenias were associated with worse outcome but multivariate analysis failed to confirm the prognostic unfavourable value of cytopenias. Forty-four low-risk MDS patients (25%) presented discordant findings: in 17 cases bi- or pancytopenia was associated with unilineage dysplasia and in 27 cases anemia was associated with multilineage dysplasia. Kaplan Meier estimates of overall and leukemia-free

survival showed that all patients with multilineage dysplasia had a significant worse outcome, independently of the number of peripheral cytopenias (p=0.001). In conclusion, our data suggest that the definition of BM dysplasia with a standardized morphological panel that improves the objectivity and reproducibility of microscopic examination is needed for a correct application of the MDS WHO classification, and enables to identify in the low-risk MDS group further subgroups with different prognosis.

0716

ANEMIA WITH RINGED SIDEROBLASTS ASSOCIATED WITH THROMBOCYTOSIS: CLINICAL AND ANALYTICAL FEATURES ACCORDING TO THE PRESENCE OR ABSENCE OF THE JAK2 V617F MUTATION

J.M. Raya,[1] L. Arenillas,[2] A. Domingo,[3] E. Alonso,[3] B. Bellosillo,[2] M. Rozman,[4] G. Gutierrez,[4] E. Luño,[5] C. Sanzo,[5] M.A. Piñan,[6] G. Letamendi,[6] M. Barbon,[7] M.L. Perez-Sirvent,[8] J. Cervera,[8] M.J. Muruzabal,[9] J.I. Olalla,[9] L. Yanez,[10] L. Garcia,[11] A. Lemes,[12] M.T. Molero,[12] F. Milla,[13] J.T. Navarro,[13] A. Elosegi,[14] T. Hernandez-Santamaria,[14] M.A. Cortes,[15] M.L. Gonzalez-Ponte,[15] A. Villegas,[16] M. Mateo,[16] MA Duran,[17] M Ardanaz,[18] L Morabito[1]

[1]Hospital Universitario de Canarias, LA LAGUNA; [2]Hospital del Mar, BARCELONA; [3]Hospital Universitari de Bellvitge, HOSPITALET; [4]Hospital Clinic i Provincial, BARCELONA; [5]Hospital Central de Asturias, OVIEDO; [6]Hospital de Cruces, BARACALDO; [7]Hospital de Leon, LEON; [8]Hospital La Fe, VALENCIA; [9]Hospital de Sierrallana, TORRELAVEGA; [10]Hospital Marques de Valdecilla, SANTANDER; [11]Hospital de Getafe, MADRID; [12]Hospital Doctor Negrin, LAS PALMAS; [13]Hospital Germans Trias i Pujol, BADALONA; [14]Hospital de Zumarraga, ZUMARRAGA; [15]Hospital de Laredo, CANTABRIA; [16]Hospital Clinico San Carlos, MADRID; [17]Hospital Son Dureta, PALMA DE MALLORCA; [18]Hospital Txagorritxu, VITORIA; [19]Hospital Vall d'Hebron, BARCELONA, Spain

Background. The JAK2 V617F mutation has been identified as a pathogenic factor in typical chronic myeloproliferative diseases. Several authors have found a high frequency of the JAK2 V617F mutation in patients with refractory anemia with ringed sideroblasts associated with marked thrombocytosis (platelet count > 600×10^9/L), provisional entity considered as mixed myelodysplastic/myeloproliferative, unclassifiable (RARS-MT; WHO, 2001). Some controversies exist concerning the cut-off value for platelet count, and other authors consider a lower value for diagnosis. It is not elucidated if patients with RARS associated with thrombocytosis (RARS-T) and the JAK2 mutation significantly differ from those without this mutation, from a clinical and analytical point of view.

Table 1.

	RARS-T with JAK2 V617F mutation (n=17)	RARS-T without JAK2 V617F mutation (n=30)	*p* value
Age (years)	72.9 ± 11.1	73.6 ± 8.8	N.S.
Sex (M:F)	11:6	17:13	N.S.
Platelet count (x10^9/L)	845 ± 278	591 ± 239	<0.001
WBC count (x10^9/L)	9.2 ± 3.5	6.7 ± 2.2	0.018
Hemoglobin (g/L)	109.2 ± 16.6	97.7 ± 16.0	0.029
MCV (fL)	97.3 ± 8.7	102.4 ± 6.4	0.037
Basophil count (x10^9/L)	0.108 ± 0.082	0.063 ± 0.060	N.S.
Bone marrow blasts (%)	0.9 ± 1.2	1.2 ± 1.2	N.S.
Type III sideroblasts (%)	31 ± 27	32 ± 23	N.S.
Ringed sideroblasts (%)	48 ± 21	48 ± 19	N.S.
Uric acid	5.3 ± 2.4	5.8 ± 1.8	N.S.
Lactate-dehydrogenase (U/L)	429 ±191	324 ± 106	N.S.
Ferritin	656 ± 674	560 ± 984	N.S.
Vitamin B_{12}	579 ± 391	677 ± 577	N.S.
Splenomegaly	4/14 (29%)	3/23 (13%)	N.S.
Karyotype aberrations	2/15 (13%)	2/22 (9%)	N.S.
BM Megakaryocytic hyperplasia	7/8 (87%)	4/5 (80%)	N.S.
BM reticulin fibrosis	5/7 (71%)	2/5 (40%)	N.S.
Transfusional dependence	1/14 (7%)	8/27 (29%)	N.S.

Methods. We have retrospectively studied the main clinical and analytical differences in a group of 47 patients with diagnosis of RARS-T (platelet count above 400×10^9/L), taking into account the presence or absence of the JAK2 V617F mutation. DNA was extracted from peripheral blood or bone marrow smears at diagnosis. Allele-specific PCR for the detection of JAK2 V617F was performed. *Results.* The JAK2 V617F mutation was present in 17 cases (36%). In those patients with a platelet count > 600×10^9/L (RARS-MT 23/47, 49%) the JAK2 mutation was found in 14 cases (61%), while only in 3 cases (12.5%) below 600×10^9/L. All these three cases had a platelet count between 500×10^9/L and 600×10^9/L. Patients carrying the mutation had a significant higher platelet count (p<0.001) and a higher WBC count (p=0.018), while patients without the mutation had a lower haemoglobin level (p=0.029) and a higher MCV (p=0.037) (Table 1). Although not significant, the frequency of splenomegaly was higher in the JAK2 mutated group, and transfusion requirements were more elevated in the JAK2 non-mutated group. We did not find statistically significant differences in age, sex, motive of consultation, basophil count, bone marrow blasts, ringed sideroblasts, lactate-dehydrogenase and uric acid levels, serum ferritin and vitamin B12, presence of cytogenetic aberrations, bone marrow cellularity and megakaryocytic hyperplasia, bone marrow fibrosis, and treatment strategies (all of them, p>0.05). There were also no differences in terms of survival (p=0.38). *Conclusions.* In our study, patients with RARS-T carrying the JAK2 V617F exhibited a higher WBC and platelet count, and splenomegaly was more frequently found (*myeloproliferative behaviour*), while those without the mutation showed a lower haemoglobin level, a higher MCV, and more elevated transfusion requirements (myelodysplastic features). All the JAK2 mutated patients had a platelet count above 500×10^9/L. Further studies are needed to confirm these findings.

0717

THE IMMUNOPHENOTYPE OF DIFFERENT IMMATURE, MYELOID AND B-CELL LINEAGE COMMITTED CD34$^+$ HEMATOPOIETIC CELLS ALLOWS DISCRIMINATION BETWEEN NORMAL/REACTIVE AND MYELODYSPLASTIC SYNDROME PRECURSORS

S. Matarraz Sudon,[1] A. Lopez,[2] S. Barrena,[3] C. Fernandez,[4] E. Jensen,[4] J. Flores,[4] P. Barcena,[4] A. Rasillo,[4] J.M. Sayagues,[4] M.L. Sanchez,[4] J.M. Hernandez Rivas,[5] C. Salvador,[6] N. Fernandez Mosteirin,[6] L. Perdiguer,[7] A. Orfao[4]

[1]Centro de Investigación del Cancer, CSIC-USAL, SALAMANCA; [2]Centro de Investigación de Cancer CSIC-USAL, SALAMANCA; [3]Centro de Investigación del Cáncer, USAL-CSIC, SALAMANCA; [4]Centro de Investigación de Cancer, CSIC-USAL, SALAMANCA; [5]Servicio de Hematologia, Hospital Universitario de Salamanca, SALAMANCA; [6]Servicio de Hematologia, Hospital Miguel Servet, ZARAGOZA; [7]Servicio de Hematologia, Hospital Alcañiz, TERUEL, Spain

Aims and Methods. The aim of the study was to analyze the numerical and phenotypic abnormalities of different maturation-associated subsets of bone marrow (BM) CD34$^+$ HPC from 50 newly diagnosed MDS patients in comparison to normal/reactive BM (n=29). The subsets identified were mainly composed of: 1) the phenotypically more immature CD34hi/CD45int/HLA-DRhi/CyMPO-/nTdT-/CD117hi compartment of CD34$^+$ HPC; 2) CD34$^+$ B-cell precursors displaying a CD34int/CD45int/dim/HLA-DRhi/CyMPO-/nTdTint/CD117-immunophenotype and; 3) CD34hi/CD45int/dim/HLA-DRhi/CyMPOint/hi/nTdT-/CD117hi neutrophil precursors. Additionally, CD34$^+$ HPC committed to other cell lineages were identified in normal/reactive BM, but at smaller percentages: plasmacytoid DC, monocytic lineage, basophil, mast cell and, nucleated erythroid CD34$^+$ precursors. For the individual abnormalities detected, a score was defined. Accordingly, a score of 0.5, 1 or 2 was given when the value obtained for each of these parameters was between the mean±2 SD and the mean±3 SD, between the mean ± 3 SD and the mean±4 SD and, over the mean ±4 SD of the values found for that parameter among the normal/reactive BM samples analyzed, respectively. *Results*. Our results confirm the existence of heterogeneously altered phenotypes among CD34$^+$ HPC from MDS and indicate that such variability depends both on the relative distribution of the different subsets of CD34$^+$ HPC committed into the different myeloid and B-lymphoid compartments, and their immunophenotype (e.g.: higher reactivity for CD117 and CD13 and lower expression of CyMPO, CD64 and CD65 on CD34$^+$ immature and neutrophil precursors), a clear association existing between the accumulation of CD34$^+$ HPC and that of immature CD34$^+$ HPC. Interestingly, expansion of erythroid and neutrophil lineage CD34$^+$ cells is detected in low-grade MDS

at the expense of CD34+ plasmacytoid dendritic cell and B-cell precursors, while expansion of immature CD34+ precursors occurs in high-grade MDS. Based on the marked differences observed in the distribution and phenotypic characteristics of different subsets of CD34+ HPC in MDS *vs* normal/reactive BM, a multivariate hierarchical cluster analysis was performed. This analysis showed that while CD34+ HPC are clearly different from normal precursors in high grade MDS, there appears to be a significant degree of overlap between reactive BM and low grade MDS cases. In line with this, the predictive value of the most informative independent phenotypic parameters analyzed -overall number of CD34+ cells, CD34+ B-cell precursors, and CD34+ plasmacytoid DC precursors, together with the expression of CD13, CD117 and CyCD79a- reached an efficiency of close to 80% and 100% on identifying normal/reactive *vs* LOW-R and HIGH-R MDS patients, respectively. However, once a phenotypic scoring system in which the number of phenotypic abnormalities and their degree of deviation from normal values were considered, a 100% efficient discrimination was obtained between normal/reactive BM and MDS cases, the mean score increasing from low to high grade MDS patients. *Conclusions*. The scoring system proposed, devised as a means of condensing multiple flow cytometry abnormalities into numerical scores efficiently discriminates between normal/reactive and MDS CD34+ HPC.

Table 1. Flow cytometry immunophenotypic scores of normal/reactive bone marrow (BM) and MDS patients grouped according to WHO and IPSS classifications.

	IPSS subgroups of MDS						WHO subgroups of MDS					
Score	Normal/reactive BM (N=28)	LOW-R (N=10)	INT-1-R (N=14)	INT-2-R (N=9)	HIGH-R (N=4)	TOTAL (N=37)	RA (N=9)	RCMD (N=11)	RAEB-1 (N=7)	RAEB-2 (N=16)	MD/MPD (N=5)	TOTAL (N=48)
0 to 0.5	100%	0%	0%	0%	0%	0	0%)	0%	0%	0%	0%	0%
1 to 4	0%	6(60%)	5(36%)	1(10%)	0%	32%	4(44%)	10(91%)	0%	1(6%)	0%	30%
≥ 5	0%	4(40%)	9(64%)	8(90%)	4(100%)	67%	5 (55%)	1(9%)	7(100%)	15(94%)	5(100%)	70%
Mean Score	0.08±0.1	3.5±2	5±3	9±4	14±3	7±5	6±6.5	2.4±1.5	6.5±1.6	10.5±4.5	9±3	7±5

0718

TELOMERE/CENTROMERE FLUORESCENCE IN-SITU HYBRIDIZATION (T/C-FISH) IN PATIENTS WITH SECONDARY ACUTE MYELOID LEUKEMIA ARISING FROM MYELODYSPLASTIC SYNDROME WITH COMPLEX KARYOTYPES

G. Göhring, K. Lange, B. Schlegelberger

Hannover Medical School, HANNOVER, Germany

Background. Telomere shortening and genomic instability play an important role in the development of myeloid neoplasia. Published data are only available on the average telomere length in MDS and AML but not on telomere length of individual chromosomes. *Aims.* The aim of this study was to validate and perform T/C-FISH on 18 patients with secondary AML after MDS with characteristic aberrations and a complex aberrant karyotype and in a cohort of 18 age- and gender-matched healthy controls. *Methods.* Using T/C-FISH, a centromere-calibrated method generating fluorescence signals proportional to the number of telomere repeats (Perner *et al.*, Am J Pathol 2003), the telomere length of each chromosome in a metaphase spread can be measured. T/C-FISH was validated by comparing the telomere length profile and the shortest chromosomes of 5 healthy controls with published data: the measured T/C values, the standard deviation and the telomere length profile, especially the telomere length expected according to the patient's age, agreed with Mayer *et al.* (Cytogenet Genome Res 2006). In order to identify the individual chromosomes, T/C-FISH was established after R-banding of the same metaphases. *Results.* The mean telomere length and the average value of each chromosome arm of the AML patients were significantly shorter than those of the healthy controls (mean length in patients: 7.5 kb, controls: 9.6 kb, $p<0.005$). The telomeres of 17p, 18p, 19p, 19q and 21p as well as the telomeres of the short arms of acrocentric chromosomes were critically short. Neo-telomeres were found in 2 patients. However, there was no correlation between telomere length and structural aberrations. *Conclusions.* T/C-FISH is currently being performed on further cytogenetic subgroups of MDS including patients with normal karyotype, with an isolated monosomy 7 and with 5q- syndrome. Potential differences between the telomere lengths within these groups may provide new insights into the pathomechanism responsible for the development of these cytogenetic and morphological subgroups of MDS.

0719

IMMUNOPHENOTYPIC DESCRIPTION OF PATIENTS WITH A MYELODYSPLASTIC SYNDROME AND 2 SPECIFIC CYTOGENETICS ABNORMALITIES: DELETION OF 5Q AND TRISOMY 8

M. Subirá,[1] P. Font,[2] E. Arranz,[3] C. Serrano,[1] R. Gonzalo,[1] S. Castañón,[1] R. Mata,[1] C. Soto,[1] E. Olaso,[4] A. Román,[1] P. LLamas[1]

[1]Fundación Jiménez Díaz, MADRID; [2]Clínica Moncloa, MADRID; [3]Gemolab Laboratory, MADRID; [4]Clínica Santa Elena, MADRID, Spain

Multiparametric flow cytometry immunophenotyping (FCI) has recently become a new co-criterion to assist in the diagnosis of myelodysplastic syndromes (MDS). Deletion of 5q (5q-) and trisomy 8 (+8) are two chromosome abnormalities often described in MDS. *Aims.* To describe the immunophenotype of MDS patients with isolated 5q- and trisomy 8, and to determine whether they show any recurrent profile. *Patients*. In a series of 104 MDS patients, 9 (1 male, 8 female; median age 57, range 21-86) had a 5q-, and 15 (10 male, 5 female; median age 79, range 67-93) had a trisomy 8. According to cytological data, 4 patients with 5q- had multilineage dysplasia (1 with ringed sideroblasts), 1 had isolated megakaryocytic dysplasia, and 4 had mainly erythroid dysplasia. According to the WHO classification, patients with +8 were classified as 1 refractory anaemia, 2 refractory anaemia with ringed sideroblasts, 3 refractory cytopenia with multilineage dysplasia, 7 refractory anaemia with excess of blasts, and 1 unclassified MDS. The patient left had a chronic myelomonocytic leukaemia. *Methods.* Conventional karyotype and FISH analysis were used to detect 5q- and +8. FCI in bone marrow (BM) samples was used to study the following data: 1 - In the myeloid lineage: abnormal granularity, CD45 distribution, phenotypic pattern of maturation (CD16/CD11b/CD13), and absence of CD10 expression on mature granulocytes (CD10-). 2 - In the monocytic lineage, decreased (<2%) or increased (>10%) percentage of monocytes, and aberrant antigenic expression. 3 - In myeloblasts, identification of >5% CD34+ cells, abnormal distribution of CD34 and CD117, and evaluation of CD7 and TdT expression (positive expression was described when >10% of CD34+ cells were positive for any of these antigens). 4 - In B-cells, detection of a low percentage of CD10+/TdT+ B-cells (<1% of BM B-cells). *Results.* Table 1 summarises FCI findings. The percentage of FCI abnormalities ranged from 20-71% in 5q- patients, and 18-86% in +8 patients. In both groups, patients with early stages of MDS had a lower number of abnormalities than patients with advanced MDS. No consistent abnormal myeloid pattern was found in 5q- patients. In contrast, +8 patients showed 2 recurrent abnormalities: absence of CD13 in early stages of maturation associated with a very low expression on mature neutrophils, and CD11bhigh in early stages of maturation combined with a very low CD13 expression in mature granulocytes. The first pattern was described in 5/15 patients with trisomy 8, and 18/104 patients within the whole MDS group ($p>0.05$). The second pattern was described in 8/15 patients with +8, and 20/104 within the MDS group ($p<0.05$). *Conclusions.* All patients from both groups have abnormalities in the myeloid lineage, and the number of abnormalities seems to be related to the severity of BM dysplasia. With the number of cases studied, no common immunophenotypic profile was found in patients with 5q-. These data might reflect changes in the immunophenotype over the course of a dynamic disease. The specific distribution of CD11b and CD13 described, should lead to a search of trisomy 8.

Table 1. FCI findings: abnormal cases/parameters studied.

	Abnormal SSC/CD45	Abnormal myeloid maturation pattern	CD10- neutrophils	Abnormal monocytes	>5% CD34+	Abnormal CD34/CD117 distribution	CD34+ CD7+cells	CD34+ TdT+ cells	<1% early B-cell stages
5q-	9/9	9/9	2/6	2/9	3/9	2/5	7/9	0/9	3/9
+8	15/15	15/15	7/15	7/15	5/15	6/11	9/15	2/15	8/15

0720

BONE MARROW EXPRESSION OF CD33, CD34 AND CD117 FOR THE PROGNOSTIC ASSESSMENT OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES

G. Nador,[1] A. Molteni,[1] C. Cesana,[2] M. Nichelatti,[1] B. Scarpati,[2] R. Cairoli,[2] L. Barbarano,[1] M. Riva,[3] A. Nosari,[3] E. Morra[3]

[1]Department of Hematology, Niguarda Ca' Granda Hospital, MILAN; [2]Immunohematology and Transfusional Medicine, Niguarda Ca' Granda Hospital, MILAN; [3]Department of Hematology, Niguarda Ca' Granda Hospital, Milan, Italy, MILAN, Italy

Background. Blast cell population is relatively scarce in bone marrow (BM) of myelodysplastic syndromes (MDS) patients, so that few data on its immunophenotypic characterization have been published. Even if there is some evidence that markers of cell immaturity such as CD7 and CD117 may be associated with poor outcome (Ogata *et al.*, Blood 2002), the prognostic role of markers of immaturity and myeloid commitment remains largely unexplored. *Aims.* To preliminarily evaluate if blast immunophenotyping of a not-enriched bone marrow sample may represent a further simple and reproducible tool for prognostic assessment in MDS patients. *Methods.* Expression of CD33, CD34 and CD117 antigens in not-enriched BM samples of 69 newly diagnosed MDS was compared with both IPSS score and BM blast percentage stratified into three groups in accordance to WHO classification: <5%, from 5 to 9%, from 10 to 19%. Immunophenotypic analysis was carried out by using the panel of quadruple monoclonal antibodies CD34/CD117/CD45/CD33, conjugated with the fluorochromes FITC, PE, PerCP, APC, respectively. Acquisition of information on 1×10^5 stained cells corresponding to the whole BM cellularity was assessed on a dual-laser FACSCalibur flow cytometer using the CellQUEST software (Becton Dickinson, San José CA USA). Multiple group comparison was made using general linear model with Wald's test and Kruskal Wallis' confirmation test (KW) for IPSS and using non parametric ANOVA for BM blast percentage. *Results.* According to IPSS, 10 (14.5%) low risk, 34 (49.3%) intermediate-1 risk, 19 (27.5%) intermediate-2 risk and 6 (8.7%) high risk pts were identified. Expression of markers of myeloid cell commitment (i.e. CD33 and CD34) and a marker of myeloid cell immaturity (i.e. CD117) significantly correlated with both IPSS and blast WHO category, as shown on Table 1. Interestingly, by analyzing the subset of 43 pts with BM blasts <5%, a correlation was found between IPSS and the expression of both CD33 (p=0.010) and CD34 (p=0.033); CD117 expression also increased in pts with higher IPSS, however not reaching statistical significance (p=0.680). *Conclusions.* In our series expression of markers of myeloid commitment (CD33 and CD34) showed a significant correlation with IPSS score and blast percentage according to WHO. Furthermore, of interest is the correlation of CD33 and CD34 expression with IPSS within the subset of pts with <5% BM blast. The immaturity marker tested (CD117) also correlated with risk score in the general population. These preliminary data suggest a possible correlation between CD33, CD34 and CD117 expression at diagnosis and outcome. This simple and reproducible immunophenotypic test may be candidate as an adjunctive prognostic tool in MDS setting, but needs to be previously confirmed in survival-oriented studies on larger populations.

Table 1.

Surface marker	IPSS category				Blast percentage (WHO)		
	Low	Int-1	Int-2	High	<5	5-9	10-19
Mean CD33 expression (%)	1.81	1.65	6.45	11.05	2.16	3.22	9.93
	p=0.0001 (KW)				p= 0.0013 (ANOVA)		
Mean CD34 expression (%)	0.96	1.54	5.75	12.13	1.49	3.39	10.18
	p=0.0001 (KW)				p= 0.0001 (ANOVA)		
Mean CD117 expression (%)	1.87	1.89	7.16	16.58	1.97	3.62	14.48
	p=0.0001 (KW)				p=0.0001(ANOVA)		

0721

FOUR-COLOR FLOW CYTOMETRY IS USEFUL IN DIAGNOSIS AND PREDICTING PROGNOSIS OF MYELODYSPLASTIC SYNDROME

C.H. Cha,[1] C.J. Park,[2] J.W. Chung,[2] H.Y. Chung,[2] Y.U. Cho,[2] E.J. Seo,[2] H.S. Chi,[2] J.H. Lee,[2] J.H. Lee,[2] K.H. Lee[2]

[1]Asan Medical Center, SEOUL; [2]University of Ulsan College of Medicine and Asan Medical Center, SEOUL, South-Korea

Background. Myelodysplastic syndrome (MDS) is a clonal disorder characterized by peripheral cytopenia, ineffective hemopoiesis and increased risk of acute myeloid leukemia. The diagnosis is based on the morphology of peripheral blood and bone marrow (BM), and cytogenetics. However, morphologic evaluation is subjective and often difficult, and cytogenetic results are normal in some MDS. Recently several approaches have been made to detect abnormal immunophenotypes in MDS by flow cytometry (FCM) because of their reproducibility and objectiveness. The purpose of this study was to find the usefulness of 4-color FCM in the diagnosis and predicting prognosis of MDS. *Methods.* The immunophenotypes of BM aspirates from 30 MDS patients (6 RA, 7 RCMD, 3 RCMD-RS, 8 RAEB-1, 4 RAEB-2, 2 MDS-u) and 11 patients with non-clonal hematologic disorders (controls, 4 lymphoma without BM involvement, 1 ITP, 1 FUO, 2 AA, 3 normal marrow) were tested by 4-color FCM (FACSCanto, Becton Dickinson, San jose, CA). Twelve panels composed of 15 kinds of monoclonal antibodies were used; 1 erytrhoid panel (CD71/-/glycophorin A/-CD45); 1 myeloblast panel (CD34/CD33/CD38/CD45); 5 granulocyte panels (CD16/CD11b/-/CD45, CD16/CD13/-/CD45, CD64/CD33/-/CD45, CD15/CD10/-/CD45, CD56/CD19/CD7/CD45) and 5 monocyte panels (same as granulocyte panels, but gating of monocytes different from gating of granulocytes). The immunophenotypes of MDS were compared with those of controls. We also analyzed the correlation of numbers of panels showing abnormal immunophenotypes with International Prognostic Scoring System (IPSS) in MDS. *Results.* MDS revealed more abnormal immunophenotypes than controls (4.3±2.0 *vs* 0.1±0.3, p<0.001). In erytroid panel, MDS showed partial losse of CD71 and/or glycophorin A expression (p=0.064). In myeloblast panel, the ratios of $CD33^+CD34^+$ cells/$CD33^-CD34^+$ cells were not significantly different between MDS patients and controls (p=0.569). In granulocyte panels, MDS showed the decreased expression of CD10, CD11b, CD15 and CD16, and the increased expression of CD13 (p<0.001). In monocyte panels, MDS showed the increased expression of CD10, CD11b, CD15 and CD16 (p<0.001). MDS patients showed the positive correlation between the number of panels showing abnormal immunophenotype and IPSS score (R2=0.151, p=0.0045). *Conclusions.* Four-color FCM using 15 monoclonal antibody combinations, especially CD15/CD10/-/CD45 and CD16/CD13/-/CDCD45 panels for granulocytes, helps to differentiate MDS from non-clonal hematological disorders. And the abnormality of immunophenotypes in MDS correlates with IPSS score. Four-color FCM could be a useful tool in the diagnosis and predicting prognosis of MDS.

0722

URINARY HEPCIDIN LEVELS ARE SUPPRESSED IN LOW GRADE MYELODYSPLASTIC SYNDROME AND MAY BE ASSOCIATED WITH MARKERS OF FUNCTIONAL IRON DEFICIENCY

T. Murphy,[1] S. Mitra,[1] C. Gleeson,[1] P. Desmond,[1] W. Swinkels[2]

[1]Beaumont Hospital, DUBLIN, Ireland; [2]Radboud University Nijmegen Medical Center, NIJMEGEN, Netherlands

Background. In low grade myelodysplastic syndrome (MDS) (refractory anemia (RA) and refractory anemia with ring sideroblasts (RARS)), we have previously found a correlation between percentage hypochromic cells (PHC) and red cell zinc protoporphyrin (ZnPP), suggesting that these measurements may reflect functional iron deficiency. Data on urinary hepcidin levels in low grade MDS are limited, although hepcidin suppression due to ineffective erythropoiesis might be expected, contributing to iron overload. *Aims.* To correlate urinary hepcidin levels in RA and RARS with other parameters, especially PHC, ZnPP and iron levels. *Methods.* 17 MDS patients (12 RA, 5 RARS, 15/17 with multilineage dysplasia) had the following investigations: complete blood count, reticulocytes, liver blood tests, CRP, LDH, plasma viscosity, serum iron, transferrin saturation and ferritin, serum erythropoietin (EPO), PHC, ZnPP and urinary hepcidin. Statistical significance was calculated by Spearman rank correlation and Mann-Whitney U. *Results.* For the 17 cases, median (range) urinary hepcidin, PHC and ZnPP were 6.11 Mint/mmol creat (0.63-35.6), 2.9% (0.5-23.8) and 3.2 ug/g Hb (0.9-13) respectively. 8 cases had a hepcidin level <4 Mint/mmol creat. There was a statistical-

ly significant correlation between urinary hepcidin and serum iron (rs 0.574, $p<0.05$), although the slope of the correlation was 0.22, indicating suppression of hepcidin relative to serum iron. A positive correlation between PHC and ZnPP (rs 0.483) and a negative correlation between urinary hepcidin and ZnPP (rs -0.434) just failed to reach statistical significance. For the RA patients, urinary hepcidin also correlated with serum iron ($p<0.01$) and transferrin saturation ($p<0.01$). A strong correlation between serum EPO levels and serum LDH was seen for the total group ($p<0.01$), as well as the RA ($p<0.01$) and RARS ($p<0.02$) subgroups and both patients receiving (n=8, $p<0.02$) and not receiving (n=9, $p<0.02$) recombinant EPO therapy. The higher levels of PHC in the RARS group compared to the RA group just failed to reach statistical significance ($p=0.057$). *Conclusions.* Our results show a correlation between urinary hepcidin and serum iron in low grade MDS with the slope of the correlation indicating suppression of hepcidin. Functional iron deficiency, as reflected by raised PHC and ZnPP, may be contributing to ineffective erythropoiesis and thus suppression of hepcidin, with subsequent iron overload. A strong correlation between serum EPO levels and serum LDH for both patients receiving and not receiving recombinant EPO therapy is of interest, given that serum LDH has been associated with poor prognosis in MDS.

0723

EXTERNAL VALIDATION OF WHO CLASSIFICATION-BASED PROGNOSTIC SCORING SYSTEM(WPSS)

M.J. Park, J.H. Jang, C.W. Jung, K.H. Kim, S.J. Kim

Samsung medical center, SEOUL, South-Korea

Background . Based upon the classification of FAB criteria, International Prognostic Scoring System(IPSS) has been a standard prognostic model to predict survival and progression in MDS. In 2000, the WHO has formulated a new classification of MDSs. The aim of this study was to evaluate the prognostic value of WHO classification-based prognostic scoring system(WPSS) in MDS, suggested by Malcovati L *et al.* in 2007(J Clin Oncol 25:3503-3510). *Patients and Methods*. One hundred forty-nine patients who were diagnosed as having de novo MDS at the Division of Hematooncology, Samsung medical center(Seoul, Korea), between Dec. 1994 and Feb. 2007, were evaluated retrospectively for clinical and hamtologic features at diagnosis, transfusion dependence, overall survival(OS), and progression to leukemia(LFS). Risk group stratifications in MDS patietns were done according to IPSS and WPSS. *Results*. 18 patients(12.1%), 93 patients (62.4%), 29 patients(29%) and 9 patients(6%) had IPSS risk scores of low, intermediate1, intermediate2 and high, respectively. According to WPSS risk scores, 8 patients(5.4%), 30 patients(20.1%), 41 patients(27.5%), 57 patients(38.3%) and 13 patients(8.7%) were classified to very low, low, intermediate, high and very high risk group, respectively. In IPSS, median OSs of low, intermediate1, intermediate2 and high subgroup were 65.2, 32.9, 14.3 and 9.1 months respectively ($p<0.001$). According to WPSS, median OSs of very low, low, intermediate, high and very high risk subgroup were not reached, 55.4, 27.4, 19.0 and 6.2 months respectively ($p<0.001$). Between subgroups classified according to WPSS, significant differences in OS were noted in low *vs* intermediate risk group ($p=0.047$), in intermediate *vs* high risk group ($p=0.046$) and in high *vs* very high risk group($p=0.003$) but statistically not significant difference in OS was observed between very low and low risk group ($p=0.08$). The mean and median OS of the lowest risk group(low risk) in IPSS are 65.33 and 55.43 months, respectively. The mean and median OS of the lowest risk group(very low risk) in WPSS are 102.8 months and not reached, respectively. *Conclusions*. These data show that WPSS with five risk groups might provide more refined prognostic stratifications of MDS than IPSS with four risk groups. Especially, new prognostic system appears to discriminate a subset of patients with very low risk, who could have long term survival.

0724

IMMUNOLOGICAL BACKGROUND OF MDS, AN IMPACT ON PROGNOSIS AND PATHOGENESIS OF MDS

R. Neuwitova,[1] V. Cukrova,[1] J. Bartunkova,[2] J. Karban,[1] K. Siebertova,[1] A. Jonasova,[1] J. Cermak,[3] M. Belickova[3]

[1]*University Hospital, PRAGUE 2;* [2]*Institute of immunology Faculty Hospitla Motol, PRAGUE;* [3]*Insitute of Hematology and Blood Transfusion, PRAGUE, Czech Republic*

Research of immunological background is warranted in MDS not only because of decreased antiinfection immunity but also of possible compromised antitumor immunity. Methods. MDS-RA, RARS, RAEB patients and controls (hematologically normal elderly persons and patients with other hematological diseases, mainly MPD) had examined: 1) Monocyte-derived dendritic cells (DC) in 16 patients. 2) Clonality of separated B and T lymphocytes, granulocytes and monocytes (n=12 females) with Humara test. 3) By means of flow cytometry (n=53) basic antigens of T cell and B cells, regulatory T lymphocytes (T regs) identified with $CD25^+CD4^+$ and finally γσ T ($CD8^+$ TCR (γ,σ)+) cells. 4. In 5 days culture of effector and allogeneic target cells cytotoxic test was performed as standard cell mediated cytotoxic reaction using allogeneic cells labeled with 51 CR in 12 RA a 6 RARS patients. 5. In 6 days culture of patient´s cells with irradiated third party person cells one-way mixed lymphocyte reaction (MLR) was done in 13 MDS patients using 3 H-thymidine incorporation. *Results*. DC manifested defective maturation in 70% MDS patients. T and B cells were polyclonal in all but one patient. There was no statistically significant difference in CD3 subpopulations. Percentage and absolute number of B cells was decreased in RA and RARS ($p<0,01$), not in RAEB. T regs were decreased in RA and RARS in comparison with hematological controls($p<0,001$). Contrary to published data increased T regs were observed only in 2 of 10 RAEBs probably because of not staining for Foxp3؟ γσ T lymphocytes, important in the innate immunity, were decreased in MDS patients in comparison with both controls ($p<0,01$). Cytotoxic test, demonstrating in particular final effector cytotoxic phase of cell mediated immunological reaction, was defective in 68% MDS, predominantly in RA. Cytotoxic test was positive in all 12 controls. Initial, proliferative phase of immunological reaction, presented by MLR, was strongly positive in all MDS patients. Discussion and conclusion. The results of tested immunological parameters and reactions are unfavorable in the majority of cases. Polyclonality of T and B cells do not guarantee normal function of these cells. The decrease of T regs may have a favorable prognostic significance. Defective function of DC was not observed in MDS patients in contact with a strong antigen (allogeneic cells in MLR), but can appear in meeting weak antigens of dysplastic cells. The decreased cytotoxicity in the effector phase of cell mediated immunological reaction can lead to a compromised antitumor surveillance in MDS. It is assumed that premature apoptosis of hemopoietic cells is inherent to the dysplasia itself, but apoptosis can be also induced by immunologic attack of T cells with the aim to eliminate dysplastic cells, possessing features of tumor cells (hypermethylation of DNA, angiogenic activity, growth advantage). However, immunologically induced apoptosis will be incomplete in MDS because of impaired cytotoxic reaction, in particular in RA patients. Especially young cells resist the immunological attack, survive and due to their growth advantage replenish soon the bone marrow again with dysplastic cells.

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Myeloproliferative disorders - Biology

0725

CHRONIC PHASE POLYCYTHEMIA VERA EVOLVES BY ACCRUING GENETIC LESIONS WITH SUBTLE CONSEQUENCES

M. Scott,[1] W. Tong,[2] P.A. Beer,[1] P.A. Futreal,[3] M.R. Stratton,[3] H.F. Lodish,[4] A.R. Green[1]

[1]University of Cambridge, CAMBRIDGE, UK; [2]University of Pennsylvania, PHILADELPHIA, USA; [3]Wellcome Trust Sanger Institute, HINXTON, UK; [4]Whitehead Institute for Biomedical Research, CAMBRIDGE, USA

Background and Aims. Malignancies are thought to undergo Darwinian selection of mutant subclones, but little is known about the initial stages of this process. The myeloproliferative disorders provide an accessible paradigm for the earliest steps of tumorigenesis. *Methods*. Levels of different JAK2 mutations were monitored by sequencing and allele-specific PCR using T-cells, buccal cells, and granulocytes sampled 5, 9, 12 and 19 years after presentation. The *in vitro* consequences of various JAK2 alleles were explored by retrovirally expressing each in IL3-dependent BaF3 cells co-expressing the erythropoietin receptor (BaF3/EpoR). Transduced cells were tested for factor-independence by growing in the absence of IL3, and for activation of the JAK/STAT and RAS/MAPK signaling pathways by Western blot analysis. Erythroid colonies, produced by plating patient peripheral blood mononuclear cells in methylcellulose assays, were genotyped by sequencing JAK2 exon 14. *Results*. An acquired V615L mutation was identified in a patient with V617F-positive polycythemia vera. The V615L mutation occurred in cis with, and subsequent to, the V617F mutation. In BaF3/EpoR cells, the V615LV617F and V617F JAK2 alleles conferred similar rates of cytokine-independent proliferation; levels of phospho-JAK2, phospho-STAT5 and phospho-ERK were also comparable. The lack of a detectably different phenotype raised the possibility that V615L might represent a non-functional *passenger* mutation. However, the level of the V615L mutation in the patient progressively increased over fourteen years, with eventual evolution to homozygosity for the V615LV617F allele. Furthermore, sequential analysis of individual erythroid colonies over a two year period identified intermediate genotypes and demonstrated a significant increase in the proportion that were V615LV617F-homozygous ($p<0.0001$). Taken together, the data support a model in which at least four distinct *driver* events occurred during chronic phase disease. *Conclusions*. Our results show that, during chronic phase PV, the neoplastic clone evolves by accruing genetic lesions with subtle consequences. Furthermore, our data demonstrate the existence of functional tyrosine kinase mutations that are not detectable in conventional BaF3 assays.

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CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED MICRO-RNAS IN HEMATOPOIETIC CELLS OF CHRONIC MYELOPROLIFERATIVE DISORDERS

P. Guglielmelli,[1] L. Tozzi,[1] C. Bogani,[1] R. Zini,[2] A. Bosi,[1] R. Manfredini,[2] A.M. Vannucchi[1]

[1]University of Florence, FLORENCE; [2]University of Modena and Reggio Emilia, MODENA, Italy

Background. Notwithstanding the discovery of recurrent molecular abnormalities in JAK2 or MPL, additional molecular defects associated with Chronic Myeloproliferative Disorders (MPDc), and possibly responsible for their variable phenotype, remain still largely undefined. Previous study of transcriptome highlighted a complex pattern of aberrantly regulated genes in Primary Myelofibrosis (PMF) CD34$^+$ cells [Guglielmelli *et al.*, Stem Cells, 2007:165-73]. microRNAs emerged as important regulators of gene expression, and we recently reported on miRNA profile in PMF granulocytes [Guglielmelli *et al.*, Exp. Hematol, 2007:1708-18]. AIMS. To identify abnormally expressed miRNAs in CD34$^+$ cells from patients with PMF, polycythemia vera (PV) or essential thrombocythemia (ET). METHODS. The study involved granulocytes from 50 patients with PMF, 40 PV and 25 ET, and CD34$^+$ cells Selected miRNAs were assayed using stem-looped primers for reverse transcription, followed by quantitative real-time PCR in granulocytes, CD34$^+$ cells and erythroid colonies. miRNA precursor' expression was analyzed using SYBR Green RTQ-PCR. Sequencing technology was utilized to evaluate abnormalities in mature miR-16 and precursors, while FISH analysis were performed to search for abnormalities in chromosome 13q14. *Results*. PMF granulocytes could be differentiated from both PV and ET cells based on the expression of a set of four miRNAs, of which miR-31, -150, and -95 were downregulated and -190 was upregulated. We found increased expression level of miR-182, -183 and -96 in granulocytes from all cMPDs compared to cells from controls or subjects with idiopathic or secondary erythrocytosis (n=15); these genes are included in the same cluster on chromosome 7 and their level was correlated with JAK2V617F allelic burden. In-silico analysis indicated that potential target of this miRNA cluster is Bcl-2, whose expression levels were actually found increased in cMPD cells. Overexpression of miRNA16 was observed in CD34$^+$ cells and in erythroid colonies, both EEC and Epo-dependent BFU-E, of patients with PV; the level measured in patient with post-polycythemia myelofibrosis were significantly higher than in all other conditions. To address potential mechanisms for miR16 overexpression we performed FISH and sequencing analysis of the microRNA precursors (mir16-1 and miR-16-2 that are located on chromosome 13 and chromosome 3, respectively), but no abnormality was found. Also expression level of miR- 15a and miR-15b, that are included in the same cluster with miR-16, were found unchanged compared to controls, suggesting a specific up-regulation of miR-16 due to a still uncharacterized mechanism. *Conclusions*. These data indicate that specific abnormalities in the expression of selected miRNAs can be detected in both, or either, granulocytes and CD34$^+$ cells from cMPD patients, that in some instances are correlated with JAK2V617F mutational status; in particular, hsa-miR-16 might represent a potential novel prognostic factor in PV patients since its expression level correlated with the progression of the disease towards myelofibrosis.

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IDENTIFICATION OF NOVEL GENETIC ABERRATIONS IN BCR/ABL-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS USING HIGH-RESOLUTION SNP ARRAYS

F. Stegelmann, L. Bullinger, S. Kuhn, S. Schauer, S. Miller, M. Griesshammer, H. Dohner, K. Dohner

University Hospital of Ulm, ULM, Germany

The discovery of the gain-of-function mutation JAK2V617F represents the genetic hallmark in patients (pts) with polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). However, about 30% of the pts lack the mutation and previous studies on ET and PV demonstrated that clonality exceeds the percentage of V617F mutated cells. Therefore, additional genetic alterations may be involved in the pathogenesis of these diseases. To identify novel disease-related aberrations we applied genome-wide single nucleotide polymorphism (SNP) arrays in 108 BCR/ABL-negative myeloproliferative neoplasm (MPN) pts classified according to the 2008 WHO criteria: ET, n=36; PV, n=36; post-ET myelofibrosis (MF), n=6; post-PV MF, n=6; PMF, n=24. In our study, DNA from granulocytes was hybridized on GeneChip® Mapping 250K Nsp arrays (Affymetrix) to identify copy number alterations (CNAs) and regions with copy neutral loss of heterozygosity (uniparental disomies, UPDs). As SNP call rates > 90% were reached in all experiments, average SNP spacing was approximately 10 kb over the whole genome. Genotypes were analyzed using Copy Number Analyzer for Affymetrix GeneChip® Mapping arrays software (CNAG 2.0). Data were normalized against an own set of 30 reference samples (unmatched-pair analysis). Regions recently detected as copy number polymorphisms were excluded from data analysis. CNAs were found in 22% of ET, 14% of PV, 50% of secondary MF (SMF), and 46% of PMF cases. In ET, recurrent CNAs were small gains of 10q11 (2.5-2.6 Mb, n=5) encompassing the ANXA8 gene, whereas gain of 1q12-q23 (62 Mb), 3q27-q28 (1.2 Mb), and 16p13 (1.9 Mb) and loss of 20q11-q13 (15 Mb) were restricted to single cases. In PV, we identified gain of 9p / trisomy 9 and loss of 20q11-q13 in two cases each, as well as gain of 1q and 7p21 (0.2 Mb) in single pts. Of note, in this cohort one additional case with 10q11 gain (1.7 Mb) was identified. Recurrent CNAs in SMF pts were trisomy 9 (n=3) and microdeletion of 17q11.2 harboring the NF1 locus (1.4-2.4 Mb, n=2); in both cases monoallelic NF1 loss was confirmed by FISH. In PMF pts trisomy 8 and loss of 20q11-q13 (n=2, each) represented the most frequent genomic abnormality. In addition, twelve more MF pts exhibiting single losses ranging from 0.3-32 Mb in size were identified. Among others, genes such as SKIP (2q36, 0.5 Mb), FOXP1 (3p13, 2.6 Mb), SDC2 (8q22, 0.2 Mb), and TCF1 (12q24, 1.3 Mb) were affected. UPDs were detectable in 10% of ET, 61% of PV, and 33% of MF cases (5.1-41.2 Mb). While there were no recurrent UPDs in ET and PMF pts, 50% or more of PV and SMF cases exhibited 9p UPDs (6.4-38.7 Mb) encompassing the JAK2 locus (n=19 and n=6, respectively). In addition, two PV pts had 4q UPDs with a common affected segment in 4q25-q26 (6.8 Mb). In conclusion, our data on a large series of well defined MPN cases suggest that array SNP-mapping is an excellent

tool to identify known and novel genetic aberrations in MPN, thereby pinpointing to regions that may harbor disease-relevant genes.

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ANAGRELIDE DOWN-REGULATES THE EXPRESSION OF THE HAEMATOPOIETIC TRANSCRIPTION FACTORS GATA-1 AND FOG-1 DURING MEGAKARYOCYTE DIFFERENTIATION

D. Erusalimsky, M. Ahluwalia, N. Singh, H. Donovan

University of Wales Institute Cardiff, CARDIFF, United Kingdom

Background and Aims. Anagrelide is a potent and selective inhibitor of megakaryocytopoiesis used for the treatment of essential thrombocythaemia. While the effectiveness of this drug in lowering platelet counts is now firmly established, the molecular mechanisms that underlie this effect are poorly understood. We have previously demonstrated that anagrelide inhibits the development of megakaryocytes in cell cultures derived from human CD34$^+$ haematopoietic progenitors undergoing thrombopoietin (TPO) -induced terminal differentiation (Wang *et al.* Br. J. Pharmacol. 2005;146:324). In the present study we have exploited this cell culture system to examine the effects of anagrelide on the mRNA expression levels of GATA-1 and Friend of GATA-1 (FOG-1), two transcription factors that play an essential role in the control of megakaryocytic and erythroid cell development. *Methods.* Human umbilical cord blood-derived CD34$^+$ cells were expanded in IMDM-based serum free medium (Stem Cell Technologies) supplemented with haematopoietic growth factors and then induced to undergo megakaryocytic or erythroid terminal differentiation by further culture with 40 ng/mL TPO or 8 U/mL erythropoietin (EPO), respectively. Relative expression levels of selected transcripts were quantified by Real Time PCR with gene-specific probes (Applied Biosystems), using β-glucuronidase or TATA box binding protein as internal references. *Results.* Culture of haematopoietic cells for 4 days with TPO led to a 2.5-5.0-fold increase in the mRNA levels of the erythroid/megakaryocytic transcription factors GATA-1 and FOG-1 and to a ~15 fold increase in the expression of the megakaryocyte-specific gene GpIIb. Addition of 0.3 μM anagrelide at the beginning of the differentiation period reduced markedly the increase in the levels of these transcripts but had no effect on the house keeping gene glyceraldehyde-3-phosphate-dehydrogenase. Furthermore, the phosphodiesterase type III inhibitor cilostamide, had no discernible effect on the expression of any of these genes. Consistent with their dual role in megakaryocytic and erythroid differentiation, GATA-1 and FOG-1 mRNA levels also increased by > 2.5-fold when cells were cultured in the presence of EPO. However, in sharp contrast to the inhibition observed during megakaryocyte differentiation, anagrelide did not suppress the expression of these transcripts during erythroid differentiation. *Conclusions.* These findings indicate that anagrelide suppresses megakaryocyte development, at least in part by reducing the expression levels of the transcriptional co-regulators GATA-1 and FOG-1, and via a mechanism that does not involve the associated phosphodiesterase III activity of the drug. The fact that anagrelide does not affect the expression of GATA-1 and FOG-1 during erythroid cell development suggests that the inhibitory action of the drug is differentiation-context specific. Our findings also suggest that the molecular target of anagrelide lies upstream of GATA-1 and FOG-1 regulation.

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MOLECULAR RESPONSE OF POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA PATIENTS TREATED WITH HYDROXUREA

F. Girodon,[1] C. Schaeffer,[1] C. Cleyrat,[2] M. Mounier,[3] I. Lafont,[4] F. Dos Santos,[5] A. Vidal,[5] M. Maynadié,[5] S. Hermouet[6]

[1]CHU Bocage, DIJON; [2]INSERM U892, Centre de Recherche en Cancérologie Nantes/Angers, NANTES; [3]Registre des Hémopathies Malignes de Côte d'Or, EA Université de Bourgogne, DIJON; [4]Service d'Hématologie Clinique, CHU de Dijon, DIJON; [5]Laboratoire d'hématologie, CHU Bocage, DIJON; [6]Laboratoire d'hématologie, CHU Nantes, NANTES, France

Background. Detection of the JAK2-V617F mutation has become a main diagnostic test of BCR-ABL-negative myeloproliferative neoplasms (MPN). A high molecular response rate has been reported in Polycythaemia Vera (PV) patients treated with interferon alpha; however, the effect of hydroxyurea (HU), an inexpensive drug widely used in MPN, on the JAK2-V617F mutation load is still mostly unknown. *Aims.* To analyse the effect of HU on the JAK2-V617F allelic ratio (%JAK2-V617F) of patients with PV and Essential Thrombocythaemia (ET). *Methods.* Two groups of patients were examined at a single time point at the time of diagnosis (99 PV, 178 ET) or while receiving HU treatment (36 PV, 98 ET). In a second series of studies, 38 patients were examined sequentially prior to and after receiving HU (9 PV, 17 ET) or while remaining untreated (2 PV, 10 ET). For all patients, the %JAK2-V617F was determined in purified blood granulocytes using sensitive allele-specific, quantitative real-time polymerase chain reactions. *Results.* The mean %JAK2-V617F was significantly lower in HU-treated patients (24.5%) than in patients at diagnosis (33.5%, $p<0.01$). However, when analysed by diagnosis and gender, the decrease in %JAK2-V617F was significant only in female patients; this was true for PV (39.1% *vs* 52.7%, $p=0.0249$) and ET (12.8% *vs* 17.5%, $p=0.0364$). When analysis was individual and sequential, we found that HU-treatment (mean duration: 17 months) reduced the %JAK2-V617F by >50% in 12/26 patients (3 PV, 9 ET). In 4 of the 12 patients (4 ET, 3 males), JAK2-V617F was not detectable for a 3-27 months period of follow-up (mean %JAK2-V617F prior to treatment: 43.8%; with HU: 24.2%, $p<0.0001$). In contrast, there was no significant change in %JAK2-V617F for the 12 patients who received no treatment. *Conclusions.* HU frequently reduces the JAK2-V617F allelic ratio in PV and ET patients and may render the JAK2-V617F-mutated clone undetectable

0730

TARGETED ARRAY CGH INDICATES THAT CYTOGENETICALLY CRYPTIC TYROSINE KINASE FUSION GENES ARE RARE IN ATYPICAL MYELOPROLIFERATIVE DISORDERS

T. Ernst,[1] A. Chase,[1] F. Grand,[1] A. Reiter,[2] N.C.P. Cross[1]

[1]Wessex Regional Genetics Laboratory, SALISBURY, UK; [2]III. Medizinische Klinik, Universitätsklinikum Mannheim, MANNHEIM, Germany

Background. Activation of tyrosine kinases (TKs) by mutation or gene fusion is of major importance for the development of many haematological malignancies, particularly myeloproliferative disorders (MPDs). In general, TKs activated by mutation are associated with less aggressive MPDs, whereas TK fusions are seen in more aggressive diseases. Currently, more than 40 TK fusions have been identified in atypical BCR-ABL negative MPDs, the great majority of which are associated with visible chromosome rearrangements. However cytogenetically cryptic TK fusions have also been identified that arise from small deletions (e.g. FIP1L1-PDGFRA in chronic eosinophilic leukaemia) or episomal amplification (e.g. NUP214-ABL in T-cell acute lymphoblastic leukaemia). *Aims.* We hypothesized that hitherto unrecognised cryptic tyrosine kinase fusions may be common in atypical MPDs. To detect genomic copy number changes associated with such fusions, we performed a systematic search using custom designed, targeted high-resolution array comparative genomic hybridisation (array CGH). *Methods.* Pretreatment genomic DNA from 68 patients (44 males, 24 females; median age 62 years, range 16-86) with atypical MPD was studied: atypical MPD with eosinophilia, n=17; CEL/HES, n=17; CMML, n=10; unclassified atypical MPD, n=9; atypical CML, n=6; MDS/MPD, n=5; CNL, n=3; acute basophilic leukaemia, (n=1). Nine HES patients showed a significant response to imatinib treatment in the absence of any known imatinib-sensitive abnormality. All patients were negative for BCR-ABL, FIP1L1-PDGFRA, JAK2V617F and none had karyotypic abnormalities suggestive of other known TK fusions. We designed custom Agilent oligonucleotide arrays containing 44,000 oligonucleotide probes that targeted all TKs (n=90) plus a further 450 genes encoding downstream TK signalling components, other translocation targets plus receptors and other factors known to be important for myelopoiesis. For each target, 50-100 probes were selected that spanned the gene plus flanking sequences of up to 200kb, providing a resolution of approximately 5-10kb for each target. *Results.* Control experiments indicated that the arrays were readily able to identify FIP1L1-PDGFRA in a background of 50% normal cells. For analysis of the patient cohort, known polymorphic copy number variants (CNV) were excluded, as were regions not in CNV databases but showing both loss and gain in different patients (a feature of CNVs). Six cytogenetically cryptic abnormalities were detected in five (7%) patients: Pt 1 with atypical CML: 0.5 Mb del(21q22.12) (including RUNX1) plus 1 kb del(19p13.11) (JUND); Pt 2 with HES: del(19p13.11) as seen in Pt 1; Pt 3 with CMML: 53 kb dup(19p13.3) (in 5' proximity to MATK); Pt 4 with unclassified atypical MPD: 44 kb del(5p12) (FGF10) together with a 25 kb del(15q21.1) (FGF7); Pt 5 with eosinophilia: 44 kb dup(22q13.2) (L3MBTL2). No abnormalities involving TKs were detected. *Summary and Conclusions.* We conclude that cytogenetically cryptic TK fusion genes are rare in patients with atypical MPD, even in cases who responded to imatinib. Other abnormalities were identified in a minority of cases that warrant further investigation.

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FREQUENCY OF JAK2 EXON 12 MUTATIONS IN JAK2 (V617F) NEGATIVE PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISORDERS (CMD)

M. Bernardi, M. Ruggeri, E. Albiero, D. Madeo, F. Rodeghiero

San Bortolo Hospital, VICENZA, Italy

Background. V617F JAK2 exon 14 mutation has been reported in about 95% of patients with Polycythemia Vera (PV) and 50% of patients with Essential Thrombocythemia (ET). The underlying pathogenetic mechanism in V617F negative patients remains unclear. Recently, mutations in exon 12 have been found in some cases of V617F negative patients with PV or with Idiopathic Erythrocytosis (IE). *Aims.* To estimate the frequency of exon 12 mutations in V617F negative patients with PV, IE and ET. Patients and *Methods.* In a group of 366 patients with CMD followed at our center, 127 found negative for V617F were investigated: 3 PV, 80 ET and 44 IE; DNA was extracted from isolated granulocytes. All samples were previously screened for V617F mutation by ASO-PCR and found negative. Exon 12 was amplified using the couple of JAK2exon12F (5'-ctcctctttggagcaattca-3') and JAK2exon12R (5'-caatgtcacatgaatgtaaatcaa-3') primers. The amplicons were submitted to denaturing high performance liquid chromatography (dHPLC) analysis and runs were performed at 53° C. The amplicons showing a heteroduplex profile were sequenced directly in both strands. Erythroid colony culture was prepared isolating peripheral blood mononuclear cells on Ficoll-Hystopaque density gradient (Sigma-Aldritch), washed and risospended in RPMI medium solution (Sigma-Aldritch). Mononuclear cells were plated at final concentration of 2.5×10^5 cells/mL in Methocult medium with 3 units/mL Epo (Methocult GF H4434) and without Epo (Methocult GF H4534), (StemCell Technologies). Cultures were incubated at 37° C in a humidified atmosphere of 5% CO2. Erytroid colonies were scored on day 14 for burst forming unit-erythroid (BFU-E). Individual colonies were plucked, resuspended in 300 µL of 10% Chelex 100 chelating resin (Sigma) and heated at 95° C for 20 min to release genomic DNA. All endogenous erythropoietin-independent erythroid colonies (EECs) were genotyped by direct sequencing of exon 12. *Results.* JAK2 mutations in exon 12 were detected in 5 out 127 patients negative for the V617F mutation, all with a clinical phenotype of IE consisting of increased hematocrit requiring regular phlebotomy therapy, absence of spleen enlargement, increased white blood cell count or platelet count and normal or low serum erythropoietin over several years of follow up. Three different exon 12 heterozygous mutations were found: the previously described N542-E543del in 3 cases, the 547insL+I540-F547dup8 mutation in one and a new I540-N542delinsS mutation. The presence of the mutations was confirmed in EECs in all cases. *Conclusions.* Among our V617F negative patients, exon 12 mutations (including a new one) were found only in cases with clear IE phenotype, suggesting the hypothesis that these patients represent a distinctive clinical syndrome. Our results are in keeping with the observations of other investigators that found an increased frequency of exon 12 mutations in these cases.

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INCREASED BIOAVAILABILITY OF TRANSFORMING GROWTH FACTOR β1 IN PLASMA FROM PATIENTS WITH PRIMARY MYELOFIBROSIS

R. Campanelli, V. Rosti, G. Bergamaschi, E. Bonetti, L. Villani, S. Lo Pò, V. Perfetti, G. Barosi, M. Massa

Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy

Background. TGF, is made in excess in most organs and is stored in a latent inactive form that becomes active by proteolitic cleavage *in vivo* and by acidification *in vitro*. It is one of the most powerful regulators of hematopoiesis; moreover, in a number of diseases has been shown to induce the abnormal deposition of extracellular matrix proteins resulting in tissue fibrosis. Previous studies in primary myelofibrosis (PMF) showed an increased TGFβ1 mRNA expression in peripheral blood (PB) mononuclear cells and increased circulating levels of total (latent+active) TGFβ1; on the contrary, the attenuation of the TGFβ1 signalling through a down-regulation of TGFβRII on $CD34^+$ cells was described. *Aims.* To investigate the presence of abnormal amounts of active circulating TGF,1 in patients with PMF, in disease controls with polycythemia vera (PV) or essential thrombocytemia (ET), and in healthy controls (CTRLs). *Methods.* Platelet-poor plasma samples were obtained from 41 PMF, 15 PV, 6 ET, and 17 CTRLs. Active circulating TGFβ1 was assessed in 1/50 diluted plasma samples, based on the capability of active TGFβ1 to inhibit the *in vitro* growth of CCL64 mink lung cells. The inhibition was calculated as percentage of the proliferation obtained with 1/1000 diluted plasma samples. The inhibition induced by total TGF,1 was tested on 1N HCl treated plasma samples. Similarly, we tested active and total TGF, in the lysates of 2.5×10^9 platelets/mL from patients with PMF and CTRLs. *Results.* CCL64 cell growth inhibition induced by plasma of patients with PMF (43.7%±4.3SE) or PV (28.1%±7.8SE) was higher ($p<0.0001$ and $p<0.04$, respectively) than that of plasma from CTRLs (6.4%±3.3SE). After plasma acidification, the mean increase in the percentage of CCL64 cell growth inhibition was 24.3% in PMF, 30.6% in PV, 27.4% in ET and 69.1% in CTRLs indicating that active TGFβ1 predominates in the PB of patients with myeloproliferative diseases compared with CTRLs. Total TGFβ1 plasma levels, evaluated by ELISA, were higher ($p=0.024$) in patients with PMF (5718pg/mL±627SE) than in CTRLs (2904pg/mL±470SE), but did not correlate with the CCL64 proliferation inhibition induced by the active form of TGFβ1. To investigate the source of active/bioavailable TGFβ1, we evaluated the growth inhibition of CCL64 cells incubated with platelet lysates of 9 patients with PMF and 8 CTRLs. The percentage of inhibition induced by platelet lysates from patients (36.4%±10.5SE) was not significantly different from that by lysates from CTRLs (24.5%±7.4SE). Similarly, both CCL64 cell growth inhibition due to the total amount of TGFβ1 in platelet lysates and the levels of total TGFβ1 in the lysates were comparable in patients and CTRLs (*data not shown*). *Conclusions.* Our data indicate that the bioavailable/activated form of TGF,1 predominates in the PB of patients with myeloproliferative diseases; its ability to inhibit CCL64 cell growth is not related to the total TGFβ1 plasma levels. Abnormal activation of TGFβ1 in the PB of PMF suggests a defective regulatory mechanism that does not involve its form or levels investigated in platelet lysates.

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COOPERATING MUTATIONS OF RECEPTOR TYROSINE KINASES/JAK2/RAS SIGNALING PATHWAYS AND HEMATOPOIETIC TRANSCRIPTION FACTORS IN PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA

L.Y. Shih,[1] C.F. Huang,[1] Y.S. Shih,[2] J.H. Wu,[1] M.C. Kuo,[1] T.L. Lin,[1] D.C. Liang[2]

[1]Chang Gung Memorial Hospital, TAIPEI; [2]Mackay Memorial Hospital, TAIPEI, Taiwan

Background. Two-hit model of leukemogenesis has been proposed for AML; class I mutations that drive proliferation and survival, and class II mutations that block differentiation. The cooperation of class I and class II mutations has recently been described in therapy-related myelodysplastic syndrome and AML. The cooperating mutations in chronic myelomonocytic leukemia (CMML) have not been systematically examined. *Aims.* We sought to determine the collaboration of class I mutations including receptor tyrosine kinases/JAK2/Ras signaling pathways and class II mutations involving hematopoietic transcription factors in patients with CMML. Patients and methods. Bone marrow samples obtained from 84 patients with CMML at diagnosis were analyzed for FLT3-LM, FLT3-TKD, c-KIT (exons 1-21), c-FMS (exons 5-22), JAK2V617F, N-Ras and K-Ras (codons 12, 13 and 61) and PTPN11 (exons 1-15) of class I mutations and RUNX1 (exons 3-8), CEBPalpha , NPM1 (exon 12) and PU.1 (exons 1-5) of class II mutations. Mutations were all confirmed by repeated assays with direct sequencing in both directions. Results. Class I mutations were detected in 3 of 84 patients for FLT3-LM, 3/84 for FLT3-TKD, 1/81 for c-KIT(D816V), 4/76 for c-FMS (T621M and 3 silent mutations), 3/83 for JAK2V617F, 16/84 for N-Ras, 3/84 for K-Ras, and 4/82 for PTPN11. Class II mutations were present in 30/83 for RUNX1, 7/83 for CEBPalpha(2 silent mutations), 2/82 for NPM1, and 0/20 for PU.1 mutations. The cooperation of class I and class II mutations (excluding silent mutations) were detected in 11 patients (13%): 3 N-Ras and RUNX1, 1 K-Ras and RUNX1, 2 N-Ras and CEBPalpha, one each for N-Ras and NPM1, PTPN11 and CEBPalpha, JAK2V617F and RUNX1, FLT3-TKD and RUNX1, PTPN11 and RUNX1. In addition, one patient had two class I mutations, FLT3-LM and PTPN11; 4 patients had two class II mutations, RUNX1 and CEBPalpha; and another one had 3 class I mutations, FLT3-TKD, c-FMS and N-Ras mutations. Conclusions. In CMML, 13.4% of patients had collaboration of class I and class II mutations, and additional 7.3% of patients had combined mutations of either class I or class II.

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A NEW VARIANT OF BPGM DEFICIENCY (ARG89HIS) ASSOCIATED WITH CONGENITAL ERYTHROCYTOSIS

C.V. Vercellati,[1] A.P. Marcello,[1] E. Fermo,[1] P. Bianchi,[1] W. Barcellini,[1] E. Rumi,[2] F. Passamonti,[2] A. Zanella[1]

[1]Fondazione IRCCS OMPMARE, MILANO; [2]Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy

Background. Erythrocytosis is generally defined as an absolute increase in red cell mass. The potential etiology is multifactorial and can be divided into primary and secondary causes. Primary causes of erythrocytosis are polycytemia vera (PV) and more rarely mutations in the erythropoietin receptor gene. Secondary hereditary erythrocytosis is caused by haemoglobin variants (either high oxygen-affinity or haemoglobin M variants) or, in a few cases, by red cell bisphosphoglycerate mutase (BPGM) or phosphofructokinase (PFK) deficiency. Erythrocyte BPGM deficiency is a rare disease associated with a decrease in 2,3-bisphosphoglycerate (2,3-BPG) concentration. BPGM gene is localised on chromosome 7. So far only 3 molecular variants have been reported in two unrelated patients with congenital erythrocytosis (Arg61Gln/ Arg61Gln and Arg89Cys/delC205). *Aims.* We describe a new variant of BPGM deficiency associated with congenital erythrocytosis. Case. The propositus was a 24 yrs male of Southern Italian origin. He reported a family history of erythrocytosis in the mother and two maternal uncles. These latter were diagnosed as PV and treated one with hydroxyurea for 7 years, and the other with phlebotomy for 14 years. *Methods*. Erythrocyte BPGM activity and 2,3 DPG concentration were determined according to Beutler. The codifying region and flanking intronic regions of BPGM gene were amplified and sequenced on an ABIPRISM 310 capillary sequencer to identify the molecular defect. *Result*. At the time of the study the propositus displayed Hb 17.4 g/dL, Hct 0.51, RBC 6.2×10^{12}/L, PLTs 214×10^{9}/L. Serum erythropoietin was 10.9 mU/mL, search for JAK2 mutations negative, oxygen saturation 100% on room air. The carboxyhaemoglobin level was 1.5% (n.v. < 1.5) and the oxygen dissociation curve of whole blood was left-shifted with a p50 of 18.9 mmHg (ref. range 21-27). No haemoglobin variants were detected and screening test for unstable haemoglobins was negative. 2,3DPG was decreased (6.2 umoles/gHb, ref. range 8.8-12.2) and ATP slightly increased (5.6 umoles/gHb, ref. range 3.6-4.8). The activity of the most important red cell enzymes was normal excepted for a decrease of BPGM (2.5 UI/gHb, ref. range 4.64-6.46). Propositus's mother showed similar haematologic findings. The sequence of BPGM gene showed the presence of a new missense mutation Arg89His (CGT-CAT) at heterozygous level. Arg89 is an highly conserved aminoacid and has been reported to be mutated in another BPGM deficient patient, suggesting that it is located at or near the active site of the enzyme and is probably involved in the binding of monophosphoglycerates. *Conclusions*. This is the first case of dominant BPGM deficiency associated with congenital erythrocytosis characterized at molecular level. Investigation of these rare erythrocytic defects may be useful in diagnostic definition of patients with isolated erythrocytosis and in differential diagnosis of cases with PV JAK2 V617F negative.

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RCE-1 DEFICIENCY ACCELERATES MYELOPROLIFERATIVE DISEASE IN CONDITIONAL NF1 MUTANT MICE

C. Karlsson, B. Cutts, A. Wahlström, A.K. Sjögren, M. Liu, O. Kahn, K. Andersson, M. Bergö

Gothenburg University, GOTHENBURG, Sweden

Background. RAS proteins are targeted to the plasma membrane following three posttranslational processing steps at a carboxyl-terminal CAAX motif. RAS-converting enzyme 1 (RCE1) is responsible for cleaving the -AAX residues from the cysteine residue (i.e., the *C* in CAAX) after this cysteine has been farnesylated. We previously showed that inactivation of the Rce1 gene in mouse fibroblasts results in mislocalisation of the RAS proteins away from the plasma membrane and reduces oncogenic RAS transformation *in vitro*.[1] Consequently, we tested the hypothesis that inactivation of Rce1 would reduce the development of a K-RAS-induced myeloproliferative disease *in vivo*. Contrary to our expectations, the inactivation of Rce1, although it mislocalized RAS proteins in myeloid cells, actually accelerated the development of MPD, dramatically increased white blood cell counts and tissue infiltration, and reduced survival.[2] The simplest potential explanation for this finding is that RCE1 processes a CAAX protein, aside from RAS, that normally suppresses cell proliferation in myeloid cells and that this protein is dysfunctional in the absence of RCE1-mediated proteolytic processing. If this explanation were true, then inactivating Rce1 should accelerate the development of MPD caused by other mutations. *Aims*. The aim of this study is to test the hypothesis that inactivation of Rce1 would accelerate the development of a MPD induced by a deficiency in the neurofibromatosis type 1 gene (Nf1) in mice. *Methods*. We use mice with a conditional Nf1 allele (N) and an Mx1-Cre transgene (M). Injection of pI-pC into NM mice inactivates Nf1 in haematopoietic cells and results in a progressive and lethal MPD.[3] We have bred NM mice on a background of homozygosity for a conditional Rce1 knockout allele (Rfl/fl). Control mice are Rfl/+. Injections of pI-pC into Rfl/flNM mice inactivated Nf1 (which initiated MPD development), and simultaneously inactivated Rce1. With these mice we are now in a position to assess the impact of inactivating Rce1 on the development, progression, and lethality of the MPD induced by Nf1 deficiency. *Preliminary Results.* The inactivation of Rce1 did indeed accelerate the development of MPD induced by N1 deficiency: white blood cell counts and spleen size were increased and the sensitivity of hematopoietic cells to GM-CSF was dramatically increased. Over the next few months, we should be able to conclude whether the absence of Rce1 would also reduce survival in pI-pC-injected Rfl/flNM mice. *Conclusions.* This study should allow us to confirm that inactivation of Rce1 accelerates the development of myeloid leukemia in a second mouse model of myeloid leukemia induced by hyperactive RAS signalling. This would strengthen the argument that RCE1 processes a farnesylated or geranylgeranylated CAAX protein that normally functions to suppress myeloid cell proliferation (or promotes differentiation). When this protein terminates with a farnesylated or geranylgeranylated cysteine residue and the *-AAX* tail (i.e., in the absence of RCE1) this protein would be dysfunctional leading to enhanced cell proliferation.

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0736

IMMUNOPHENOTYPICAL CHARACTERIZATION OF BONE MARROW MAST CELLS FROM DIFFERENT SUBTYPES OF SYSTEMIC MASTOCYTOSIS

C. Teodosio,[1] A.C. García-Montero,[1] M. Jara-Acevedo,[1] L. Sanchez-Muñoz,[2] R. Nuñez,[3] I. Álvarez,[2] L. Escribano,[2] A. Orfao[1]

[1]Cancer Research Center, SALAMANCA; [2]Centro de Estudios de Mastocitosis de Castilla la Mancha, TOLEDO; [3]Servicio de Hematología, Hospital Ramón y Cajal, MADRID, Spain

Background. Bone marrow (BM) mast cells (MC) from patients with systemic mastocytosis (SM) are known to be phenotypically different from MC from healthy subjects. Interestingly, and despite the fact that most SM patients (93%) present the D816V KIT mutation, different subtypes of SM have distinct clinical behaviour, supporting the notion that additional genetic lesions could exist, which could be associated with variable aberrant MC phenotypes. *Aims.* To describe the immunophenotypic characteristics of BMMC of the different subtypes of SM. *Methods.* BM samples from 92 healthy donors and 123 patients with different subtypes of SM [indolent SM (ISM) (n=70), aggressive SM (ASM) (n=10), MC leukemia (MCL) (n=3), SM associated with a clonal non-MC-lineage hematopoietic disease (SM-AHNMD) (n=13), SM associated with MC activation syndrome (SMAS) (n=16), and well-differentiated SM (WDSM) (n=11)] were analysed for a broad panel of flow cytometry markers (CD2, CD16, CD22, CD25, CD32, CD34, CD59, CD63, CD64, CD69, CD117, CD123, CD203c, HLA-DR , HLA-DQ, HLA-I, FcγRI, cytBcl2, cytCA2, cytB12 and cytG5) and total tryptase serum levels. *Results.* WDSM was the only diagnostic subgroup which showed CD25 negative BMMC in association with a significantly higher expression of total cytoplasmic tryptase (cytB12). In addition only one patient suffering from WDSM expressed CD2. Interestingly, with the exception of WDSM, all SM subtypes displayed increased serum tryptase levels along with low cytB12 expression. Likewise, MC from ASM, MCL and SM-AHNMD patients showed the lowest cytB12 levels detected. In turn, BMMC from ISM, SMAS and ISM-AHNMD patients also displayed increased light scatter properties, along with higher expression of CD2 and the CD63, CD69 and CD203c activation related markers. In turn, BMMC from ASM, MCL and SM-AHNMD patients showed a significantly lower expression of FcγRI and CD117. *Summary and Conclusions.* In summary, our results show the occurrence of three distinct phenotyp-

ic patterns for BMMC which are, apparently, related to both the maturation appearance of the clonal MC and the prognosis of the disease: WDSM, mild-prognosis SM (SMAS and ISM) and intermediate/poor prognosis patient (ASM, MCL and SM-AHNMD) subgroups.

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0737

RECURRENT DER(9;18) IN ESSENTIAL THROMBOCYTHEMIA WITH JAK2 V617F IS HIGHLY LINKED TO MYELOFIBROSIS DEVELOPMENT

K. Ohyashiki, A. Kodama, J. Ohyashiki

Tokyo Medical University, TOKYO, Japan

Background. The JAK2 V617F mutation is a consistent change in myeloproliferative neoplasia (MPN): approximately 50% of essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIM) show this mutation, resulting in constitutive up-regulation of JAK2. However, it is unknown why this single mutation may exhibit various phenotypes within different types of among MPN. *Aims.* Myelofibrosis is a major complication of MPN, thus factors predicting myelofibrosis in MPN are important in patient management, therefore we attempted to obtain more insight into the correlation between additional cytogenetic changes and JAK2 mutational status. *Methods.* We studied JAK2 mutational status, in combination with cytogenetic analysis, in 54 patients with essential ET, and attempted to obtain greater insight into the correlation between clinicohematologic features and genetic abnormalities. The JAK2 V617F mutational status was determined using the semiquantitative sequence-specific primer single-molecule fluorescence detection (SSP-SMFD) assay. We also performed spectral karyotyping (SKY) or FISH analysis using the CEP 8 and CEP 19 probes, to identify the translocation segment. *Results.* We found that 6 ET patients developed myelofibrosis and 4 of them had JAK2 V617F mutation. Of note is that 3 of the 4 ET with JAK2 V617F had add(18)(p11). In contrast, the remaining 2 ET patients developing myelofibrosis had neither JAK2 V617F nor add(18)(p11). Moreover, none of ET with JAK2 V617F and chromosome changes, other than add(18)(p11), developed myelofibrosis. *Conclusions.* The current results indicate that add(18)(p11), possibly due to der(9;18), may be linked to myelofibrosis development in JAK2 V617F-positive ET patients, while those with wild-type JAK2 may use another pathway towards myelofibrosis.

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EVI-1 OVER-EXPRESSION, AND PROGNOSTIC VALUE IN PH NEGATIVE CMPD

M.T. Gomez Casares,[1] P. Martin-Cabrera,[1] C.E. Lopez Jorge,[1] G. Santana,[1] J.D. Gonzalez-San Miguel,[2] H. Luzardo,[1] J. Lopez Brito,[1] T. Molero[1]

[1]Hospital Dr. Negrin, LAS PALMAS DE GRAN CANARIA; [2]Hospital Insular, LAS PALMAS DE GRAN CANARIA, Spain

Background. To our knowledge, there are no studies that have analyzed EVI-1 expression in Ph negative CMPD. Previous studies have demonstrated that EVI-1 is associated to the development of certain types of leukemia. Although high levels of EVI-1 have been reported in these specific cases, they don't always have to be associated to 3q26 abnormalities. EVI-1 over-expression has a high prevalence in acute myloid leukemia (AML) and myelodisplastic syndromes (MDS), suggesting a lack in the cells ability to accomplish final differentiation. Other studies have analyzed EVI-1 expression without 3q26 abnormalities in chronic myeloid leukemia (CML) patients , demonstrating a high expression during blastic crisis. *Aims.* To analyze EVI-1 expression in Ph negative CMPD, and relate it to diagnostic subtypes and to JAK2 V617F mutation. *Methods.* JAK2 mutations were studied in bone marrow (BM) and/or peripheral blood (PB) samples belonging to the following groups of patients: 9 myelofibrosis (1 of them could be studied before and after leukemic transformation), 27 polyglobulia, 25 thrombocytosis, and 14 non labeled CMPD (one of them during accelerated phase). QRT-PCR was used for the relative quantification of EVI-1 levels. Positive results were considered when EVI-1 expression levels were situated above a pre-established cut-off point by our laboratory. *Results grouped by diagnostic subtypes*. Myelofibrosis (9 patientes).6 patients showed EVI-I over-expression (66%), and 5 of them were JAK2 positive. Another patient was positive either before and after developing AML. Out of the 3 patients without EVI-1 expression, 2 were JAK2 positive and 1 JAK2 negative. Polyglobulia(27 patients). EVI-1 over-expression was found in 4 out of the 17 JAK2 positive patients (23.5%). There was no EVI-1 over-expression in the group of JAK2 negative polyglobulia. Thrombocytosis (25 patients). EVI-1 over-expression was found in 7 cases (28%), 3 of them belonged to the group of 14 JAK2 positive patients (21.4%) and another 4 to the group of 11 JAK2 negative patients (36.3%). Unclassified Ph negative CMPD (14). There were no JAK2 positive patients in this group; however 2 of them did show EVI-1 over-expression. One of them was in accelerated phase at the time the determination was carried out, and eventually developed AML. *Conclusions.* 1. We have described for the first time EVI-1 over-expression in a group of patients diagnosed of Ph negative CMPD. 2. The group of patients suffering from Myelofibrosis showed a higher incidence in EVI- 1 over-expression, which could argue in favor of a higher rate in malignant transformation in these patients. 3. EVI-1 over-expression seems not to be associated to the JAK2 mutational state, because EVI-1 over-expression has been documented both in positive and negative JAK2 patients, except for the polyglobulia subgroup, in which JAK2 negativity rules out polycythemia vera. 4. EVI-1 over-expression could help tell apart CMPD from other reactive states, when JAK2 happens not to be mutated.

0739

EXTREME VARIABILITY OF FIP1L1-PDGFRALPHA TRANSCRIPTS IN CEL: ANALYSIS OF 30 PATIENTS TREATED WITH IMATINIB AND CORRELATION WITH CLINICAL AND MOLECULAR RESPONSE

M. Rondoni,[1] E. Ottaviani,[2] F. Messa,[3] S. Paolini,[2] C. Papayannidis,[2] I. Iacobucci,[2] S. Merante,[4] F. Buccisano,[5] P.P. Piccaluga,[2] D. Cilloni,[3] F. Pane,[6] A. Zaccaria,[1] G. Saglio,[3] M. Baccarani,[2] G. Martinelli[2]

[1]Hematology Unit Ravenna Hospital, RAVENNA; [2]Institute of hematology and Oncology Seràgnoli, BOLOGNA; [3]Division of Hematology, San Luigi Gonzaga Hospital, TORINO; [4]Hematolgy Department, Policlinico S. Matteo, PAVIA; [5]5Department of Hematology, Policlinico Tor Vergata, ROMA; [6]Department of Biochemistry and Medical Biothecnology, University of Naples Feder, NAPOLI, Italy

Background. an interstitial deletion in the long arm of chromosome 4 leads to the formation of a the fusion gene FIP1L1-PDGFRalpha coding for a constitutively activated form of PDGFRalpha. The fusion gene has become the molecular marker of clonal hypereosinophilic syndrome (CEL) and it predicts a dramatic response to imatinib mesylate. Different FIP1L1-PDGFRalpha transcript has been described, and it has been recognized in other hematologic disease than CEL. Extreme variability of the breakpoints and different transcript could be the base of different disease phenotype. *Aims.* to investigate the biological variability of FIP1L1-PDGFRalpha transcript in an homogeneous CEL population to evidence possible clinical differences. In particular we focus our analysis on correlation with kinetic of molecular response to imatinib mesylate, with presence or not of organ involvement at diagnosis, with sex and with history of disease. *Methods.* total RNA was obtained from bone marrow or peripheral blood cells taken at diagnosis from 30 consecutive CEL patients enrolled in the Italian multicenter prospective study of imatinib treatment in HES. The RNA was subsequently reverse transcribed in cDNA and then amplified by reverse transcription-PCR. The samples were purified and sequenced using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems). Imatinib was administered at daily dose of 400 mg. Patients were 29 males and one female. Median age was 48 years (range 25-72). All 30 patients achieved a complete hematologic response (CHR) in less than one month, and PCR negativity in a median time of 3 months (range 1-9). Patients in continuous imatinib therapy have remained PCR negative as of today. *Results.* 28 samples of the totality were evaluable for molecular analysis. All delections of 4q12 generates in-frame chimeric fusion gene. FIP1L1 breackpoints scattered between exon 9 to 18, with several splicing variants. All breackpoints in PDGFRA are located within exon 12. Fusion gene sequencing demonstrate an extreme variability, with lack of whole exons of FIP1L1, deletion of exons, with the presence of introns in a minority of cases. Region of FIP1L1 varies in length from 109 to more than 500 nucleotides. The more conserved regions are exon 10 and exon 11 of FIP1L1, that are repeated together in 13 out of 28 analyzed transcripts. Transcript of the only female patient is the same of one of the males. No evidence of correlation was noted with kinetic of molecular response or with the presence at diagnosis of peculiar organ involvement. More complexity of transcript is noted in patients with longer history of disease prior to imatinib therapy. *Conclusions.* with this large series of patients we can confirm more complexity and variability in FIP1L1-PDGFRalpha transcripts than data reported in other series, but clinical correlation between this heterogeneity and phenotype of disease

and response to the imatinib therapy is not clear with present data and require largest studies.

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0740

GATA1 IS UP-REGULATED IN PATIENT AFFECTED BY ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA BUT NOT IN PRIMARY MYELOFIBROSIS OR CHRONIC MYELOGENOUS LEUKEMIA

C.R. Rinaldi,[1] C.R. Rinaldi,[1] R. Ciancia,[1] V. Martinelli,[1] L. Del Vecchio,[1] P. Rinaldi,[1] T. Cicchetti,[1] G. Giagnuolo,[1] L. Brunetti,[1] G. Nucifora,[2] F. Pane,[1] B. Rotoli[1]

[1]Federico II University, NAPOLI, Italy; [2]University of Illinois at Chicago, CHICAGO, USA

Background. In vitro studies, murine models, and sporadic reports on human samples suggest that a connection exists between the transcription factor GATA1 and myeloproliferative disorders (MPDs). *Aims.* We measured the GATA1 expression to determine the differences among the chronic myeloproliferative disorders in order to prove a role of GATA1 in the pathogenesis of the diseases and propose it as a new molecular marker useful in diagnosis and molecular follow-up. *Patients and Methods.* We collected 68 bone marrow aspirates from newly diagnosed patients affected by myeloproliferative disorders (MPDs) according to the WHO criteria: 40 essential thrombocitemia (ET) patients, 8 polycythemia vera (PV), 8 chronic phase of chronic myeloid leukaemia (CML) and 8 primary myelofibrosis (PMF). Six aspirates from healthy donors, and 6 from patients with reactive myeloproliferation (3 idiopathic thrombocytopenic purpura and 3 secondary erythrocytosis) were used as controls. We performed Syber-Green Real Time PCR for GATA1 detection. The relative GATA1 quantification was calculated according to the ΔCt method with GAPDH as internal control. *Results.* We found GATA1 overexpressed in ET (median 89.48; range 7.03-282,636.84) and PV patients (median 167.73; range 68.12-270.6), but not in PMF (median 3.8; range 0.13/12.12), or CML (median 2.1; range 0.39/6.82), comparing with 6 aspirates from healthy donors (median 1.109; range 0.06-20.73), (p<0,003) and with reactive thrombocytosis (median 0.8; range 0.53-0.93) or erythrocytosis (median 2.42; range 0.8-2.7) (p<0,001). In the ET group, no significant differences were found in GATA1 expression in patients harbouring a JAK V617F mutation (median 77.64; range 1.46/282636) and in those with wild type JAK2 alleles (median 213; range 9.69/71047) (p>0,3). *Conclusions.* GATA1 expression could be identified as a new approach for better understanding the molecular mechanisms of MPDs. It might become an additional marker of the diseases, useful to detect the residual clone after and during treatment of ET and PV, especially when there are no other molecular markers for following the minimal residual disease, such as in ET patients without the JAK2 mutation. Finally, we could imagine GATA1 as a new possible target for specific treatment.

0741

EFFECTS OF HSP90 INHIBITION BY 17-ALLYLAMINO-17-DEMETHOXYGELDANAMYCIN (17-AAG) ON CELL SURVIVAL AND SIGNALING PATHWAYS IN CHRONIC MYELOID LEUKEMIA (CML)

D.G. Guyotat,[1] P. Flandrin,[2] E. Tavernier,[1] N. Nadal,[2] C. Manissolle,[2] L. Campos[2]

[1]Institut de Cancérologie de la Loire, SAINT-PRIEST-EN-JAREZ; [2]CHU de Saint-Etienne, SAINT-ETIENNE, France

Background. Imatinib mesylate (IM), a Bcr-Abl kinase inhibitor, has improved the treatment of patients with chronic myeloid leukemia (CML). However primitive leukemic cells are resistant to imatinib. Heat shock protein 90 (HSP90) is a highly conserved, constitutively expressed molecular chaperone that facilitates folding and affects the stability of client proteins like bcr-abl. 17-AAG inhibits the function of Hsp90 and destabilizes the complex Hsp90/ bcr-abl resulting in the degradation of bcr-abl by the proteasome. The aims of our study were to evaluate the effects of 17-AAG alone or in combination with IM on survival of CML cells and on activation of AKT and ERK signaling pathways. *Methods.* Bone marrow samples were obtained from 10 patients with CML at diagnosis (CP) and from three patients in imatinib-resistant chronic phase (IR) (one with T315I mutation). Three normal bone marrows (NBM) were used as control. Mononuclear cells (MNC) were isolated by Ficoll density gradient. In addition, primitive $CD34^+CD133^+$ and $CD34^+CD38^-$ were selected by magnetic beads in the 10 CP, in 1 IR patients, and in the 3 controls. MNC and selected cells were cultured in liquid medium for 1 and 2 days with or without 17-AAG at different concentrations. The 3 IR samples were cultured with imatinib alone, 17-AAG alone and both drugs in combination. Apoptosis was assessed by activated Caspase 3 expression and annexin V binding. All cell types were also plated in semi-solid media (with or without growth factors) containing or not 17-AAG. *Results.* in liquid culture, 17-AAG induced apoptosis in a dose dependant fashion in MNC: at 5μM we found 100% apoptosis in 3/10 CP, 3/3 IR, after 1 day and in all 13 samples after two days of culture. At 10μM, cells from the 13 patients were apoptotic after 1-day culture. Imatinib at 1 or 2 μM had no effect in the 3 IR samples, while 17-AAG at 5μM induced 100% of apoptosis. When both drugs were added in combination at lower doses (Imatinib 0.5μM and 17AAG 2μM) all cells were apoptotic after 1 day. 17-AAG induced 100% apoptosis in 6/10 $CD34^+CD133^+$ and $CD34^+CD38^-$ cells at 2μM and in all samples at 5 μM. NBM cells were not affected. In semi-solid media the growth of progenitors from MNC or from $CD34^+CD133^+$ and $CD34^+CD38^-$ CP and IR samples was completely inhibited at 5 μM. $CD34^+CD133^+$ and $CD34^+CD38^-$ from the 3 NBM were not inhibited. We also studied the ability of IM and 17-AAG to induce down-regulation of signaling proteins in the three IR samples. IM did not suppress AKT and ERK activity. By contrast, 17-AAG alone induced a down-regulation of pAkt and pERK as assessed by flow cytometry. *Conclusion*. Inhibition of HSP90 by 17-AAG provokes apoptosis in CML cells even in IM-resistant cases, and down regulates AKT and ERK activation. 17-AAG may also restore sensitivity to IM. HSP90 inhibitors could be a novel therapeutic strategy to inhibit primitive malignant progenitors in CML either as single agents or in combination with other targeted therapies.

Myeloproliferative disorders - Clinical

0742

IMATINIB MESYLATE INDUCES COMPLETE AND DURABLE RESPONSES IN ALL PATIENTS WITH THE FIP1L1-PDGFRALPHA POSITIVE HYPEREOSINOPHILIC SYNDROME. CLINICAL AND MOLECULAR FOLLOW-UP OF THE ITALIAN MULTICENTER PROSPECTIVE STUDY

M. Rondoni,[1] D. Cilloni,[2] S. Paolini,[3] E. Ottaviani,[3] P.P. Piccaluga,[3] F. Messa,[2] C. Papayannidis,[3] S. Merante,[4] F. Buccisano,[5] M. Tiribelli,[6] A. De Vivo,[3] F. De Rosa,[3] E. Messa,[2] E. Gottardi,[2] E. Giugliano,[2] I. Iacobucci,[3] S. Soverini,[3] G. Rosti,[3] A. Zaccaria,[1] F. Pane,[7] G. Saglio,[2] G. Martinelli,[3] M. Baccarani[3]

[1]Hematology Unit Ravenna Hospital, RAVENNA; [2]Division of Hematology, San Luigi Gonzaga Hospital, TORINO; [3]Institute of hematology and Oncology Seràgnoli, BOLOGNA; [4]Hematolgy Department, Policlinico S. Matteo, PAVIA; [5]Department of Hematology, Policlinico Tor Vergata, ROMA; [6]Division of hematology, General Hospital, UDINE; [7]Department of Biochemistry and Medical Biothecnology, University of Naples Feder, NAPOLI, Italy

Background. The hypereosinophilic syndrome (HES) may be associated with the fusion of the PDGFRalpha gene with the FIP1L1 gene in chromosome 4 coding for a constitutively activated tyrosine kinase. This condition delineate a clonal chronic myeloproliferative disorder and it is usually referred to as chronic eosinophilic leukemia (CEL). These cases of FIP1L1-PDGFRalpha (F/P) rearranged CEL have been reported to be very sensitive to the TK inhibitor Imatinib mesylate (IM). *Aims*. The aim of this analysis is to evaluate the duration of reponse to IM in FIP1L1-PDGFRalpha positive (F/P^+) HES patients and to evaluate the prevalence of HES-related organ damage, its relation to F/P status, and the response to imatinib therapy. *Methods*. A prospective multicenter study of the HES was established in 2001. Hypereosinophilic syndrome was defined according to Chusid criteria. The FIP1L1-PDGFRalpha transcript was identified and monitored every three months on bone marrow cells using a nested retrospective reverse transcriptase polymerase chain reaction (RT-PCR). Patients were systematically screened for organ damage with instrumental evaluation (chest radiography, echocardiogram, abdomen ultrasonography) and for the presence of symptoms. 72 patients were treated with IM 100 to 400 mg daily. The observation period ranges between 12 and 72 months (median 28 months). *Results*. 33 patients (46%) were found to carry the F/P rearrangement, while 39 (54%) were negative. Notably, gender was male in all but one rearranged patients *vs* 26 of 39 in negative cases. Other minor differences concerned the age, with a median of 50 years in positive patients *vs* 60 in negative ones; the total eosinophil count (median 4.9×10^9/L in positive patients *vs* 3.4 in negative patients). Organ involvement was recorded in 42% of F/P^+ and in 51% of F/P^-. Skin involvement was recorded only in 6 negative patients, and splenomegaly in 7 rearranged patients and in only one negative. To date, soft tissue was peculiar site of F/P^+ patients. After imatinib therapy, F/P^+ patients became negative for organ localization and free of symptoms. All 33 achieved a complete hematologic remission (CHR) and became $RT\text{-}PCR^-$ for the fusion transcripts. All 29 patients who continue imatinib therapy remain in CHR and RT-PCR negative, with a dose of 100 to 400 mg daily. In four patients IM treatment was discontinued for few months, and the fusion transcript became rapidly detectable. CHR was maintained. The transcript was again undetectable upon treatment resumption. In the group of 39 patients who not carry the rearrangement, only 5/39 (13%) achieved a CHR, that was lost in all cases after 1 to 15 months. Interpretation and conclusion. All patients fitting the criteria for the HES should be screened for the FIP1L1-PDGFRalpha rearrangement and for rearranged patients chronic IM treatment ensures complete and durable responses. Organ involvement do not seems to be a constant characteristic of HES, irrespective to F/P status, but there are differences between F/P^+ and F/P^- patients. Organ damage in F/P^+ subset is reversible before fibrosis development. In the whole population observed, no deaths were recorded in more than five years.

0743

BONE MARROW FEATURES OF ERYTHROCYTOSIS WITH JAK2 EXON 12 MUTATIONS

N. Erber

Cambridge University Hospitals NHS Foundation Trust, CAMBRIDGE, UK

Background. The JAK2 V617F mutation is found in 95% of patients with polycythemia vera (PV) but rarely in patients with idiopathic erythrocytosis (IE). Recently novel JAK2 gain-of-function mutations in exon 12 of the gene have been described in the majority of cases of V617F-negative PV, as well as some cases of IE. These mutations alter residues about 80 amino acids upstream of V617, confer erythropoietin-independent growth on erythroid progenitors and manifest with isolated erythrocytosis. *Aims.* The revised 2008 WHO criteria for Myeloproliferative Neoplasms will require bone marrow histology for JAK2 V617F-negative erythrocytosis with subnormal erythropoietin. The aim of this study was to assess the bone marrow morphology of PV and IE patients with erythrocytosis and the JAK2 exon 12 mutations, F537-K539delinsL, H538QK539L, K539L or N542-E543del. *Methods.* JAK2 exon 12 mutations were detected by sequencing patient peripheral blood granulocyte DNA. Diagnostic bone marrow trephines (BMT) of 9 patients, and a 10-year follow-up BMT of one patient, all with JAK2 exon 12 mutations, were reviewed. The BMT biopsies, either formalin-fixed and decalcified (n=7) or resin-embedded (n=3), were assessed for morphology, using haematoxylin and eosin stained sections, and reticulin. Immunophenotyping was performed on the formalin-fixed trephines to assess erythroid (CD235), granulocytic (myeloperoxidase) and megakaryocytic (CD61) components. An automated immunostainer (BondmaX™, Leica Biosystems, UK) was utilised with the Bond™ Polymer Refine indirect polymer based peroxidase system, diaminobenzidine substrate and haematoxylin counterstain. *Results.* Patients presented with elevated hemoglobin levels (mean±SD, 197±14g/L), and normal leucocyte (mean±SD, $7.7\pm3.1\times10^6$/mL) and platelet (mean±SD, $304\pm69\times10^6$/mL) counts. All patients had suppressed serum erythropoietin levels at diagnosis (3.7±2.5 IU/L), and, had erythropoietin-independent erythroid progenitors. Three patients met the PVSG criteria for PV and 6 were classified as IE. The diagnostic BMT of all 9 patients were hypercellular with marked erythroid hyperplasia with normal granulopoiesis. The myeloid:erythroid ratios were reversed, ranging from 1:1 to 1:6. In 5 cases megakaryocytes were present in normal number and location and had normal morphology. Mild megakaryocytic hyperplasia was seen in 4 cases, with some atypia in 3 of these. Megakaryocyte clusters were not a feature. A small number of loose megakaryocyte clusters were seen in only one case. Reticulin was normal (grade 0-1) in 6 patients, and mildly increased (grade 2) in 3. The 10-year follow-up sample showed evidence of disease progression. Marrow cellularity had increased from 60-80%, there was greater erythroid hyperplasia and granulopoiesis was reduced. There was megakaryocytic hyperplasia with marked morphological atypia (large with abnormal nuclear lobation) and reticulin had increased from grade 1 to 3. *Conclusions.* The bone marrow of erythrocytosis with JAK2 exon 12 mutations at diagnosis is characterised by marked erythroid hyperplasia, normal granulopoiesis and normal or only mild megakaryocyte abnormalities. This differs from classical JAK2 V617F-positive PV which characteristically has a pan-myelosis and significant megakaryocytic morphological abnormalities, including clustering. The 10-year post-diagnosis case shows that, over time, the bone marrow can progress to a more myelofibrotic appearance with megakaryocytic abnormalities and increased reticulin.

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IMPACT OF THE JAK2 (V617F) MUTATION AND THE MUTANT ALLELE BURDEN ON DISEASE PROGRESSION IN CHRONIC MYELOPROLIFERATIVE DISORDERS

F. Passamonti,[1] E. Rumi,[1] D. Pietra,[1] E. Boveri,[2] C. Pascutto,[1] M. Lazzarino,[1] M. Cazzola[1]

[1]Division of Hematology, Fondazione Policlinico San Matteo, University of Pavia, PAVIA; [2]Department of Surgical Pathology, Fondazione Policlinico San Matteo, PAVIA, Italy

Background. An identical somatic mutation (V617F) of JAK2 is found in most patients with polycythemia vera (PV) and in 50 to 60% of those with essential thrombocythemia (ET) and primary myelofibrosis (PMF). Previous studies suggested that the mutant allele burden might influence both clinical phenotype and clinical course of these conditions. *Aims.* We conducted a prospective study to evaluate whether granulocyte the JAK2 (V617F) mutant allele burden has an impact on disease progression in

chronic myeloproliferative disorders (CMD). *Methods*. We enrolled 830 patients with CMD in a prospective, observational cohort study. At baseline, the JAK2 (V617F) allele burden was assessed through a quantitative evaluation of granulocyte mutant alleles by real-time polymerase chain reaction. All patients were diagnosed according to the revised WHO criteria, while the IWG-MRT criteria were employed for definition of disease progression. Patients with PV and ET received treatments according to their risk categorization, while those with PMF were treated on the basis of their clinical manifestation. *Results*. Overall, 276 patients had PV (124 at diagnosis and 152 at follow-up), 338 had ET (188 at diagnosis and 150 during follow-up), 131 had PMF (80 at diagnosis and 51 at follow-up), 45 had post-PV MF and 40 had post-ET MF. Clinical and hematologic data prior to enrolment were collected. All events occurring after JAK2 (V617F) assessment were regularly recorded along a total follow-up of 21.036 person/years (mean follow-up: 26 months). The median allele burden was 32% (range, 1.3-100%) in PV, 6.5% (range, 1.1-64%) in ET, 20% (range, 1.8-99%) in PMF, 85% (range, 9.5-100%) in post-PV MF, and 41% (range, 2.2-87%) in post-ET MF. Disease progression includes evolution into myelofibrosis and leukemia in PV and ET, and into leukemia in PMF. During follow-up, progression occurred in 14 patients with PV, in 8 with ET and in 6 with PMF. Within PV patients, Kaplan-Meier analysis showed a significant worse progression-free survival (PFS) (p=0.0001) in those with more than 50% mutant alleles than in those with up to 50%. This result retained statistical significance in multivariate Cox regression analysis accounting for both left censoring of the observation and baseline age (Hazard Ratio 5.4; p=0.03). Few patients with ET and PMF had more than 50% mutant alleles (2 with ET and 14 with PMF). Therefore, in these conditions we compared JAK2 (V617F)-positive and JAK2 (V617F)-negative patients. Concerning ET, JAK2 (V617F)-positive patients had a trend toward worse PFS (p=0.09). When a multivariate Cox regression accounting for left censoring of the observation and baseline age was applied, JAK2 (V617F)-positive patients had significant worse PFS (Hazard Ratio: 3.45; p=0.03). With respect to PMF, JAK2 (V617F)-positive patients had significant worse PFS (p=0.035). As no progression occurred in JAK2 (V617F)-negative patients, multivariate Cox regression analysis was not feasible in this condition. *Conclusions*. Overall, carrying the JAK2 (V617F) mutation involves a higher risk of disease progression in CMD. In particular, the mutant allele burden represents a risk factor for hematologic transformation in patients with polycythemia vera.

0745

INFLUENCE OF THE JAK2 V617F HOMOZYGOUS OR HETEROZYGOUS MUTATION AND OF INHERITED THROMBOPHILIA ON THE THROMBOTIC RISK AMONG PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

V. De Stefano, T. Za, A. Fiorini, E. Rossi, A. Ciminello, P. Chiusolo, S. Sica, G. Leone

Institute of Hematology, Catholic University, ROMA, Italy

Background. It is uncertain whether the JAK2 V617F mutation is associated with an increased risk of thrombosis in patients with Philadelphia-negative chronic myeloproliferative diseases (CMD). It is unknown whether inherited thrombophilia is an additive risk factor in the patients with the JAK2 mutation. *Aims.* The present study is aimed to investigate the thrombotic risk associated with the JAK2 mutation and thrombophilia in ET patients. Patients and *Methods.* We studied 132 patients with ET (M/F 46/86, median age 53 years, range 20-92). Forty-five patients had had a major thrombotic event (34%). Arterial vessels were involved in 27 cases (cerebrovascular disease in 16, acute coronary syndrome in 7, peripheral arterial thrombosis in 4); thrombosis involved venous vessels in 18 cases (splanchnic veins in 9, deep veins of the legs in 7, cerebral veins in 1, retinal vein in 1). All patients were investigated for the presence of the JAK2 V617F mutation by PCR and sequencing analysis, defining homozygosity (Homo) or heterozygosity (Hetero) as a mutant allele burden higher or lower than 50%. Laboratory investigation for inherited thrombophilia (deficiency of antithrombin, proteins C and S, factor V Leiden [FVL], prothrombin G20210A [PT-A]) was carried out in all patients. *Results.* The JAK2 mutation was detected in 83 patients (62.8%), with Homo in 8 cases (6%). Seven patients carried thrombophilia (4 FVL and 3 PT-A). The relative risk (RR) for thrombosis was 2.1 (95%CI 1.1-3.8) in JAK2 mutated patients in comparison with wild-type (WT) patients; in Homo and Hetero the RR was 3.7 (95%CI 1.8-7.2) and 1.9 (95%CI 1.0-3.5) in comparison with WT patients. The RR of Homo in comparison with Hetero was 1.9 (95%CI 1.2-3.2). The patients with mutation had a RR in comparison with WT patients without thrombophilia of 4.4 (95%CI 2.2-8.8) in the presence of thrombophilia and of 2.1 (95%CI 1.1-4.0) in the absence of thrombophilia. Among the patients with mutation, those with thrombophilia had a RR of 2.1 (95%CI 1.3-3.4) in comparison with those without thrombophilia. *Conclusions.* In ET patients the thrombotic risk is higher in the presence of the JAK2 mutation. The magnitude of the increase in risk is dependent on the mutant allele burden, being higher in homozygotes. The concomitant presence of inherited thrombophilia produces a further increase in the thrombotic risk, yet further studies on larger patient cohorts are needed to confirm this finding.

0746

LONG TERM EFFICACY AND TOLERABILITY OF ANAGRELIDE IN MYELOPROLIFERATIVE DISORDERS

M.E. Ejerblad,[1] B. Andreasson,[2] M. Björkholm,[3] E. Löfvenberg,[4] B. Markevärn,[5] L. Nilsson,[6] J. Palmblad,[3] J. Samuelsson,[7] G. Birgegård[1]

[1]Uppsala University Hospital, UPPSALA; [2]Sahlgrenska Hospital, GOTHENBURG; [3]Karolinska Institute, STOCKHOLM; [4]Karolinska Institute, STOCKHOLM; [5]Umeå University, UMEÅ; [6]University of Lund, LUND; [7]Södersjukhuset, STOCKHOLM, Sweden

Background. Anagrelide, a non-cytostatic drug, selectively reduces the production of platelets by inhibiting megakaryopoiesis. It is frequently used to reduce platelet counts in myeloproliferative disorders (MPD) However, few studies have investigated the long-term efficacy and clinical toxicity of anagrelide. *Aims*. To evaluate the long-term efficacy and tolerability of anagrelide in patients with thrombocytosis of MPD. *Methods*. The Swedish Myeloproliferative Disorder Study Group conducted a prospective multicenter study of anagrelide treatment in 60 patients with MPD; 42 with essential thrombocythemia (ET), 17 with polycythemia vera (PV) and 1 having primary myelofibrosis (PMF). The median age was 53 years (27-75). One patient was lost to follow-up after two years, all others were followed for a minimum of 7 years. Results after 2 years of treatment have been previously reported1. *Results*. After 1 year of treatment 50% of the patients (30/60) had stopped anagrelide treatment, mostly due to side effects or lack of efficacy at a tolerable dose.[1] During the following 6 years, another 8 out of the 30 remaining patients stopped treatment. The reasons were transformation to myelofibrosis (n=4) and acute leukaemia (n=1) and side-effects (n=3). However, 4 of the patients who during the first year had withdrawn from anagrelide restarted the treatment. Thus, at the end of the 7-year follow-up 26 patients were treated with anagrelide (22 ET, 4 PV). Four of these patients also received other cyto-reductive drugs. Ten of the other 34 enrolled patients had died during the study period; causes of death will be presented in detail. Most commonly reported side-effects were tachycardia, fatigue, oedema, headache and gastrointestinal disturbances. Considerably fewer and less severe side-effects were reported during year 3-7 than during the first two years. At follow-up study end patients and doctors rated their satisfaction with the anagrelide treatment on a 10-grade scale as 9.1 and 8.2, respectively. Complete remission (defined as a platelet count $<400\times10^9$/L in symptomatic patients and $<600\times10^9$/L in asymptomatic patients) was maintained in 81 % of patients still treated with anagrelide. During the study period 19 patients experienced 24 tromboembolic or bleeding events. Among the 26 patients who had continued their anagrelide treatment, 6 thromboembolic and 3 bleeding complications occurred. One patient had a worsening of cardiac insufficiency during the year two and was taken off anagrelide. Two patients died from heart failure; both had stopped anagrelide treatment more than 3 years before death. Fibrosis grade 2 or higher occurred in 9 patients, 8 of whom were on anagrelide treatment. Transformation to MDS or AML was reported in 4 cases and from ET to PV in 1 case. *Conclusions*. After a high dropout rate during the first 6 months of therapy, mostly due to side effects, most patients continued treatment during the 7 years, and 4 restarted therapy. Long term efficacy was good, tolerance and safety was satisfactory with regard to cardiac toxicity. Fibrosis development and thromboembolic events will be further analyzed i.e. with regard to initial bone marrow morphology.

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Reference

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0747

HIGHER JAK2 MUTATION LEVELS CORRELATE WITH MYELOFIBROSIS AND BLASTIC TRANSFORMATION OF PV

M.K.M. Koren-Michowitz,[1] J. Landman,[1] N. Rahimi-Levene,[2] Y. Cohen,[1] G. Rechavi,[1] V. Amariglio,[1] A. Nagler[1]

[1]Chaim Sheba Medical Center, RAMAT GAN, Israel; [2]Assaf Harofeh Medical Center, ZERIFFIN, Israel

Background. The JAK2 V617F mutation is found in 80-97% of polycythemia vera (PV) pts. The aim of the current study was to correlate the JAK2 mutation level with clinical symptoms and complications including vascular events and late hematological complications i.e myelofibrosis or blastic transformation. Patients and *Methods.* 101 JAK2 V617F positive PV pts diagnosed according to the revised PVSG criteria or the 2001 WHO criteria were included. JAK2 mutation was analyzed in DNA extracted from PB using a quantitative MALDI-TOF based assay (Leuk Res 2007) and the JAK2 mutation level calculated as the AUC of the mutated allele x100/ (AUC of the mutated allele+ AUC of the WT allele). *Results.* JAK2 analysis was performed 96 (median, range 0-423) months from diagnosis. The mean JAK2 mutation level was 54% (range, 4-95%). There was no correlation between the JAK2 mutation level and: 1. age at the time of mutation analysis, 2.clinical symptoms, 3. the presence of splenomegaly and 4. the need for cytoreductive treatment, while there was a positive correlation between hydroxyurea dose and the JAK2 level (r=+0.23, p=0.043). JAK2 levels were not associated with thromboembolic complications. In contrast, we found a highly significant correlation between the JAK2 mutation level and late hematological complications. The median JAK2 mutation level in pts with and without hematological complications including MF and blastic tranformation was 84.4% (MF-86%;BT-80.6%) and 49.6% ,respectively (p=0.0001). The correlation remained significant for each complication alone; MF *vs* no progression p=0.0001 and blastic transformation *vs* no progression p=0.045, respectively. Since the JAK2 levels were determined in some pts after prolonged disease courses we tested the hypothesis whether the late hematological complications were more affected by the duration of disease than by the JKA2 levels. Although there was a trend towards higher JAK2 mutation levels in pts with longer disease durations (r=+0.18, p=0.064), using an ANOVA model the JAK2 mutation level remained significantly associated with the development of late hematological complications independent of disease duration. *Summary*. In conclusion, we found that late hematological complications including transformation to myelofibrosis and blastic transformation in PV pts are associated with higher JAK2 mutation levels while thrombotic events are not associated with higher JAK2 levels. These results may be of clinical and therapeutic significance in particular in the future era of JAK2 inhibitors.

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0748

EFFICACY AND SAFETY OF PRIMARY ANTITHROMBOTIC PROPHYLAXIS WITH TICLOPIDINE AND WITH ASPIRIN IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA RESULTS FROM A COHORT STUDY.

M. Ruggeri, A. Tosetto, B. Bolgan, F. Rodeghiero

San Bortolo Hospital, VICENZA, Italy

Background. Essential Thrombocythemia (ET) and Polycythemia Vera (PV) are two chronic myeloproliferative diseases with prolonged survival, but with a high rate of vascular complications, mainly arterial thrombosis (AT). For this reason, clinical guidelines recommend the use of aspirin for primary and secondary prophylaxis. There are no data on the efficacy and safety of tienopyridine antiplatelet drugs (ticlopidine, clopidogrel), which could be useful alternatives in patients with contraindication or when aspirin is unable to prevent thrombotic event. *Aims*. To estimate the frequency of thrombotic and hemorrhagic complications, in ET and PV patients treated with ticlopidine in comparison with patients taking aspirin, in single institution, prospective cohort study. *Patients and methods*. Data from 246 PV (143 males, 58%), median age at diagnosis 63 years (range 20-89) and 339 ET (114 males, 33.6%) patients, median age 62 (range 20-95) consecutively diagnosed from 1985 to 2005 were analyzed. Risk factors for arterial thrombosis (diabetes mellitus, arterial hypertension, hypercholesterolemia, smoking, cardiovascular disease, previous AT) were present in around 30% of patients. At diagnosis, median values of hemoglobin, leukocyte and platelet level in ET and PV were 137 and 180 g/L, 9.3 and 11×10^9/L, 888 and 582×10^9/L, respectively. After diagnosis, aspirin (100-300 mg daily) was given to 270 patients (155 ET, 57%), in 70 of them (25%) for secondary prophylaxis; ticlopidine (250 mg twice day) was administered to 84 patients with a previous history of gastric ulcer, gastritis or allergy to ASA (48 ET, 57%), in 19 of them (22%) for secondary prophylaxis. In 216 (137 ET, 63%) patients no antiplatelet drug was given. The two treated group had similar cardiovascular risk profile, higher than in those untreated. Cytoreductive treatment was given to 87 (32%) patients in ASA, 14 (17%) in ticlopidine and 61 (28%) in those not on antiplatelet treatment (p=0.02). An higher percentage of patients received hydroxyurea in ASA group compared with ticlopodine (19.6% *vs* 8.6%). Warfarin was administered to 10 patients for atrial fibrillation or venous thrombosis (not analyzed). 2 cases were lost from follow-up. All PV patients were phlebotomized to reduce hematocrit level. *Results*. After a median follow-up of 7.8 years (very similar in the 3 groups of patients), 29 (14.5%) thrombotic events (5 fatal) among 200 ASA patients and 18 (27.7%, 1 fatal) among 65 ticlopidine patients treated for primary prophylaxis were recorded (p=0.016). In 216 not-treated patients, 40 (18.5%) thromboses were recorded. Major hemorrhages (need of transfusions, surgical intervention or hospital admission) were 17 (8.5%) in ASA, 8 (12.3%) in ticlopidine (p= 0.299) and 25 (11.6%)) in not-treated patients (p= 0.392 between antiplatelet treated and not treated patients). Thrombotic rates for patients in primary prophylaxis were 0.4%, 0.8% and 2.5% in patients on ASA, ticlopidine and not-treated, respectively. *Conclusions*. In a cohort of ET and PV patients, ASA therapy appears more effective than ticlopidine in the primary prevention of thombosis, without a significative increase of hemorrhagic risk in comparison with ticlopidine or untreated patients. This increased efficacy should be further investigated by stratifying patients accordingly to cytoreductive treatment.

0749

EFFECTIVENESS AND SAFETY OF COMBINED CYTOREDUCTIVE THERAPY IN 56 PATIENTS WITH ESSENTIAL THROMBOCYTHAEMIA: REPORT OF THE REGISTRO ITALIANO TROMBOCITEMIE (RIT)

P.R. Scalzulli,[1] A. Tieghi,[2] A.M. Liberati,[3] M. Crugnola,[4] G. Specchia,[5] E. Cacciola,[6] R. Ciancia,[7] A. Candoni,[8] E. Balleari,[9] R. Latagliata,[10] M. Gubbiotti,[11] M.R. Valvano,[12] L. Melillo,[13] N. Cascavilla,[14] L. Gugliotta[15]

[1]IRCCS Casa Sollievo della Sofferenza, SAN GIOVANNI ROTONDO; [2]Alessia, REGGIO EMILIA; [3]Anna Marina, PERUGIA; [4]Monica, PARMA; [5]Giorgina, BARI; [6]Emma, CATANIA; [7]Rosanna, NAPOLI; [8]Anna, UDINE; [9]Enrico, GENOVA; [10]Roberto, ROMA; [11]Marta, PERUGIA; [12]Maria Rosa, SAN GIOVANNI ROTONDO; [13]Lorella, SAN GIOVANNI ROTONDO; [14]Nicola, SAN GIOVANNI ROTONDO; [15]Luigi, REGGIO EMILIA, Italy

Background. In essential thrombocythaemia (ET) patients considered at high risk for thrombosis, particularly if the JAK2V617F mutation and an increased WBC count are documented, a treatment with aspirin is usually performed and a cytoreductive therapy is recommended. Hydroxyurea (HU), Interferon alpha (IFN) and Anagrelide (ANA) are largely used, but their side effects/toxicity or inefficacy are not rarely cause of drug withdrawal. Since HU, IFN, and ANA have different activity and toxicity patterns, the combined use of two drugs at lower dose may result very useful to overcome the limits of a single drug at conventional or high dose. *Aims*. To evaluate the ET patients enrolled into the RIT who received a combined cytoreductive therapy, with particular interest for feasibility, efficacy and toxicity. *Patients.* Fifty-six ET patients, 18 males and 38 females, diagnosed in the years 1998-2008 according to the PVSG or WHO criteria (25 and 31 cases, respectively) , are object of this study. The patients at diagnosis had a median platelet count of 792 ×10^9/L (600-2009), with disease related symptoms in 18 cases (32%) and previous thrombotic or haemorrhagic events in 6 (12 %) and 1 (2%) cases, respectively. *Results*. The patients received as first cytoreductive drug HU (n 32, 57%) at a median dose of 1 g/day (0.5-3.0), or ANA (n 13, 23%) at a median dose of 1.5 mg /day (0.5-4.0), or IFN (n 11, 20%) at a median dose of 9 MU/week (3-9). The 32 patients receiving HU started a combined therapy with ANA as a consequence of haematological toxicity (n 23), fever (n 4), cutaneous ulcers (n 2), inefficacy (n1), other causes (n 2); the 13 patients receiving ANA started a combined therapy ANA + HU (n10) or ANA + IFN (n 3) as consequence of cardiovascular side effects (n 10), anemia (n 2), or GI side effects (n 1); the 11 patients receiving IFN started a combined therapy IFN + ANA as consequence of unaccepted side effects. All patients who started the combined therapy had reduced the dosage of the initial drug. The median follow-up of the patients on combined therapy was 11 months (1-75). A haematological response (platelet count ≤ 500×10^9/L, WBC ≥ 4×10^9/L,

Hb ≥12.5 g/dL) was registered in 21 of 42 (50%) patients on HU + ANA and in 11 of 14 (79 %) patients on IFN + ANA. Disease-related or drug-related symptoms (headache, dizziness, palpitation, abdominal pain) were registered in 7 of 56 (12 %) patients; no thrombotic or haemorrhagic complications occurred. *Conclusions.* In these ET patients the combined therapy HU + ANA, IFN + ANA, or ANA + IFN was well tolerated and able to control the symptoms and to obtain a satisfactory haematological response (particularly with ANA + IFN). These promising results could be due to the low dosage and to synergyc effect of the combined drugs, but controlled studies are needed to prospectively evaluate the role of the combined therapy in ET patients.

0750

THE V617F JAK 2 MUTATION IS NOT A FREQUENT EVENT IN PATIENTS WITH CEREBRAL VENOUS THROMBOSIS WITHOUT OVERT CHRONIC MYELOPROLIFERATIVE DISORDER (MPD)

S. Bellucci,[1] B. Cassinat,[2] N. Bonnin,[3] C. Marzac,[4] I. Crassard[5]

[1]APHP, Hopital Lariboisiere, PARIS; [2]APHP, Hopital Saint Louis, Unité de Biologie Cellulaire, PARIS 10; [3]3AP-HP, Hôpital Saint Louis, Unité de Biologie Cellulaire, PARIS 10; [4]4AP-HP, Hôpital Saint-Antoine, Service d'immunologie et hématologie biologiques,, PARIS 12; [5]AP-HP, Hôpital Lariboisière, Service de Neurologie, 2 rue Ambroise Paré, PARIS 10, France

Background. Thrombosis is a main cause of morbidity and mortality in patients with chromosome Philadelphia negative chronic MPD. Venous thromboses at unusual sites are not exceptional: thus thromboses in the splanchnic territory have been described in 5-10% of the patients with PV or ET; cerebral venous thromboses (CVT) have also been associated with PV and ET reaching up to 1% of patients with ET. Conversely, for many years, the diagnosis of MPD, based upon the results of endogenous erythroblastic colonies and/or on bone marrow data, has been documented at an early stage or even at a latent stage in a high proportion of patients with splanchnic thromboses. These data have been confirmed by several teams when considering the presence of the V617F JAK2 mutation which was initially reported to be associated with PV and ET in about 95% and 60% of cases respectively. *Aims.* In this study, we wanted to assess the proportion of patients with the V617F JAK2 mutation in a greater series of 87 patients with CVT and no overt MPD referred in the department of neurology from October 2003 to July 2007. *Patients*. Diagnosis of CVT was based on magnetic resonance imaging (MRI) combined with MR venography and/or helical cerebral CT venography. MPD was carefully ruled out on conventional updated criterias. Thus, hematocrit was checked to be always below 52% in men and 48% in women permitting to rule out PV and the platelet count below 450×10^9/L as recommended recently to rule out ET. Nevertheless, in 3 patients the platelet count was increased at 607, 485 and 644×10^9/L but the patients were not excluded since the thombocytosis was attributed to a high inflammatory state. *Methods.* The V617F JAK2 mutation was detected on genomic DNA extracted from peripheral granulocytes on an ABI7700 apparatus using a real time PCR-based mutation detection with allele specific Taqman probes and with a sensitivity of about 2%. *Results.* Among our 87 patients the mutation was only detected in 1 patient (1.1%). This patient (male, 55 years old) had a thrombocytosis at 607×10^9/L associated with a high inflammatory state. He had no other thrombophilic abnormality. In the two other patients with thrombocytosis related to inflammation we did not detect the V617F JAK2 mutation. In our population, the median age + sd was at 35.4 + 12.9 y and the M/F sex ratio at 22%. An hormonal transient risk factor in relation with oestroprogestative therapy, pregnancy or puerperium was noticed in 53 of our 71 female patients (75%). Last, inherited thrombophilic anormalities were found in 23% of our patients, in agreement with the rate of 22% reported in the International Study of cerebral venous thromboses (ISCVT). *Discussion and Conclusions.* Concerning the detection of the V617FJAK2 mutation with a sensitive technique our data showed a quite low incidence (1.1%) which do not justify for us the systematic research of this mutation in patients with CVT, but suggest that this research may be discussed in cases with thrombocytosis (even when initially considered as secondary).

0751

LONG-TERM FOLLOW-UP OF 386 CONSECUTIVE PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA: EFFICACY AND SAFETY OF CYTOREDUCTIVE THERAPY

F. Palandri, L. Catani, E. Ottaviani, N. Testoni, A. De Vivo, M. Fiacchini, N. Polverelli, F. Salmi, A. Lucchesi, M. Baccarani, N. Vianelli

Department of Hematology Seragnoli, BOLOGNA

Background. Despite the recent identification of the Jak2 V617F mutation in 40%-60% of the patients with Essential Thrombocythemia (ET), which will probably allow a better evaluation and management of these patients, cytotoxic agents like Hydroxyurea (HU), Busulfan (BU), Anagrelide (ANA) and Interferon-alpha (IFN-α) are to date the only therapeutic strategies available. *Aims.* We evaluated the efficacy and safety of cytoreductive therapy, including the leukemogenic risk in the long-term, and explored the potential correlation of baseline characteristics and outcome. *Methods.* We report updated results of the long-term outcome of 386 consecutive ET patients, followed at our Institution for a median follow-up of 9.5 years (range, 3-28.5). Median age was 64 years; 62% were female. Correlation of haemoglobin, leukocytes, platelets, age, thrombotic risk factors at diagnosis with clinical evolution and with presence of JAk2 mutation were examined. *Results.* Cytoreductive therapy was administered to 338 patients (88%), obtaining a response in 86% of cases, with only 7% of patients treated with HU or BU discontinuing the therapy because of toxicity. Forty-five patients (12%) experienced a thrombotic event. Incidence of thrombosis was not correlated with baseline parameters nor by the response to cytoreductive therapy (non-responding patients showed the same risk of complete responders, p=0.3). Evolution in acute leukemia (AL) and myelofibrosis (MF) occurred in 8 (2%) and 18 (5%) patients, respectively, and was significantly higher in patients receiving sequential cytotoxic therapy. Presence of JAK mutation displayed higher haemoglobin level (median values: 14.96 ± 0.2 *vs* 13.76 ± 0.3, p=0.001), but did not influence the risk of evolution to AL/MF or of developing thrombotic events. The overall survival rate was 74% and 35% at 12 and 25 years after diagnosis, respectively (Figure 1).

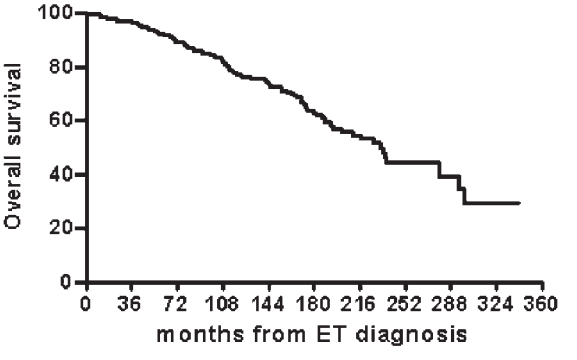

Figure 1. Overall survival

Conclusions. Cytoreductive therapy was effective in decreasing platelet number in high risk ET patients with negligible toxicity; however, thrombocytosis control did not significantly influence long-term outcome, both in terms of incidence of thrombotic events and overall survival.

0752

CORRELATION BETWEEN LEUKOCYTOSIS AND THROMBOSIS IN PHILADELPHIA NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES.

D. Caramazza, A. Malato, G. Saccullo, I. Abbene, S. Maisano, R. Palazzolo, L. Lo Coco, G. Quintini, S. Siragusa

Policlinico Universitario di Palermo, PALERMO, Italy

Introduction. Recent investigations suggest that leukocytosis may cause thrombosis in Philadelphia negative (Ph-neg) MyeloProliferative Diseases (MPD)1,2. We investigated the relationship between leukocytosis and the occurrence of arterial and venous thromboembolic events in Ph-neg MPD patients over a period of two years. *Material and methods*. Seventy-five patients [46 females and 29 males, median age at diagnosis: 54 years; 42 with Essential Thrombocytemia (ET), 25 with Polycythaemia Vera (PV) and 9 with Idiopathic Myelofibrosis (IM)] were evaluated during the period 2000-2005; all of them received at least 2-years of follow-up. Patients were treated with cytoreductive therapy, anagrelide or α-IFN

accordingly to age and type of MPD. Twenty-one patients had at least one episode of objectively confirmed thrombosis (arterial or venous) at the moment of diagnosis, or six months prior to diagnosis or during the follow-up. *Results*. A total of 28 vascular events were observed: 12 (42.8%) occurred in patients with PV (3 in the follow-up), 13 (46.4%) in patients with TE (3 in the follow up) and 2 (7.1%) in patients with IM(1 in the follow up). A leukocytes count above 8.5×10^9/L (median value) was statistically associated with an increased risk of thrombosis (p= 0.03). The multivariate analysis, evaluating the interaction between conventional risk factors for thrombosis and leukocytosis, showed that the increased leukocytes counts was the most important risk factor for thromboembolic events. The relation between standard risk factors and leukocytosis is reported in Table 1. *Conclusions*. The presence of a median leukocytes count >8.5×10^{9}L confers a high risk of thromboembolic events in Ph-negative MPD. These data may suggest the choice of cytoreductive therapy, but this approach must be confirmed in properly designed clinical trials, specially in young patients.

Table 1. Interaction between standard risk factors for thrombosis and leukocytes count.

Risk factors*	Hazard ratio (95% CI)
Low risk with low WBC, n° 1/11 (9%)	1 (Reference)
Low risk with high WBC**, n° 4/7, (57%)	6.3 (4.1-8.4)
High risk and low WBC, n° 6/28, (21%)	2.3 (1.2-3.4)
High risk with high WBC, n° 18/29, (62%)	6.8 (2.9-10.7)

* Patients > 60 years and/or previous thrombosis
** Leukocytes > 8.5 x 10^9/L (median)

0753

CARDIOVASCULAR EVALUATION IN 232 PATIENTS WITH ESSENTIAL THROMBOCYTHAEMIA (ET) TREATED WITH ANAGRELIDE: REPORT OF THE REGISTRO ITALIANO TROMBOCITEMIA (RIT)

L. Gugliotta,[1] A. Tieghi,[1] G. Tortorella,[2] P.R. Scalzulli,[3] R. Ciancia,[4] M. Lunghi,[4] E. Cacciola,[4] R. Cacciola,[4] A. Candoni,[4] M. Crugnola,[4] E. Usala,[4] G. Specchia,[4] V. Martinelli,[4] F. Palmieri,[4] C. Santoro[4]

[1]Arcispedale S. Maria Nuova, REGGIO EMILIA; [2]Cardiology Unit, Arcispedale S. Maria Nuova, REGGIO EMILIA; [3]Hematology Unit, San Giovanni Rotondo, FOGGIA; [4]Hematology Unit, NAPOLI, Italy

A series of 232 ET patients registered in the RIT and treated with anagrelide has been retrospectively analyzed to evaluate the effects of this molecule on the cardiovascular system. The patients, 89 males and 143 females, had at diagnosis a mean age of 47 years (range 13-88) and a mean platelet (PLT) count of 1043×10^9/L (range 412-3009). A previous treatment with cytoreductive drugs (mainly Hydroxyurea and IFN alpha) and with antiplatelet agents (mainly aspirin) was performed in 62.5% and 69% of cases, respectively. The anagrelide mean dose was 1.2 mg/day at start and 1.6 mg/day in the maintenance phase, when the median PLT count was 478×10^9/L. During the follow-up (median 61 months) 5 major thrombotic complications (0.42/100 pt-yrs) and 5 minor haemorrhages (0.42/100 pt-yrs) were registered. Moreover, 74 of 232 patients (32%) showed 98 non-lethal cardiovascular events: palpitation (n56), angina (n16), cardiac failure (n9), AMI (n4), arrhythmia (n4), other (n9). Sixty-five patients (28%) withdrew the treatment due to the following reasons: side effects (n27), inadequate response (n15), cardiovascular toxicity (n9), compliance loss (n7), pregnancy (n3), other (n4). In detail, the nine cardiovascular withdrawals were associated to palpitation (n5), angina (n2), AMI (n1) and arrhythmia (n1). Aspecific abnormalities of the ECG and of echocardiography (ECHO) were reported before the anagrelide treatment start in 11 of 194 (5.7%) and 19 of 193 (9.8%) cases, respectively. During the anagrelide treatment the ECG and the ECHO showed aspecific abnormalities in 5 and 5 cases, respectively, being the ejection fraction (EF) always ≥ 51%. The clinical and instrumental cardiovascular evaluation in ET patients receiving anagrelide is now routinely performed and is object of study by the Registro Italiano Trombocitemia (RIT).

0754

REAL TIME DETECTION OF JAK2 V617F IN THE MYELOPROLIFERATIVE DISORDERS

A. Goday-Fernandez, E. M. Boyd, A.J. Bench, W.N. Erber

Addenbrooke's Hospital, CAMBRIDGE, United Kingdom

Background. An acquired valine to phenylalanine mutation of Janus kinase 2 (JAK2) at codon 617 is present in the majority of patients with polycythaemia vera (PV) and approximately 50% of patients with essential thrombocythaemia (ET) and idiopathic myelofibrosis (IMF). Some patients, particularly ET patients, may carry a low burden of the JAK2 V617F allele in peripheral blood especially if treated with cytoreductive therapy. Current methods may therefore be insufficiently sensitive. Allele specific PCR is often used in a diagnostic setting to screen for the JAK2 V617F mutation. However, the sensitivity of conventional allele specific PCR of peripheral blood DNA may not identify all JAK2 V617F positive patients. We therefore explored the use of real time PCR for JAK2 V617F detection, specifically to assess its sensitivity. *Aims.* To compare allele specific PCR assay with a real time PCR detection system for the detection of JAK2 V617F mutation in 250 MPD patients (PV, ET and IMF). To evaluate the applicability of real time to assess JAK2 V617F in air dried Romanovsky stained bone marrow smears. *Methods.* Peripheral blood DNA samples from 250 well characterised patients with PV, ET or IMF were analysed for the presence of the JAK2 V617F mutation by allele specific PCR and real time PCR. Finally, DNA was extracted from archived air dried Romanovsky stained bone marrow smears of MPD patients and assessed by real time PCR. *Results.* For PV, allele specific PCR of peripheral blood DNA identified the JAK2 V617F mutation in 97% of patients. In 2% of PV patients the mutation was not detectable by allele specific PCR but was detected by real time PCR. Fifty three per cent of ET patients were JAK2 V617F positive by allele specific PCR and 60% by real time PCR. For IMF there was 100% concordance between the two methods. Real time PCR was also able to identify a JAK2 V617F mutation in DNA extracted from archived bone marrow aspirate smears despite being of poor quality; allele specific PCR was unsuccessful on this material. *Conclusions.* Real time PCR detection of JAK2 V617F is more sensitive than conventional allele specific PCR. Real time PCR also eliminates the chance of PCR acquired contamination. Furthermore, it is applicable to poor quality DNA extracted from archived bone marrow smears. This method is sensitive for use in routine and diagnostic laboratories and could be used for retrospective analysis of stored material.

0755

HEPARIN INDUCED-THROMBOCYTOPENIA IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

M.L. Randi, F. Tezza, M. Scapin, E. Duner, S. Vettore, R. Scandellari, F. Fabris

University of Padua, PADUA, Italy

Background. Treatment with both unfractionated (UFH) or low molecular weight (LMWH) heparin can cause heparin induced thrombocytopenia (HIT). The typical presentation of such complication includes the development of thrombocytopenia (platelet fall greater than 50% from baseline) at 5-10 day of treatment and the occurrence of thrombotic complications (HITT). However, in patients with consumptive thrombocytopenia or thrombocytosis, HIT can not be recognized on the basis of the absolute value of platelet count. This can be the case of patients with Philadelphia -negative myeloproliferative disorders (MPD) who can be treated with heparin because thrombotic events. *Patients, Material and Methods.* Recently, we observed a 54 years old female affected by polycythemia vera (PV) (platelets number 436×10^9/L) and a 38 years old female with essential thrombocythemia (ET) (plts n° 500×10^9/L) who received LMWH for a Budd-Chiari syndrome. They both developed pulmonary embolism respectively at 10th and 18th day of treatment and a concurrent decrease in platelet count. The research of heparin-related IgG (polyanion PF4/ELISA) confirmed the hypothesis of HITT. Therefore, we revaluated our records of MPD patients with unusual sites thrombosis. *Results.* We found 3 other cases who developed pulmonary embolism during heparin treatment (2 UFH, 1 LMWH) for 1 suprahepatic, 1 portal and 1 cerebral sinus thrombosis. None of them develop a platelet count lower than the normal value but the platelet decrease in 2 cases was higher than 50% in respect to the basal value. Even if the plasma samples were unavailable for the assay of PF4/ELISA, an high

4T's clinical score suggested that also in these patients pulmonary embolism should be related to HIT. The main data of our patients are summarized in Table 1. In the literature we found 9 case reports of MPD (5 ET, 4 PV) who developed HIT and in 4, the syndrome occurred in the absence of thrombocytopenia. *Conclusions.* HIT, in patients with normal-high platelet count, represents a diagnostic challenge. We think that all MPD patients receiving heparin both for treatment or for prevention of thrombosis have to be carefully monitored and suspicion of HIT has to be performed when platelet count decline or a thrombosis occurs.

Table 1.

Patients MPD/sex	Platelet nadir X 10^9/L	Platelet decrease %	Day of platelet nadir	Other causes of thrombocytopenia	4 T's score	PF4/ELISA OD
PV/F	94	80	18	No	7	>2.0
ET/F	78	56	10	No	8	>2.0
ET/F	200	64	25	No	7	
ET/F	328	50	8	No	8	
PV/M	380	35	5	No	7	

0756

THE MUTATION LEVEL OF JAK2 V617F STRONGLY CORRELATED WITH VASCULAR EVENTS IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE NEOPLASM

S.M. Bang,[1] J.S. Lee,[1] J.Y. Ahn,[2] J.H. Lee,[2] M.S. Hyun,[3] B.S. Kim,[4] M.R. Park,[5] H.S. Chi,[6] H.Y. Kim,[7] H.J. Kim,[7] M.H. Lee,[8] H. Kim,[9] J.H. Won,[10] H.J. Yoon,[11] D. Oh,[12] E.M. Nam,[13] S.H. Bae[14]

[1]Seoul National University Bundang Hospital, SEONGNAM-SI; [2]Gachon University Gil Hospital, INCHEON; [3]Yeungnam University Hospital, DAEGU; [4]Seoul Veterans Hospital, SEOUL; [5]Wonkwang University School of Medicine, IKSAN; [6]University of Ulsan College of Medicine, SEOUL; [7]Hallym University Sacred Heart Hospital, ANYANG; [8]Inha University Hospital, INCHEON; [9]Ulsan University Hospital, ULSAN; [10]Soonchunhyang University Hospital, SEOUL; [11]Kyunghee University Hospital, SEOUL; [12]Pochon CHA University College of Med., SEONGNAM; [13]Ewha Women's University Hospital, SEOUL; [14]Daegu Catholic Uni. Medical Center, DAEGU, South-Korea

Background. Janus kinase 2 (JAK2) V617F mutation is useful for the diagnosis of chronic myeloproliferative neoplasm (CMPN). But its prognostic relevance to clinical outcome is inconclusive now. *Aims.* We investigate the impact of JAK2 V617F on vascular complications in patients with CMPN. *Methods.* We investigated 266 patients from 15 centers, who were diagnosed as CMPN. JAK2 V617F was examined by allele-specific PCR and sequencing.

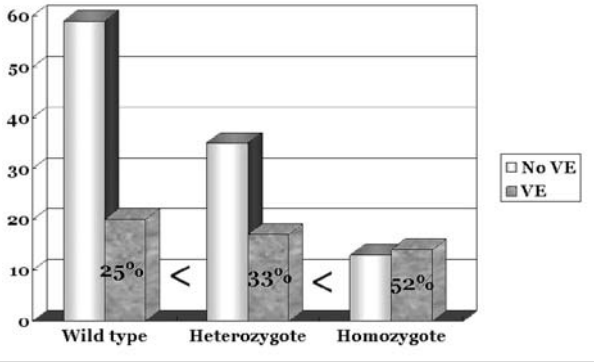

Figure 1.

Results. Their diagnoses were essential thrombocythemia (ET; n=133), polycythemia vera (PV; n=116), primary myelofibrosis (n=12) and unclassifiable CMPN (CMPNu; n=6). JAK2 V617F was detected in 80 (60%) patients with ET, 99 (85%) with PV, 4 (33%) with myleofibrosis, and 4 (80%) with CMPNu. Higher WBC and hemoglobin, panmyelosis of BM, and older age were significantly related with JAK2 V617F. Thrombotic events (25%) occurred more frequently than bleeding of grade 3 or more (11%). Vascular events occurred in 34% of patients with JAK2 V617F, and 25% of those without mutation. In 158 patients whose samples were available for sequencing, patients with homozygous JAK2 V617F experienced more vascular events (52%) than those with heterozygote (33%) or wild type (25%) (Figure 1, p=0.044). *Conclusions.* JAK2 V617F should be tested using more differential method. Also aggressive cytoreduction and careful monitoring for vascular events should be performed for the CMPN patients with JAK2 V617F, especially homozygous mutation.

0757

FIRST-TIME ANAGRELIDE IN PATIENTS WITH HIGH-RISK ESSENTIAL THROMBOCYTHAEMIA (ET): RESULTS OF A MULTICENTRE, PROSPECTIVE OBSERVATIONAL STUDY IN 198 PATIENTS

S. Schmitz

Gemeinschaftspraxis fuer Haematologie und Onkologie, COLOGNE, Germany

Background. Essential thrombocytemia (ET) is generally associated with a more favourable outcome than other myeloproliferative disorders; however, thrombotic and haemorrhagic complications are a key feature of this disease, contributing heavily to morbidity. Anagrelide is a platelet-selective cytoreductive therapy that achieves defined treatment goals in the majority of patients, leading to a reduction of disease-related complications. *Aims.* The objectives of this post-marketing surveillance study were to assess the efficacy and tolerability of anagrelide administered for the first time to patients with high-risk ET, and collect data on disease-related complications in these patients. *Methods.* Patients with ET who had not received anagrelide previously were recruited from private practices across Germany. ET-related complication rates prior to anagrelide treatment were assessed retrospectively. Anagrelide was administered according to European prescribing information. Each patient was observed for at least 6 months. Descriptive statistics were used to evaluate results. *Results.* A total of 198 patients treated at 73 centres were available for analysis. Diagnosis of ET was performed in 82% of patients according to the WHO criteria (including bone marrow biopsy). Mean age of patients was 61 (range: 19-88) years and the mean body mass index was 25 kg/mÇ at enrolment. Almost half of patients entering the study had received hydroxycarbamide previously (49%). The mean starting and maintenance doses of anagrelide were 1.03 and 1.65 mg/per day, respectively. The mean platelet count was reduced from 908×10^9/L at the enrolment examination to 506×10^9/L at the final examination, a mean reduction of 402×10^9/L (95% CI; 329×10^9/L-474×10^9/L; p<0.001). The treatment objective (final count of <400×10^9/L or <600×10^9/L) was achieved in 55% of the patients; 76% of patients were classified as responders (platelet count lowered by half or to below 600×10^9/L). Adverse events (AEs) were experienced by 23% of patients (87 events in 46 patients); only 6% (n=12) discontinued anagrelide treatment due to AEs (n=14). The most frequent AEs were headache (5%), palpitations (4%), dizziness (3%) and tachycardia (3%). Ten events in eight patients were considered serious. More than 85% of patients and doctors rated the overall tolerability as good or very good. Compared with the number of complications that were observed during the 6 months preceding the start of treatment, the frequency of thromboses and transient ischaemic attacks (TIAs) was reduced with anagrelide therapy. Thirteen disease-related complications were reported for 11 patients. These included TIAs (two events in one patient) and haemorrhages (four events in three patients). There were no cases of thrombosis during the study. In the first 6 months of treatment, haemorrhages were observed with the same frequency as that seen prior to anagrelide treatment. *Summary and Conclusions.* This post-marketing surveillance study confirms the efficacy and tolerability of anagrelide in high-risk ET, showing that the platelet count was reduced in the majority of patients, and that thromboses and TIAs were decreased compared with those recorded in the 6 months prior to anagrelide therapy.

0758

INCIDENCE AND SURVIVAL OF MYELOID MALIGNANCIES IN 1102 PATIENTS IN PROVINCE OF MODENA, NORTHERN ITALY

A. Bari,[1] I. Rashid,[2] R. Marcheselli,[3] G. Bonacorsi,[3] G. Leonardi,[3] R. Marasca,[3] P. Zucchini,[3] F. Giacobbi,[3] M. Federico,[3] S. Sacchi[3]

[1]University of Modena, MODENA; [2]Modena Cancer Registry, MODENA; [3]Department of Oncology and Hematology, MODENA, Italy

Background. Making a research on incidence and survival of myeloid malignancies we realised that few well documented population-based studies exist on epidemiology of myelodisplastic syndromes (MDS) and chronic myeloproliferative disorders (CMPD). *Aims*. The goal of our population-based study was to add and improve information about epidemiology of myeloid malignancies. In collaboration with Modena Cancer Registry (MCR) we focalized our attention above all on those haematological malignancies which until few years ago were not recorded in cancer registry because not considered neoplastic. *Methods*. We examined all new cases of AML, MDS, chronic myeloid leukemia (CML) and CMPD diagnosed in the Province of Modena (population 633.993 at 2001 Census) between 1997 and 2006. Death certificate, cytology and histology report, both local and national reports of hospital admission, ICD-9 code reported in medical records were used as sources for identifying new cases and their outcome. All cases were checked and validated by a haematologist (AB) and a pathologist (GB) by a review of the original pathology report. Clinical and follow-up data were retrieved by active search of discharge letters, review of hospital records and interview of general practitioners. Information on vital status was achieved from official population registries. Age-Standardized Rates (ASR) were calculated according to the World Standard population (Doll *et al.*, 1966). The dates of diagnosis and death or the closing date of study (January 2008) were used to estimate survival. Relative survival was calculated according to Hakulinen approach. *Results*. A total of 1102 myeloid malignancies were identified of which 304 AML, 238 MDS, 29 CMML, 417 CMPD and 114 CML. The ASR (per 100,000 people) was calculated as 2.4 for AML, 1.3 for MDS, 0.1 for CMML, 3.2 for CMPD and 1 for CML. When reported to European Standard Population the incidence was 3.2 for AML, 2 for MDS, 0.2 for CMML, 4.4 for CMPD and 1.3 for CML. Compared with reports from other European countries our series seems to be characterized by a higher incidence of CMPD, by a lower incidence of MDS and similar incidence of AML. After a median follow-up of 55 months (range 7-128) the median survival for AML was 5 months, for MDS 23 months, for CMML 26 months; median survival for CMPD and CML was not reached. Relevant differences were observed among median survival of different subtypes of AML, MDS and CMPD. The 5-year relative survival for AML was 20% (for AML M3 74%), for MDS 27%, for CMML 23%, for CMPD 87% and for CML 53% (for CML Ph1+ 80%). *Conclusions*. Our population-based study provides the first analysis of incidence, survival and subtypes distribution of myeloid malignancies performed in Northern Italy. Our results may contribute to better understand the true epidemiology of these diseases, avoiding bias related to referral pattern to myeloid malignancy registries and due to recruiting patients into clinical trials. In the time of emerging innovative treatments the availability of precise epidemiological data could help clinicians in choosing the most appropriate and cost-effectiveness treatment.

0759

ABILITY OF THE WHO 2008 CRITERIA TO ACCURATELY DIAGNOSE THE POLICYTHEMIA VERA AND ESSENCIAL TROMBOCYTHEMIA AND THE CLINICAL SIGNIFICANCE OF THE JAK2 STATUS ACCORDING TO THE NEW CLASSIFICATION

M. Sobas,[1] M. Pérez-Encinas,[1] C. Quinteiro,[2] T. González,[2] E. Ansoar,[1] S. Ordoñez,[1] A. Bendaña,[1] M.J. Rabuñal,[1] S. González,[1] N. Alonso,[1] J. Díaz,[1] I. Abuin,[1] C. Aliste,[3] J.L. Bello[1]

[1]Hospital Clínico Universitario de Santiago de Compostela, SANTIAGO DE COMPOSTELA; [2]Fundación Galega de Medicina Xenómica, SANTIAGO DE COMPOSTELA; [3]Departament of Patholgy, SANTIAGO DE COMPOSTELA, Spain

Background. Diagnosis of PV and ET according to WHO 2001 classification was based on bone marrow histology and exclusion criteria. Discovering of V617F mutation in JAK2 (65-99% in PV and 23-72% in ET) instigated the revision of diagnostic criteria (WHO 2008) which have been published recently. *Aims.* to evaluate clinical and laboratory features and their relation to V617F mutation in PV and ET patients classified according to WHO 2008. *Methods.* all patients were followed-up in our Department in a period of 32 years (1975-2007) and were re-diagnosed according to new WHO 2008 criteria. We have applied the JAK2 MutaScreenTMKit (IPSOGEN) for screening of V617F mutation in 127 patients with clinical diagnosis of PV (27) and ET (100) *Results.* All PV patients (including one V617F negative patient) have fulfilled the new criteria. In ET 95 of 100 cases fulfilled the criteria. The JAK2V617F screening was positive in 26/27 (96%) of PV, 62/95 (65%) of ET and 4/5 (80%) in clinically diagnosed ET that not fulfilled WHO 2008 criteria. Consequently, we have selected and compared patients with platelet count >= than 450×10^9 / L (new threshold for ET) with V617F mutation according to the WHO diagnosis. The PV group (n=20) compared with the ET group (n=62) showed a significantly elevated leucocytes and granulocytes count, mean platelet volume (MPV), platelet distribution width (PDW), LDH, and B12 level (apart of higher haemoglobin and hematocrit level), and a decreased of seric EPO, ferritin and mean platelet count. The pruritus was more frequently in PV patients but we did not found differences in level of leukocyte acid phosphatases (LAP), splenomegaly, thrombosis or myelofibrosis transformation. When we compared the ET V617F positive (n=62) and ET V617F negative (n=33) patients, the $V617F^+$ group showed a higher value for haemoglobin, hematocrit, MPV, PDW, and LAP, and a lower value for platelet count. We did not found differences in leucocytes and granulocytes count, EPO level, neither in clinical features. Five patients with a clinical but very probably diagnosis of ET did not fulfilled the criteria because they had a platelet count lower than 450×10^9 / L (n=2) and or they had a *normal* bone marrow histology (n=3). *Conclusions.* 1. WHO 2008 criteria seems to detect correctly all PV patients, including cases V617F negatives. 2. The classification its not usuful for patients with an initial phase of ET, even if they have the JAK2 mutation. 3. Also we suggest that histological criteria must be interpreted according to the JAK2 status and clinical data. 4. As it's reported with the WHO 2001, also with the WHO 2008 criteria some laboratory differences can be found between the JAK2 positive and negative ET, and between the PV and ET with a similar threshold for platelets and $JAK2^+$ status. More cases will be necessary for demonstrate a clinical different groups of ET and PV according the JAK2 status and the WHO 2008 diagnostic criteria0

Non-Hodgkin's lymphoma - Clinical (aggressive)

0760

OUTCOME OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA RELAPSING AFTER AN AUTOLOGOUS STEM CELL TRANSPLANT

J.M. Calvo-Villas,[1] A. Martín,[2] A. Pascual,[3] I. Heras,[4] J. De la Rubia,[5] R. Varela,[6] M.J. Ramírez,[7] R. Carrión,[8] J. Sarra,[9] M.J. Pascual,[10] M.J. Rodríguez-Salazar,[11] E. Donato,[12] S. Nistal,[13] A. Salar,[14] R. Andreu,[15] J. Sancho,[16] J. Briones,[17] R. Arranz,[18] J.N. Rodríguez,[19] E. Pardal,[20] S. Duran,[21] M. Tapia,[22] F.J. Peñalver,[23] J.A. García-Marco,[24] E. Conde,[25] T. Bernal,[26] M.J. Requena,[27] E. González-Barca,[9] M.D. Caballero[28]

[1]Hospital General de Lanzarote, ARRECIFE DE LANZAROTE [2]Hospital Virgen de la Concha, ZAMORA; [3]Hospital Clínico Universitario San Carlos, MADRID; [4]Hospital Morales Meseguer, MURCIA; [5]Hospital La Fe, VALENCIA; [6]Hospital Juan Canalejo, LA CORUÑA; [7]Hospital General de Jerez, JEREZ; [8]Hospital Gregorio Marañón, MADRID; [9]Institut Català d'Oncología-Hospital Duran i Reynals, BARCELONA; [10]Hospital Carlos Haya, MÁLAGA; [11]Hospital Universitario de Canarias, TENERIFE; [12]Hospital General de Castellón, CASTELLÓN; [13]Hospital de Getafe, MADRID; [14]Hospital del Mar, BARCELONA; [15]Hospital Dr. Peset, VALENCIA; [16]Hospital German Trias i Pujol, BARCELONA; [17]Hospital de la Santa Creu i Sant Pau, BARCELONA; [18]Hospital Universitario La Princesa, MADRID; [19]Hospital Juan Ramón Jiménez, HUELVA; 20Hospital San Pedro Alcantara, CÁCERES, Spain

Background. Although about 50-65% of the patients with chemosensitive relapsed diffuse large B-cell non-Hodgkin's lymphoma (DLBCL) will relapse or progressed after autologous stem cell transplantation (ASCT), there is few published data on the outcome of this population. *Aims.* To evaluate the clinical outcome and identify prognostic variables in DLBCL patients relapsing after ASCT. *Methods*. Eighty DLBCL patients were included in this restrospective multicenter study; 47 were males and 33 females and median age was 49 years (range 18-70). Inclusion criteria were: to achieve at least a partial response (PR) after transplant and to receive treatment after relapse. Patients were included in the database of the Spanish Group for Lymphoma and Autologous Transplantation (GEL/TAMO) Registry between July 1993 and July 2007. After transplant 63 patients (78.8%) had achieved complete remission (CR), 9 (11.3%) uncertain CR, and 8 (10%) were partial responders. Characteristics at relapse or progression were: age older than 60, 12 patients (15%); Ann-Arbor stage III or IV, 54 patients (67.5%); 32.5% presented with B symptoms; 23 (28.8%) and 45 (56.3%) had an elevated LDH or a high beta 2 microglobulin respectively, 15% had a bulky mass and 35 (43.8%) had an age-adjusted IPI 2 or 3. Regarding treatment at relapse or progression, forty patients (50%) received rituximab, either alone (n=4) or in combination with chemotherapy (n=36), 36 chemotherapy, 2 involved-field radiotherapy and 2 patients palliative treatment. Finally, 19 patients received a second transplant, (9 autologous, and 10 allogeneic). *Results*. Median time from ASCT to subsequent relapse was 265 days (43 to 2703). Overall response (OR) rate was 52.6% (37.5% CR). Among the 40 patients who received rituximab-based protocols, the OR rate was 70.6% (55% CR). With a median follow-up after relapse/progression of 32 months (2.6 to 121.8) for surviving patients, the median overall survival (OS) from ASCT failure was 272 days with a median time to progression of 140 days. The actuarial 3-year OS and event-free survival (EFS) were 31% and 6.2%, respectively. Prognostic factors significantly influencing OS and EFS in multivariate analyses were: haemoglobin at salvage treatment (OS; relative risk (RR): 2.7, 95% CI 1.4-5.4, p=0.03); (EFS; RR 3.2, 95% CI 1.4-5.4, p=0.01) and achievement of response after relapse/progression (OS; RR: 12.2, 95% CI 5.3-27.9, p=0.00); (EFS; RR 5.2, 95% CI 2.4-11.2, p=0.00). The IPI at progression (RR: 2.4, 95% CI 1.0-5.5, p=0.04) and rituximab-based regimens as salvage treatment (RR: 2.0, 95% CI 1.1-3.6, p=0.02) also influenced OS. In addition, those lymphoma patients who underwent a second transplantation fared significantly better than patients who were not re-transplanted: 65.2% *vs* 25.7% (p=0.002) OS at 2 years, and 22.2% *vs* 6% (p=0.05) EFS at 2 years. *Conclusions*. Although prognosis of relapsed DLBCL after ASCT is generally poor, some patients with chemosensitive disease treated with rituximab-based regimens and/or a second autologous or allogeneic transplantation experienced prolonged survival. Given the limitations of this retrospective series, larger prospective studies are required in order to confirm the role and feasibility of these therapeutic approaches in patients with relapsed DLBCL progressing after ASCT.

0761

A PHASE II STUDY ON INTENSIVE ALL TYPE CHEMOTHERAPY WITH MINIMAL RESIDUAL DISEASE (MRD) ORIENTED POSTREMISSION STRATEGY IN ADULT PATIENTS WITH LYMPHOBLASTIC LYMPHOMA (PROTOCOL NILG-ALL NO. 09/00)

S.C. Cortelazzo,[1] T. Intermesoli,[2] E. Oldani,[2] F. Ciceri,[3] G. Rossi,[2] E. Pogliani,[2] A. Gallamini,[2] C. Romani,[2] A. Cortelezzi,[2] A. Rambaldi,[2] R. Bassan[2]

[1]Division of Hematology and BMT, BOLZANO; [2]Hematology, BERGAMO; [3]HSR, MILAN, Italy

Background. Lymphoblastic lymphoma (LBL) is a rare and aggressive NHL for which a convincing prognostic model has not yet been defined. Regarding treatment, ALL-type chemotherapeutic regimens improved outcome of LBL, but several issues related to the management of this disease remain still controversial. *Aims.* To evaluate the clinical outcome, prognostic factors and the pattern of recurrence in adult patients with LBL treated with NILG-ALL 09/00 regimen. *Methods.* From 2000 to 2005 16 T-LBL and 5 B-LBL patients (pts), median age 27 years (range 16-57), M/F 12/9, 5 with (<20%) and 16 without BM infiltration, were enrolled in the study. The treatment consisted of induction/consolidation with Ida/V/P/Asp/Cy (blocks 1-3, 5, 6, 8), HD-MTX/Ara-C (4, 7), CNS phase and mediastinal irradiation (mRT) (Gy 24-32) for slow responders as judged by CT scan performed after induction. Eight pts (38%) were analyzed by RQ-PCR using 1 or more pt-specific probes, MRD negativity (Mneg) being defined by negative/low positive (<10-4) BM assay obtained before blocks 6 and 8. Mneg pts received maintenance, while Mpos underwent family-related/unrelated SCT or 2-4 autologous stem-cell supported hypercycles (H/C: L-PAM/VP/6MP/:HD-MTX/Ara-C) plus maintenance if without donor. The remaining 13 pts with undefined MRD were treated according to the adverse features at presentation (advanced stage, B symptoms, elevated LDH, BM infiltration). *Results.* Nineteen pts achieved CR (90%) and 2 were resistant, and died of disease. After a median follow-up of 38 months (range 10-87), 3 pts relapsed (2 BM, 1 without mRT, in mediastinum and lymph nodes) and 2 died in CR (1B-cell lymphoma, 1 sAML). Thus, 14 pts (67%) were in 1st CCR. The projected OS and DFS at 5 years were 69% and 72%, respectively. It is noteworthy that only 4/17 (23%) pts with mediastinal tumor needed mRT for residual mediastinal disease after induction and none relapsed in mediastinum. All 8 pts with known MRD study achieved CR, including 4 Mneg, who received maintenance and 4 Mpos, 2 undergoing allogeneic SCT, one who was given maintenance and 1 hypercycles. *Conclusions.* The present study showed that NILG-ALL 09/00 regimen induced an high CR rate and prolonged the survival of LBL pts, reducing the number of pts, who needed mRT after induction. Early MRD analysis supported optimal therapeutic choices in about one third of cases. A new protocol with an intensification of this regimen with PET-CT oriented mRT and an implemented MRD-oriented postremission strategy has been planned for improving the clinical outcome of LBL pts.

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RITUXIMAB WITH DOSE-DENSE MEGACEOP AND HIGH DOSE CHEMOTHERAPY (HDC) R-MAD WITH BEAM AND AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN UNTREATED HIGH RISK DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

A. Chiappella,[1] A. Tucci,[1] M.G. Cabras,[1] A.M. Liberati,[1] F. Salvi,[1] G. Ciccone,[2] E. Angelucci,[1] M. Badone,[1] B. Botto,[1] L. Falchi,[1] C. Frairia,[1] R. Freilone,[1] D. Novero,[1] L. Orsucci,[1] V. Pavone,[1] E.M. Pogliani,[1] U. Ricardi,[1] G. Rossi,[1] D. Rota-Scalabrini,[1] A. Levis,[1] U. Vitolo[1]

[1]On the behalf of GIMURELL, Hematology, San Giovanni Battista Hospital, TORINO; [2]Unit of Cancer Epidemiology, CPO Piemonte, TORINO, Italy

Background. The addition of Rituximab to dose-dense chemotherapy CHOP and to HDC seems to improve the outcome of advanced stage DLBCL. Aims and *Methods.* From August 2000 to September 2006, 120 previously untreated patients <61 years affected by aggressive DLBCL were enrolled into 12 centers of Gruppo Italiano Multiregionale per lo studio dei Linfomi e delle Leucemie. Inclusion criteria were: advanced stage II, III-IV with age-adjusted (aa)-IPI score 2-3 or BM involvement. Treatment plan was: 4 dose-dense courses R-MegaCEOP (Rituximab 375 mg/m^2 d1, Cyclophosphamide 1200 mg/m^2 + Epirubicine 110 mg/m^2 + Vincristine 1.4 mg/m^2 d3, Prednisone 40 mg/m^2 dd3-7) every 14 days with G-CSF support; patients in CR/PR received 2 courses of intensified chemotherapy R-MAD (Mitoxantrone 8 mg/m^2 + ARA-C 2000

mg/m²/12h + Dexamethasone 4 mg/m²/12h dd1-3, Rituximb 375 mg/m² d4 and before peripheral blood stem cell harvest) followed by BEAM + ASCT ± IF-RT 25-30 Gy to bulky disease. All patients received antibacterial and antifungal prophylaxis. Patients with BM and/or CNS risk involvement received 4 IT-MTX 15 mg. Germinal Center Cell (GCC) and non-GCG profile was evaluated with TMA analyzing CD10, Bcl-6, MUM1 immunohistochemistry expression. *Results.* median age 47 years (19-60); 53% at Intermediate-High and 42% at High risk aa-IPI score; PS >2 65%, 27% BM involvement, 48% bulky disease, 80% LDH >normal and stage II/III/IV 8/19/73% respectively. Complete response was achieved in 98 patients (82%), PR 5 (4%), 12 (10%) did not respond and 5 (4%) died of toxicity. With a median follow-up of 42 months, 4-yr FFS and 4-yr OS rates were: 77% and 80%. Nineteen pts were not autografted because of: 9 progressions, 5 toxicities and 5 inadequate PBSC yield. PBSC harvest was good with a median of 9.7×10^6 cells CD34/kg. All 101 patients who underwent ASCT achieved a complete hematological engraftment with a median of 9 days (3-27) to neutrophil counts $>0.5\times10^9$/L and 14 days (1-72) to a self-sustaining platelet count $>50\times10^9$/L. Few severe toxicities (WHO grade 3-4) were reported: infection 12% and mucositis 23% during R-MAD and BEAM phase. Five patients died of toxicity due to: 1 E.coli sepsis after R-MegaCEOP; 1 E.coli, 1 unspecified sepsis and one Staphilococcus pneumonia after R-MAD; one P.aeruginosa pneumonia after ASCT. There are no secondary MDS or ANLL or solid tumour. Two late infections (disseminated herpes zoster and meningitis) occurred one year off therapy and both resolved. CNS recurrence was observed in 4 patients: all of them were at CSN risk at diagnosis, but only 1 had adequate CNS prophylaxis. So far 40 patients have been characterized for GCC and non-GCC prophile: no difference in FFS rate was observed between the two groups. *Conclusions.* This study shows that Rituximab as adjuvant to dose-dense and HDC with ASCT is effective and safe in high risk DLBCL. A prospective randomized phase III trial comparing this treatment with Rituximab-dose dense chemotherapy without intensification and ASCT is currently running by Intergruppo Italiano Linfomi to properly evaluate the effectiveness of this approach in young patients with poor-prognosis DLBCL.

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IMPACT ON PROGNOSIS OF 18FDG-PET AFTER 4 CYCLES OF A CHOP OR CHOP-LIKE REGIMEN, WITH OR WITHOUT RITUXIMAB, IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

J. Dupuis,[1] E. Itti,[2] A. Rahmouni,[3] F. Hemery,[4] P. Gaulard,[5] C. Gisselbrecht,[6] K. Belhadj,[7] T. El Gnaoui,[7] I. Gaillard,[7] F. Kuhnowski,[7] C. Lin,[2] M. Meignan,[2] C. Haioun[7]

[1]CHU Henri Mondor, CRÉTEIL; [2]Nuclear Medicine, CHU Henri Mondor, CRÉTEIL; [3]Radiology, CHU Henri Mondor, CRÉTEIL; [4]Biostatistics, CHU Henri Mondor, CRÉTEIL; [5]Pathology, CHU Henri Mondor, CRÉTEIL; [6]Oncology/Hematology, CHU Saint Louis, PARIS; [7]Hematology, CHU Henri Mondor, CRÉTEIL, France

Background. 18FDG PET has emerged as a powerful tool for response assessment in aggressive lymphomas. Revised response criteria have been proposed (Cheson, J Clin Oncol, 2007) stressing the role of 18FDG PET in post treatment evaluation. The value of 18FDG PET performed during treatment, in particular after the first four treatment cycles, when decisions regarding further treatment are usually taken, remains to be established. *Methods*. 103 patients with previously untreated DLBCL were prospectively enrolled to evaluate the prognostic impact of 18FDG PET performed early after 2 treatment cycles and after 4 cycles. Responses were qualified as previously described on the basis of visual assessment (Haioun, Blood, 2005). Treating physicians were blinded to 18FDG PET results. *Results*. Median age was 53 years (19-79), and 68% of patients were male. Eighty-seven percent had advanced stage disease (Ann Arbor III or IV), 31% had a poor performance status (ECOG >=2) and 65% had elevated LDH. Repartition according to the International Prognostic Index was as follows : Low-risk (0-1 factor) = 22%, Low-intermediate (2 factors) = 19%, Intermediate-high (3 factors) = 33% and High-risk (4-5 factors) = 26%. Treatment consisted of CHOP (30%) or a dose-intensified CHOP-like regimen (70%), with Rituximab (49%) or without (51%). Four patients could not be evaluated after 4 cycles because of death from toxicity (n=2) or progression before this date (n=2). The remaining 99 patients were evaluated by 18FDG PET and conventional International Workshop Criteria (IWC). Seventy-five patients (75%) had a negative 18FDG PET, while 24 (25%) were positive. With a median follow-up of 57 months for living patients, the estimated probability of event-free survival (EFS) at 5 years was 36% for patients with a positive 18FDG PET *vs* 80% in patients with a negative examination ($p<0.0001$). Among patients with a negative 18FDG PET, the same EFS was observed whatever the response (complete vs partial) according to the IWC. Patients with a positive 18FDG PET had a 5 y EFS of 58% if in CR/CRu by IWC, and 0% if not ($p<0.0001$ for differences between all groups). The same observations could be made in patients treated with and without Rituximab. *Conclusions*. The integration of 18FDG PET in treatment evaluation offers a powerful tool to predict outcome, in particular in case of a partial response according to IWC. A negative 18FDG PET at 4 cycles doubles the probability of 5 year EFS. The relative merits of qualitative (visual) and quantitative (Standard Uptake Value -based) assessment are currently evaluated in this series.

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RESULTS FROM A PHASE II TRIAL INVESTIGATING THE EFFICACY AND SAFETY OF LENALIDOMIDE ORAL MONOTHERAPY IN RELAPSED OR REFRACTORY AGGRESSIVE NON-HODGKIN'S LYMPHOMA

H.W Peter,[1] I.S Lossos,[2] J. Tuscano,[3] G. Justice,[4] J.M. Vose,[5] D. Pietronigro,[6] K. Takeshita,[6] A. Ervin-Haynes,[6] J.B. Zeldis,[6] T. Habermann[7]

[1]New York Medical Center, NEW YORK; [2]Sylvester Cancer Center, University of Miami, MIAMI, FL; [3]University of California Davis Cancer Center, SACRAMENTO, CA; [4]Pacific Coast Hematology/Oncology Medical Group, FOUNTAIN VALLEY, CA; [5]University of Nebraska, OMAHA, NE; [6]Celgene Corporation, SUMMIT, NJ; [7]Mayo Clinic, ROCHESTER, MN, USA

Background. Lenalidomide (Revlimid®), an immunomodulatory drug of the IMiDs® class, is approved by the FDA and the EMEA, in combination with dexamethasone, for treatment of relapsed or refractory multiple myeloma and by the FDA as a monotherapy for the treatment of anemia in patients with myelodysplastic syndromes associated with a deletion 5q cytogenetic abnormality. Lenalidomide also has activity in chronic lymphocytic leukemia and cutaneous T-cell lymphoma. *Aims.* This study was designed to assess the efficacy and safety of oral lenalidomide monotherapy in patients with relapsed or refractory aggressive non-Hodgkin's lymphoma (NHL). *Methods.* Patients with relapsed or refractory aggressive NHL with measurable disease after at least 1 prior treatment regimen were eligible. Patients received 25 mg lenalidomide orally once daily on Days 1-21 every 28 days and continued therapy for 52 weeks as tolerated or until disease progression. Response and progression were evaluated using the IWLRC methodology. *Results.* As of enrollment cut-off, 50 patients were enrolled and 49 received drug. The median age was 65 (23-86) and 24 were female. Median time from diagnosis to lenalidomide was 2.7 (0.4-32) years, the median number of prior treatment regimens was 4, and 28.6% of patients had prior bone marrow transplantation. Histologies included diffuse large B-cell lymphoma [DLBCL] (n=26), follicular center lymphoma grade 3 [FLgr3-transformed] (n=5), mantle cell lymphoma [MCL] (n=15) and transformed low grade lymphoma [TSF] (n=3). Seventeen patients (35%) had an objective response, including 2 complete responses (CR), 4 complete responses unconfirmed (CRu) and 11 partial responses (PR). Eleven patients had stable disease and 15 had progressive disease. Responses were seen in each of the aggressive histologic subtypes studied: DLBCL (5/26), MCL (8/15), FLgr3-transformed (3/5), and TSF (1/3). Progression-free survival was 4.0 (0-14.5) months for all patients. The most common grade 4 adverse events were neutropenia (8.2%) and thrombocytopenia (8.2%) while most common grade 3 adverse events were neutropenia (24.5%), leukopenia (14.3%) and thrombocytopenia (12.2%).Updated results will be presented at the meeting. *Summary and Conclusions.* Lenalidomide oral monotherapy is active in relapsed/refractory aggressive NHL, resulting in a response rate of 35% with manageable side effects. Confirmatory and combination studies are underway.

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FLOW CYTOMETRY IS MORE SENSITIVE THAN CONVENTIONAL CYTOLOGY FOR THE IDENTIFICATION OF NEOPLASTIC INFILTRATION OF CEREBROSPINAL FLUID IN PATIENTS WITH AGGRESSIVE B-CELL NON-HODGKIN'S LYMPHOMA: RESULTS OF A PROSPECTIVE MULTICENTRIC STUDY

S. Quijano,[1] S. Quijano,[1] A. López,[1] J.M. Sancho,[2] C. Panizo,[3] P. Rodríguez Otero,[3] G. Deben,[4] C. Castilla,[5] E. Pérez-Ceballos,[5] J.A. García-Vela,[6] M.C. Monteserín,[6] A. Salar,[7] N. Alonso-Vence,[8] J. Arias,[8] M. Pérez,[8] E. Domingo-Domenech,[9] E. González-Barca,[9] F.J. Peñalver,[10] C. Poderós,[11] J. Plaza-Villa,[11] M. Morado,[12] M. Provencio,[13] J. Garcia-Marco,[13] C. Vallejo,[13] J. Arias,[14] J. Briones,[15] S. Ferrer,[16] E. Gómez,[16] J. Capote,[17] C. Nicolás,[18] M. De la Cruz[19]

[1]Centro de Investigación del Cáncer Universidad de Salamanca, SALAMANCA; [2]Servicio de Hematología, Hospital Universitario Germans Trias I Pujol Badalona, BARCELONA; [3]Servicio de Hematología, Clínica Universitaria de Navarra, PAMPLONA; [4]Servicio de Hematología, Hospital Juan Canalejo, LA CORUÑA; [5]Servicio de Hematología, Hospital Morales Meseguer, MURCIA; [6]Servicio de Hematología, Hospital Universitario de Getafe, MADRID; [7]Servicio de Hematología, Hospital del Mar, BARCELONA; [8]Servicio de Hematología, CHUS, SANTIAGO DE COMPOSTELA; [9]Servicio de Hematología, Instituto Catalán de Oncología, BARCELONA; [10]Servicio de Hematología, Fundación Hospital Alcorcón, MADRID; [11]Servicio de Hematología, Hospital Xeral-Cies, VIGO; [12]Servicio de Hematología, Hospital La Paz, MADRID; [13]Servicio de Hematología and Oncología, Hospital Puerta de Hierro, MADRID; [14]Servicio de Hematología, Hospital Xeral Calde, LUGO; [15]Servicio de Hematología, Hospital Sant Pau, BARCELONA; [16]Servicio de Hematología, Fundación Hospital Doctor Peset, VALENCIA; [17]Servicio de Hematología, Hospital Puerta del Mar, CÁDIZ; [18]Servicio de Hematología, Hospital Central de Asturias, OVIEDO; [19]Servicio de Hematología, Hospital Virgen de la Salud, TOLEDO; [20]Servicio de Hematología, Hospital Vall d'Hebron, BARCELONA, Spain

Background. Recent studies suggest that flow cytometry (FCM) is more sensitive than conventional cytology (CC) for detecting meningeal disease in cerebrospinal fluid (CSF) from B-NHL at high risk of CNS relapse. *Aims.* To evaluate the sensitivity and specificity of a simple 11-parameter FCM approach *vs* CC for detecting neoplastic cells in CSF samples from newly diagnosed aggressive B-NHL at high risk of CNS relapse, using a prospective, multicentric study design. In addition, we compared the distribution of different subpopulations of CSF leukocytes and of the clinico-biological characteristics in patients with CSF^+ *vs* CSF- samples, in an attempt to define new algorithms useful for predicting CNS disease. *Methods.* Since March 2006, a total of 123 CSF samples were analysed (total volume: 0.5 to 4 mL; median: 2 mL) in newly diagnosed patients with aggressive B-NHL, from a total of 29 different hospitals (Diffuse large B-cell lymphoma -DLBCL: 81; Burkitt lumphoma -BL: 31; follicular lymphoma transformed to DLBCL -tFL: 4; T-cell-rich B-NHL: 3; plasmablastic lymphoma -PL: 2; mediastinal lymphoma: 1 and intravascular lymphoma: 1). Of the 123 patients studied, 78 were men (63%) and 45 women (37%) with a mean age of 55±18 years (range: 13-92 years). In all cases, the CSF samples were analysed simultaneously by CC at the institution of origin and FCM, centrally one institution. For the FCM analysis of the CSF, stabilised samples (Transfix, Immunostep, Sl) were systematically stained with the following combination of monoclonal antibodies: CD8-sIglambda/CD56-sIgkappa/CD4-CD19/CD3/CD20/ CD45(FITC/PE/PERCPCY5. 5/PECY7/APC/APCCY7). If the FCM test showed infiltration, an additional 6-color antibody panel was used for full phenotypic characterisation of the disease. *Results.* Of the 123 cases studied, 27 (22%) showed infiltration by neoplastic B-cells by FCM, while CC was positive in only 7 patients (6%), with three other cases being suspicious (2%). CC+ samples typically had >20% neoplastic B-cells and/or >1 neoplastic B-cell/uL, while FCM^+/CC^- samples showed lower levels ($p<0.0001$) of infiltration. Overall, no statistically significant differences were found between FCM^- and FCM^+ CSF samples as regards the relative distribution of the different subsets of normal residual CSF leukocytes, except for a higher ($p<0.001$) percentage of monocytes found among CSF- samples. By contrast, the absolute number of total $CD3^+$ T-cells ($p=0.001$) -including both the number of $CD4^+$ ($p<0.0001$) and $CD8^+$ T cells ($p=0.007$)- and NK cells ($p=0.008$) was higher in patients with positive *vs* negative CSF samples. Interestingly, in BL and FLt, presence of CNS disease by FCM could be predicted with a high specificity based on coexistence of increased serum beta2-microglobulin and neurological symptoms, while PB involvement was the only independent predictive parameter in DLBCL. *Conclusions.* Our results show a significantly higher sensitivity for FCM, providing also new algorithms for selecting among aggressive B-NHL patients, cases at a higher risk of CNS disease.

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ABSOLUTE LYMPHOCYTE COUNT (ALC) IS A PROGNOSTIC FACTOR IN DIFFUSE- LARGE-B-CELL-LYMPHOMA (DLBCL)

M.C. Cox,[1] F. Saltarelli,[2] I. Nofroni,[3] G. La Verde,[2] R. Amodeo,[4] C. Tatarelli,[2] B. Veggia,[2] F. Tabacco,[4] L. Portaro,[4] M.A. Aloe-Spiriti,[2] P. Cardelli,[4] L. Ruco,[5] B. Monarca[2]

[1]A.O. Sant'Andrea, La Sapienza University, ROMA; [2]Hematology, AO Sant'Andrea, La Sapienza University, ROMA; [3]Experimenta Medicine, La Sapienza University, ROMA; [4]Clinical Pathology, A.O. Sant'Andrea La Sapienza University, ROMA; [5]Pathology, A.O. Sant'Andrea, La Sapienza University, ROME, Italy

Background. The prognostic value of Absolute Lymphocyte Count (ALC), has been a recent matter of debate in Non-Hodgkin-Lymphoma (NHL) and there are consistent data showing that ALC recovery, post auto-transplant, is predictive of relapse. *Aims*. We assessed prospectively the value of ALC at diagnosis and also after the completion of chemo-immunotherapy in 101 patients with Diffuse-Large-B-cell-Lymphoma (DLBCL). *Methods*. Analysis of prognostic factors with respect to overall survival (OS), progression free survival (PFS) and event free survival (EFS), were done by two-tailed log-rank test. The ALC cut-off value was calculated as $<0.8\times10^9$/L at diagnosis. *Results*. 23% of patients had an ALC$<0.8\times10^9$/L at diagnosis: this was a strong negative prognostic factor for OS($p=0.004$), EFS($p<0.0001$) and PFS($p<0.0001$) and in multivariate analysis was independent from the International Prognostic Index (IPI). ALC after chemo-immunotherapy was not of prognostic value nor was it related to ALC before therapy. As IPI-score(3-5) and ALC$<0.8\times10^9$/L at diagnosis, were the factors better discriminating poor prognosis patients, a new dichotomous score (ALC/IPI-score) incorporating both was built up: 1] low risk: IPI-score=0-2 and ALC$\geq0.8\times10^9$/ L; 2] high risk: patients with at least one risk factor (IPI-score=3-5 and/or ALC$<0.8\times10^9$/L). This new prognostic factor was highly significant in univariate analysis for OS($p=0.0017$), EFS($p<0.0001$) and PFS($p<0.0001$) and in multivariate analysis was the most powerful predictive factor for EFS (OR=13.302; 95% CI= 3,104-57; $p<0001$) and PFS(OR=15,997; CI=3,754-68,164; $p=0.0002$) *Conclusions*. Our data, supports the notion that ALC at diagnosis has a strong prognostic relevance and is independent from the IPI. The new score including both values proved the most powerful predictor at multivariuate analysis.

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COMBINATION OF RITUXIMAB WITH INITIAL CHEMOTHERAPY IMPROVES THE OUTCOME OF PATIENTS WITH PRIMARY MEDIASTINAL B-CELL LYMPHOMA: A RETROSPECTIVE ANALYSIS OF A SINGLE INSTITUTION COHORT

A. Avigdor,[1] T. Sirotkin,[2] N. Shemtov,[1] M. Berkowicz,[3] Y. Davidovitz,[1] A. Kneller,[1] I. Hardan,[1] A. Shimoni,[1] S. Apter,[1] I. Ben-Bassat,[1] A. Nagler[1]

[1]The Chaim Sheba Medical Center, TEL-HASHOMER; [2]Sackler Faculty of Medicine, Tel-aviv University, TEL-AVIV; [3]The chaim Sheba Medical Center, TEL-HASHOMER, Israel

Background. Primary mediastinal B-cell lymphoma (PMBCL) is a relatively rare clinico-pathologic subtype of diffuse large B-cell lymohoma. The optimal management, the prognostic factors and the role of PET/CT scan in this entity remain a matter of debate. While several retrospective studies suggested that dose-dense regimens are more effective than standard CHOP, the impact of adding rituximab (R) on the outcome of patients (pts) with PMBCL has not been fully evaluated. *Methods.* In this retrospective analysis we reviewed the clinical and radiological records of 81 consecutive pts with PMBCL treated in Sheba Medical Center between August 1985 and October 2006. *Results.* Chemotherapy in the pre-rituximab era (-R cohort) included VACOPB (n=47) or 6 courses of standard CHOP (n=5). Since October 2002, 6 cycles of R were added concurrent with the treatment in another 29 pts (+R cohort): R-VACOPB (n=21) and R-CHOP (n=8). Radiotherapy was not administered following initial chemotherapy to any of the pts. Median age at diagnosis was 31 years (yrs) (range 17-61). Stage I/II and bulky mediastinum (≥10 cm) were present in 88% and 33%, respectively, and extranodal involvement was evident in 44% of all pts. After a median follow-up of 85 months (range 9-240), the overall (OS) and progression-free (PFS) survival at 5 yrs for the entire cohort were 89% and 66%, respectively, with a plateau 2 yrs following treatment. PFS at 5 years was significantly better with +R (81%) than with -R cohort (58%, $p=0.03$). Five-year PFS in pts treated with R-VACOPB, R-CHOP, VACOP-B and CHOP were 84%,

74%, 62%, 20%, respectively (p=0.025). Yet, there was no significant difference in OS between +R and -R cohorts (96% *vs* 88% at 5 yrs, p=0.29). Direct survival comparisons demonstrated that 5-yr OS and PFS were significantly better in VACOPB than in CHOP (p=0.04 and 0.05, respectively) and that R-VACOPB was significantly superior to VACOPB in terms of PFS (p=0.05). In contrast, there was no difference in 5-yr PFS between R-VACOPB and R-CHOP (p=0.44). Univariate analysis revealed that aaIPI was not predictive of OS (p=0.51). Age above 31 yrs (p=0.02) and pericardial effusion (p=0.04) were the only predictors of reduced OS. Furthermore, beginning in 2003, 16 consecutive pts in the +R cohort, who were scanned by PET/CT-FDG before starting and after completion of therapy, were also evaluated in the middle (mid-PET) of treatment. The estimated 3-year PFS for mid-PET negative pts (n=8) and for mid-PET positive pts (n=8) was 86% and 75%, respectively (p=0.48). In terms of treatment failure, the negative predictive value of mid-PET was 100%, while the positive predictive value was only 25%. *Conclusions.* Our population-based historical comparison demonstrates that the addition of R to anthracycline-based therapy significantly improved PFS in pts with PMBCL. We observed superior PFS with VACOPB compared with CHOP, but this superiority was abrogated by the introduction of R as part of initial therapy. Randomized studies are necessary to evaluate the benefit of adding R to initial treatment and the utility of PET as a prognostic tool in pts with PMBCL.

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THERAPEUTIC COMPLIANCE OF 6 CYCLES OF DOSE-DENSE IMMUNOCHEMOTHERAPY, R-CHOP-14 PLUS PEGFILGRASTIM, IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL). PRELIMINARY RESULTS OF A PROSPECTIVE SPANISH TRIAL

E. González-Barca,[1] M. Canales,[2] A. Salar,[3] M.J. Vidal,[4] S. Ferrer,[5] J. Bargay,[6] C. Grande,[7] A. Oriol,[8] J. García,[9] S. Gardella,[10] A. López,[11] F.J. Tomás,[12] J. Briones,[13] D. Caballero[14]

[1]Institut Catalá d'Oncología, HOSPITALET DE LLOBREGAR, BARCELONA; [2]Hospital La Paz, MADRID; [3]Hospital del Mar, BARCELONA; [4]Hospital de Donostia, DONOSTIA; [5]Hospital Universitario Dr. Peset, VALENCIA; [6]Hospital Son Llàtzer, PALMA DE MALLORCA; [7]Hospital 12 de Octubre, MADRID; [8]Institut Catalá d'Oncología , Hospital Germans Trias i Pujol, BARCELONA; [9]Hospital Rio Hortega, VALLADOLID; [10]Institut Catalá d'Oncología, Hospital Josep Trueta,, GERONA; [11]Hospital Vall d'Hebrón, BARCELONA; [12]MD Anderson, MADRID; [13]Hospital de Sant Pau, BARCELONA; [14]Hospital Clínico Universitario, SALAMANCA, Spain

Background. Three-weekly R-CHOP has become the standard treatment for DLBCL. Dose-dense immunochemotherapy could improve the efficacy of treatment, however these regimens are worse tolerated due to mielotoxicity. Single dose of pegfilgrastim per chemotherapy cycle could prevent mielotoxicity and its complications and allow on-time delivery of dose-dense R-CHOP in DLBCL. *Aims.* The aim of this preliminary analysis was to evaluate the feasibility and treatment compliance of dose-dense R-CHOP supported by pegfilgrastim in patients with DLBCL. *Methods.* Prospective clinical trial in patients with DLBCL, CD20 positive disease, ECOG PS 0-2, older than 65 y with IPI 0-5 or younger than 65 y with IPI 0-2. Patients were required to have normal renal, liver and cardiac function. Treatment: R-CHOP was administered every 14 days followed by pegfilgrastim (6 mg per cycle) on day 2. *Results.* 75 patients, 25 young (median age: 46, range 18-61) and 50 old (median age 71, range 65-82) were treated, 40 (53%) were male. All 25 young patients had IPI 0-2, 22 old patients had IPI 0-2 and 28 old patients IPI 3-5. Other patient's characteristics were: stage III-IV: 47 (63%), bulky disease: 25 (33%), extra nodal involvement: 51 (68%), B symptoms: 15 (20%), ECOG 0-1: 59 (79%), elevated LDH 36 (48%), elevated b2microglobulin: 35 (47%). Sixty-four patients completed 6 cycles of treatment. Overall, 405 chemotherapy cycles were administered. Eight (5.5%) out of 146 cycles were delayed in younger patients and 22 (8.5%) out of 259 cycles in older patients, p=0.26. Among old patients with IPI 0-2, 11 (9.3%) out of 118 cycles were delayed (p=0.22), and among old patients with IPI 3-5, 11 (7.8%) out of 141 cycles were delayed (p=0.43). In young patients 3 cycles were delayed due to neutropenia, 3 due to febrile neutropenia and 2 due to fever or infection without neutropenia. In elderly patients, 4 cycles were delayed due to febrile neutropenia, 6 due to fever or infection without neutropenia, and 12 due to other adverse events. Doses of myelotoxic drugs were reduced in 4 (2.7%) cycles in young patients (all in the same patient), and in 4 (1.5%) cycles in elderly patients (in 2 patients). Considering the whole population, 375 (87.6%) cycles in 52 (69.3%) patients were delivered as scheduled. Out of 66 evaluated patients for efficacy after 130-days follow-up, 55 (83.3%) achieved complete remission (CR). CR rate in young patients with IPI 0-2 was 80%, in old patients with IPI 0-2 was 93.7%, and in old patients with IPI 3-5 was 78.2%. *Conclusions.* dose-dense immunochemotherapy with pegfilgrastim support is well tolerated; most cycles were administered as scheduled. Treatment compliance is high even in patients older than 65 years old with either low or high IPI, and remission rates do not differ between young and old patients.

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Withdrawn by the authors

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EFFECTIVENESS OF RITUXIMAB FOR THE TREATMENT OF RELAPSED DIFFUSE LARGE B- CELL LYMPHOMA: RETROSPECTIVE ANALYSIS OF 407 CASES IN A SINGLE INSTITUTE

Y. Shimazu, N. Kenji, Y. Fujiwara, T. Ito, S. Morita, A. Sato, T. Sato, T. Maeda, T. Onishi, C. Mizutani, F. Matsuyama, C. Tsukayama, Y. Ueda

Kurashiki Central Hospital, KURASHIKI, Japan

Background. The introduction of rituximab to treat Diffuse Large B cell Lymphoma (DLBCL) had improved the prognosis dramatically. However, neither the optimal use of rituximab, nor the effectiveness of rituximab for relapsed DLBCL, especially after initial chemotherapy containing rituximab, had been determined. We retrospectively analyzed cases of relapsed DLBCL treated in our institute. *Aims.* To clarify the effectiveness of rituximab for relapsed DLBCL that had been treated with or without Rituximab. *Methods.* Between January 1996 and July 2007, 407 patients were diagnosed as DLBCL in our institute, excluding primary CNS lymphoma. Before January 2008, 186 cases had relapsed or showed chemotherapy resistance. Among the relapsed cases, 159 cases received salvage chemotherapy, and we have analyzed these 159 cases. Before September 2003, patients were treated with chemotherapy without rituximab regimen, and after September 2003, patients were treated with chemotherapy plus rituximab. *Results.* Among the relapsed cases, rituximab had been administered to 101 cases. In 62 cases, rituximab had been administered before relapse, and in 81 cases, rituximab had been administered after relapse. Using the Kaplan-Meier method, we compared the over all survival of 4 different groups regarding the use of rituximab before or after relapse: (1) cases treated without rituximab before and after relapsing, (2) cases treated with rituximab before relapsing but without rituximab after relapsing, (3) cases treated with rituximab both before and after relapsing, (4) cases treated without rituximab before relapsing but with rituximab after relapsing. As a result, the overall survival rates of groups 1 and 2 were significantly lower than those of groups 3 and 4. (p=0.000) Univariate analysis for overall survival showed that treatment without rituximab after relapsing regardless of its use before relapsing (p=0.000), relapsing within one year after the diagnosis (p=0.000), above stage two at diagnosis (p=0.003), increased lactate dehydrogenase (LDH) (p=0.005) were independent factors. Multivariate Cox regression analysis indicated that not administering rituximab as the treatment after relapsing (p=0.001), relapsing within one year after diagnosis (p=0.000), above stage two at diagnosis (p=0.063) were independent predictors for overall survival. *Conclusions.* In this analysis, we demonstrated that treatment without rituximab after relapsing regardless of its use before relapsing, relapsing within one year after the diagnosis, above stage two at diagnosis were independent factors influencing overall survival. It is desirable to administer rituximab after relapsing, regardless of prior administration of rituximab before relapsing. It is important to perform biopsy again to identify CD20 expression in relapsed case. The resluts of our analysis suggest that many of relapsed cases had also expressed CD20 at the time of initial diagnosis. CD19 positive B-cells in peripheral blood were determined after relapsing in 51 cases. Multivariate analysis for these 51 cases showed that the overall survival was independent on the presence of CD19 positive cells. Because the prognosis of cases relapsing within one year after diagnosis or relapsed cases showing stage three to four disease at diagnosis show a poor prognosis, not only high dose chemotherapy but also new treatment strategies may be particularly required for these relapsed cases.

0771

RITUXIMAB DOES NOT IMPROVE SURVIVAL OF PATIENTS TREATED WITH M/VACOP-B PLUS RADIOTHERAPY IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMLBCL): A PHASE II STUDY OF INTER-GRUPPO ITALIANO LINFOMI (IIL)

M. Martelli,[1] V. Stefoni,[1] E. Russo,[1] G. Cabras,[2] A. Rossi,[3] E. Brusamolino,[2] A. Levis,[2] B. Botto,[2] V. Naso,[1] E. Finolezzi,[1] A. Di Rocco,[1] R. Foà,[1] P.L. Zinzani[1]

[1]Hematology Institute, ROMA; [2]Hematology Division, CAGLIARI; [3]Division Hematology, BERGAMO, Italy

Introduction. Third generation regimens such as MACOP-B or VACOP-B (M/VACOP-B) in combination with involved-field radiotherapy (IFRT) seem to improve lymphoma-free survival of PMLBCL. The superiority of R-CHOP over CHOP-like regimens has been demonstrated in younger low risk DLBCL. Recently, the addition of Rituximab to CHOP has also improved the survival of PMBLCL. *Aims.* To evaluate the effectiveness and safety of Rituximab added to the standard M/VACOP-B regimens (R-M/VACOP-B) ± IFRT in PMLBCL. Patients and *Methods.* At this time, a total of 45 patients with PMLBCL have been treated in six participating centers between February 2002 and July 2006. The median age was 38 years (range 17-66); 24/21 (53%) were females; 32 patients had stage II and 13 stage IV; 42 (95%) presented a bulky disease; LDH was increased in 31 (69%) and 24(55%) had a superior vena cava syndrome. According to the age-adjusted IPI score, 30 patients had an IPI = 0-1 and 15 an IPI = 2-3. All patients were treated with standard MACOP-B (35 patients) or VACOP-B (10 patients) regimens plus six cycles of Rituximab (375mg/m^2) given at weeks 3,5,7,9,11,13. Thirty-one patients (69%) received mediastinal IFRT at a median dose of 36 Gy. The response was evaluated in all patients after 6 cycles of chemo-immunotherapy, at the end of the planned chemotherapy and after IFRT. *Results.* The response rate after 6 cycles of the planned R-M/VACOP-B regimen was CR/CRu = 20 (44%), PR = 24 (53%) and NR = 1 (3%). Three/45 patients received an early intensification therapy followed by HDT-ASCT because considered low responders (less than PR or progressive disease) during M/VACOP-B chemotherapy. At the end of the chemo-immunotherapy program, 26/42(62%) patients witnessed a CR/Cru,15(36%) aPR and 1 (2%) patient progressed. Eight/15(53%) PR patients obtained a CR/CRu following IFRT for an overall CR/CRu rate of 80% (34/42). Three patients relapsed from CR/Cru and one progressed from PR at 4,12,12,19 months and died of progressive disease despite salvage therapy. After a median follow-up of 25 months, the 3-year OS and PFS were 80% and 84%, respectively. No significant differences in terms of PFS and OS were associated with the IPI score. In a our historical group of 92 pts with PMBCL treated with MACOP-B+IFRT without Rituximab the 5-yrs OS and PFS were 87% and 81% respectively. *Discussion*. R-M/VACOP-B are active therapeutic regimens devoid of severe toxicity in PMBCL. Consolidation radiotherapy seems to improve the quality of response. Further studies are required to demonstrate if the addition of Rituximab to M/VACOP-B regimens may truly improve the response rate and survival of PMBCL.

0772

COMPARISON OF CLINICAL OUTCOME AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION BETWEEN PERIPHERAL T-CELL LYMPHOMAS AND DIFFUSE LARGE B-CELL LYMPHOMA

B.S. Sohn, D.H. Lee, S. Kim, J. Huh, C.W. Suh

Asan Medical Center, SEOUL, South-Korea

Background. Peripheral T-cell lymphomas (PTCLs) are generally known to have relatively poor prognosis compared with diffuse large B-cell lymphoma (DLBCL). Although there have been several studies which showed the benefit of autologous stem cell transplantation (ASCT) in PTCLs to overcome the poor prognosis, there have been few reports comparing the outcome after ASCT between PTCLs and DLBCL so far. *Aims*. The purpose of this study is to compare the outcomes such as event free survival (EFS) and overall survival (OS) after ASCT between patients with PTCLs and DLBCL, and to identify the correlations between clinical predictive factors and prognosis after ASCT in both groups. *Methods*. We reviewed the ASCT registry of Asan Medical Center, Seoul, Korea. Peripheral T-cell lymphoma, unspecified (N=20) and angioimmunoblastic T-cell lymphoma (N=3) were included as PTCLs. From August 1993 to November 2006, we had found 23 patients with PTCLs and 54 patients with DLBCL who had undergone ASCT. The Pearson chi square test (or the Fisher's exact test), the log-rank test and the Cox proportional hazard regression model was used as appropriate. *Results*. Patient characteristics, the regimens of high-dose therapy, number of infused CD34$^+$ cells, the timing of and disease status at the time of ASCT, and each category (and score) of International Prognostic Index (IPI) showed no statistical difference between both groups except male predominance in PTCLs (p=0.034). On univariate analysis, the timing of ASCT, complete response (CR) disease status at the time of ASCT, favorable LDH/performance/stage and low/low-intermediate IPI [also same risk age-adjusted IPI (aaIPI)] were significant prognostic factors for both OS and EFS (*data not shown*). In multivariate analysis, CR status at ASCT and low/low-intermediate aaIPI were favorable for both OS (RR 2.97, 95% CI: 1.23-7.19, p=0.016 and RR 3.76, 95% CI: 1.74-8.14, p=0.001, respectively) and EFS (RR 2.60, 95% CI: 1.17-5.81, p=0.020 and RR 2.75, 95% CI: 1.30-5.84, p=0.008, respectively). But, the immunophenotype of both lymphoma still had no impact on OS (RR 0.56, 95% CI: 0.27-1.18, p=0.126) or EFS (RR 0.62, 95% CI: 0.30-1.30, p=0.206) in multivariate analysis. OS at 2-years was 46% (95% CI, 33-59%) for patients with DLBCL and 41% (95% CI, 21-61%) for PTCLs, showing no statistical difference (p=0.769). The 2-year EFS of patients with DLBCL was 40% (95% CI, 27-53%) and the 2-year EFS of PTCLs was 43% (95% CI, 23-63%), which also showed no statistical difference (p=0.833). *Conclusions*. After ASCT, PTCLs do not appear to have worse outcome compared with DLBCL. Well known prognostic factors such as disease status, clinical situation and IPI score at the time of ASCT had impact on survival following ASCT, regardless of T- or B-cell lymphoma immunophenotype. So, we conclude that the immunophenotype does not show any difference in survival especially in patients who undergo ASCT and the outcomes of PTCLs after ASCT are comparable to its counterpart, aggressive B-cell lymphoma, if both have similar clinical risk characteristics.

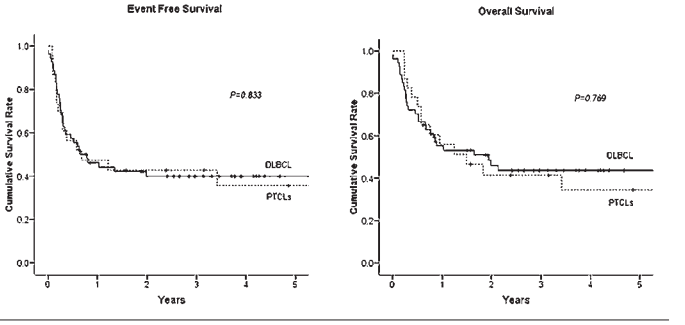

Figure 1. EFS and OS of patients with PTCLs and DLBCL.

0773

THE PREDICTION OF PROGNOSIS USING SEQUENTIAL QUANTITATIVE FDG-PET BEFORE AND AFTER 1 CYCLE OF CHEMOTHERAPY IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

H.J. Kang, S.M. Lim, G.J. Cheon, C.W. Choi, S.S. Lee, B.Y. Ryoo, B.H. Byun

Korea Cancer Center Hospital, SEOUL, South-Korea

Background and Aims. This study was undertaken to evaluate the tumor burden and metabolic response of pretreatment and early interim F-18-FDG PET in patients with diffuse large B cell lymphoma (DLBCL) using different PET parameters. *Methods.* FDG PET was performed in 34 newly diagnosed DLBCL patients at pre-treatment (preTx) and day 5 of the 1st cycle of chemotherapy. We visually scored the summed hypermetabolism (SS) in every FDG PET scan. The highest maximum SUV among involved lesions was defined as SUVmax on each PET data set. On day5 PET, maximum SUV was also measured at the region with SUVmax on preTx PET, and defined as SUVpost. Then, we acquired the decrements (%) of SS and SUVmax after the 1st cycle of chemotherapy. ROC curves for therapeutic response (CR or not) after the completion of 4 to 8 cycles of chemotherapy and progression-free survival (PFS) were analyzed using preTx SS, preTx SUVmax, SS decrement, SUVmax decrement, and clinical parameters as variables. *Results.* Initial stages were stage I in 3, II in 9, III in 12, and IV in 10 by Ann Arbor system, and median follow up period was 26.0months. 31 of 34(91.1%) patients achieved CR after the completion of chemotherapy. On day5 PET scan, SUVpost was different from SUVmax in 8 of 34(23.5%) patients, and one of them did not achieve CR. In ROC analysis, area under the curve (AUC) of preTx SS, preTx SUVmax, SS decrement, and SUVmax decrement (%) was 0.833, 0.538, 0.672, and 0.903, respectively. In multivariate analysis, PreTx SS>14 (p=0.012) and SUVmax decrement£57.5% (p=0.008) were statis-

tically significant predictor of PFS. *Summary and Conclusions.* PreTx SS and SUVmax reduction was suitable parameter for the prediction of therapeutic response in patients with DLBCL. However, further studies are needed to establishing ideal timing of FDG-PET.

0774

NODAL PERIPHERAL T-CELL LYMPHOMAS: TREATMENT OUTCOME AND PROGNOSTIC FACTORS

M.K. Angelopoulou,[1] T.P. Vassilakopoulos,[2] P. Tsirkinidis,[2] S. Masouridis,[2] M. Moschoyiannis,[2] P. Korkolopoulou,[2] S. Kokoris,[2] M. Siakantaris,[2] S. Sachanas,[2] Z. Galani,[2] E. Dimitriadou,[2] C. Kalpadakis,[2] K. Anargyrou,[2] M.N. Dimopoulou,[2] M.-C. Kyrtsonis,[2] E. Patsouris,[2] G. Vaiopoulos,[2] G.A. Pangalis[2]

[1]National and Kapodistrian University of Athens, ATHENS; [2]National And Kapodistrian University of Athens, ATHENS, Greece

Background. Peripheral T-cell lymphomas (PTCL) are uncommon entities with biological heterogeneity and challenging classification. Due to these facts there are very few large series of uniformly treated patients from single Units. *Aims.* To present the clinical features, treatment outcome and prognostic factors in patients with nodal PTCL. *Methods.* Seventy-seven consecutive patients with nodal PTCL diagnosed and treated in a single Hematology Unit between 1992 and 2007 were retrospectively analyzed. *Results.* Median age at diagnosis was 51 years (13-91), 61% were males, 65% had clinical stage III-IV, 51% B-symptoms, 55% elevated LDH and 56% extranodal involvement. The distribution of histologic subtypes was as follows: 30% anaplastic large cell lymphoma (ALCL), 29% angiommunoblastic T-cell lymphoma (AITL) and 40% PTCL unspecified (PTCL-u). Patients' characteristics according to histology are shown in Table 1. The majority of patients (83%) were treated with CHOP-like chemotherapy. Five-year progression free survival (PFS) and overall survival (OS) were 39±6% and 48±7% respectively with a plateau at 23 months. Univariate analysis revealed age >60 (p=0.02), advanced clinical stage (p=0.01), performance status ≥2 (p=0.04) and high IPI (p=0.006) as significant poor prognostic factors for PFS, while non-anaplastic histology and ≥2 extranodal sites were of borderline significance. Age (p=0.0008), clinical stage (p=0.01) and IPI (p=0.03) were significant factors for OS. The recently introduced prognostic index for T-cell lymphomas (PIT) correlated excellently with prognosis. Thus, patients with a PIT score 0-1 had a 60% 5-year PFS *vs* 0% for those with a score 2-3 (p=0.002). The corresponding 5-year OS figures were 59% and 28% (p=0.02). Multivariate analysis disclosed age as the sole prognostic factor both for PFS and OS. At a median follow-up time of 30 months, progression was observed in 43 patients. Among these, 6 are in long-lasting second complete remission with a median duration of 71+ months. *Summary and Conclusions.* Nodal PTCL are characterized by unfavorable prognostic features at diagnosis. ALCL and AITL histologies present with specific clinical characteristics. Both IPI and PIT prognostic indexes correlate significantly with prognosis of nodal PTCL patients treated with CHOP-like regimens. Approximately 40% of the patients can be cured with negligible probability of relapse at 2 years from treatment initiation.

Table 1. Patients' Characteristics according to Histology.

Characteristic	*ALCL (%)*	*AITL (%)*	*PTCL-u (%)*	*p*
Age>60	22	48	39	0.19
Male	65	46	68	0.23
Splenomegaly	13	50	24	0.02
B-symptoms	33	68	46	0.07
Clinical Stage III-IV	27	86	76	<0.001
Performance Status≥2	27	52	76	0.06
Extranodal Sites	5	57	36	0.001
IPI				
Low	86	18	29	
Low-Intermediate	5	12	29	
				<0.001
High-Intermediate	5	41	33	
High	5	29	10	

0775

BURKITT'S LYMPHOMA: A RETROSPECTIVE STUDY OF 62 cases

A.Trabzi, M. Saïdi, F.Belhadri, F. Boukhemia, N. Rahmoune, R. Sahli, F. Zerhouni, R.M. Hamladji

Centre Pierre et Marie Curie .Hematology, Algiers, ALGERIA

The prognosis of Burkitt's lymphoma is generally considered to be poor, particularly in the advanced stage of the disease. Although recent chemotherapy protocols have given high rates of cure in children, there are few such reports concerning adults. We therefore conducted a retrospective analysis of the results for treatment of 62 patients. *Objective*. the aim of our study was to present our experience in Burkitt's lymphoma. *Patients and Methods.* 41 adults, median age was 31years and 21 children, median age: 14 years (3-15), sex ratio M/F 1,1; there were included between January 1995 to December 2005. The patients were treated according to the French Multicenter Protocols for pediatric Burkitt's lymphoma LMB 95 with in adult modified dose of methotrexate: 3 g/m^2. *Results.* Clinical presentation in children was abdominal: 43% and nodal in 24%. In adults presentation was abdominal and nodal in 39%. According to Murphy's classification, there were 16 stage I (12 adults; 4 children), 17 stage II (9 adults, 8 children), 20 stage III (14 adults,6 children) and 9 stage IV (6 adults, 3 children), 8 of whom had CNS involvement. After result of COP, 43 pts (28 adults,15 children) were included in groupe B and 19 pts in groupe C (13 adults, 6 children). The res ponse to treatment could not be evaluated in 6 pts adults. In adults (35 pts),17pts(48%) achieved complete remission; 4 (11%) were refractory and died; 13 early death (37%) occurred from sepsis ; 4 pts relapsed; 13 pts (37%) are still alive in CR with a median follow- up of 56 months (2-8years) In children (21pts), 17pts (81%) achieved C R, 1pt was refractory (5%) and 3 pts died. Two pts relapsed and 1pt lost the follow up. 14pts (67%) are alive and probably cured with median follow up: 90 months (2-12years). *Conclusions.* The good tolerance of chemotherapy by children has permitted aggressive therapeutic protocols resulting in a high cure rate in children with Burkitt's lymphoma. In our study, 26% early death occurred due to septic shock and malnutrition.

Supportive care are necessary to improve our results.

0776

THE ADDITION OF RITUXIMAB TO CHOP GREATLY IMPROVES THE OUTCOME OF PATIENTS WITH PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMLBCL): MATURE RESULTS OF A RETROSPECTIVE MULTICENTER STUDY

T. Vassilakopoulos,[1] G.P Pangalis,[1] Z. Galanis,[1] S. Sahanas,[1] A. Katsigiannis,[2] E. Vrakidou,[3] C. Poziopoulos,[4] N. Konstantinou,[5] P. Repoussis,[6] M. Dimopoulou,[1] S. Kokoris,[1] E. Michali,[1] E. Dimitriadou,[1] S. Masouridis,[1] M. Siakantaris,[1] F. Kontopidou,[1] C. Kalpadakis,[1] N.A. Viniou,[1] X. Yiakoumis,[1] P. Korkolopoulou,[1] M.C. Kyrtsonis,[1] P. Panayiotidis,[1] P. Roussou,[2] M. Angelopoulou[1]

[1]University of Athens, Laikon General Hospital, ATHENS; [2]3rd Dept of Internal Medicine,University of Athens, ATHENS; [3]Hygeia Hospital, ATHENS; [4]401 General Army Hospital, ATHENS; [5]Theageneion Anticancer Hospital, THESSALONIKI; [6]Metaxa anticancer Hospital, PIREUS, Greece

Background. MACOP-B or even chemotherapy (CT) with consolidation high dose therapy and autologous stem cell support (HDT-ASCT) have been considered superior to CHOP in PMLBCL. However, in the absence of randomized trials, there is no established optimal treatment for these patients. Rituximab-CHOP (RCHOP) is superior to CHOP, being the new standard of care for patients with diffuse LBCL. In younger, intermediate/high-risk patients with aggressive lymphomas, HDT-ASCT was superior to conventional CT in the pre-rituximab era, but its role in the era of rituximab is unclear. Thus, the role of RCHOP in the particular case of PMLBCL, which usually affects young patients, is not well established yet. *Aims.* The evaluation of the efficacy of RCHOP±Radiotherapy (RT) in PMLBCL and the comparison of this approach with CHOP±RT, administered to historical controls. PATIENTS AND *Methods.* 84 patients with PMLBCL were treated in 6 centers in Greece between 1994 and 2007. R-CHOP displaced CHOP in the treatment of PMLBCL at a given timepoint in each center: 41 consecutive patients who received RCHOP±RT were compared to 43 consecutive historical controls, who had received CHOP±RT prior to that point. *Results.* The median age of the patients was 31 years (17-82) and 53/84 (63%) were females. All individual IPI parameters and B-symptoms were balanced between the two groups. The median follow-up of

currently alive patients was 33 and 88 months for patients treated with RCHOP±RT and CHOP±RT respectively. All failures occurred within 22 months from diagnosis. The 3-year failure free survival (FFS) was 82±6% *vs* 53±8% for patients who received RCHOP±RT *vs* CHOP±RT (p=0.004), with a greatly reduced early failure rate. The 3-year event free survival (EFS) was 80±6% *vs* 51±8% (p=0.005). The 3-year overall survival was 92±5% *vs* 67±7% (p=0.008), while the 3-year lymphoma specific survival (LSS) was 94±4% *vs* 67±7% (p=0.003). Within the subgroup of patients with L/LI risk IPI the corresponding 3-year FFS rates were 91±6% *vs* 52±10% (p=0.002), while they were 73±11% *vs* 53±12% (p=0.27) among patients with HI/H risk IPI. *Conclusions.* RCHOP±RT provided very good results in PMLBCL: Early progressions were minimized, long-term FFS exceeded 80%, and only 3 lymphoma-related deaths were recorded so far in 41 patients after a median follow-up of 33 months. Patients treated with RCHOP had significantly higher FFS, EFS, OS, and LSS, when compared to CHOP-treated historical controls. Based on these results we continue to treat PMLBCL patients with RCHOP±RT, avoiding more intensive strategies. Whether RT is needed after R-CHOP, especially when post-chemotherapy PET-scan is available, should be further investigated.

0777

A WORLDWIDE META-ANALYSIS ON THE USE OF ZIDOVUDINE AND INTERFERON-ALPHA FOR THE TREATMENT OF ADULT T-CELL LEUKEMIA/LYMPHOMA

A. Bazarbachi,[1] O. Hermine,[2] G. Panelatti,[3] J.C. Ramos,[4] P. Tortevoye,[5] Z. Otrock,[6] G. Taylor,[7] A. Gessain,[5] W. Harrington,[4] Y. Plumelle[3]

[1]American University of Beirut Medical Center, BEIRUT, Lebanon; [2]Necker Hospital, PARIS, France; [3]Centre Hospitalier Universitaire de Fort-de-France, FORT DE FRANCE, Martinique; [4]University of Miami Miller School of Medicine, MIAMI, USA; [5]Institut Pasteur, PARIS, France; [6]American University of Beirut, BEIRUT, Lebanon; [7]Imperial College, LONDON, United Kingdom

Background. HTLV-I associated adult T cell leukemia/lymphoma (ATL) is an aggressive T cells malignancy, with poor prognosis due to chemotherapy resistance. Multiple small phase II studies using zidovudine (ZDV) and interferon-α (IFN) showed response in ATL patients. However, the impact of this therapy on ATL prognosis remains to be determined. *Aims and Methods.* Here, we report a worldwide meta-analysis on the use of ZDV/IFN treatment for ATL in 248 patients treated from 1994 to 2008. Patients were recruited in the USA (57 patients), the UK (22 patients), Martinique (102 patients) and France metropolitaine (67 patients). Collected data included geographic origin, age, sex, type of the disease, clinical presentation, LDH levels, calcemia, and lymphocytes number. *Results*. Median age was 50 years (range 16 to 95). According to Shimoyama classification, there were 112 acute ATL, 20 chronic ATL, 10 smoldering ATL, 97 ATL lymphoma, and 9 patients with an unknown subtype. Hypercalcemia was present in 60% of patients. The data concerning the course of the disease was also collected, particularly the response status, the length of the response, the duration of ZDV+IFN therapy, as well as previous and post chemotherapy treatments. One hundred eleven patients received first line ZDV+IFN therapy. In these patients, response rate was 66%, including 40% of patients achieving complete remission (CR). Median overall survival and 5 year overall survival rate were 24 months and 50% for patients who received first line ZDV+IFN therapy, *vs* 7 months and 20% for patients who received first line chemotherapy. When analysis was performed by ATL subtype, patients with acute, chronic, and smoldering ATL significantly benefited from first line ZDV+IFN therapy, whereas no additional benefit was achieved in patients with ATL lymphoma. Achievement of CR with first line ZDV+IFN therapy resulted in prolonged survival of more than 10 years in 70% of the study population, and 75% of the acute ATL subgroup. Finally, first line ZDV+IFN therapy in chronic and smoldering ATL resulted in 100% overall survival at 10 years. *Conclusions.* In conclusion, these results confirm that treatment of ATL using ZDV and IFN results in a high response and CR rates particularly in acute, chronic and smoldering ATL, resulting in impressive prolonged survival and hence should be considered as gold standard first line therapy.

Novel therapies, drug resistance and pharmacology II

0778

TESTSTRIP-BASED GENOTYPING TO ASSIST IN THE PREDICTION OF ANTICOAGULANT DOSE REQUIREMENT

C. Oberkanins,[1] H. Puehringer,[1] Q. Berisha,[2] G. Klose,[3] B. Schreyer,[3] W. Krugluger,[2] R.M. Loreth[3]

[1]ViennaLab Diagnostics GmbH, VIENNA, Austria; [2]Department of Clinical Chemistry, Rudolfstiftung Hospital, VIENNA, Austria; [3]Clinical Haemostaseology, Westpfalz-Klinikum GmbH, KAISERSLAUTERN, Germany

Background. Coumarin derivatives, such as warfarin and phenprocoumon, are the most widespread oral anticoagulant drugs for the prevention and treatment of arterial and venous thromboembolic disorders. However, these vitamin K antagonists have a narrow therapeutic range and a wide interindividual variability in dose requirement. Despite adjustment for clinical variables, adverse events are frequently encountered during the initial phase of therapy. Genetic polymorphisms in the drug-targeted vitamin K epoxide reductase complex 1 (VKORC1) and in the drug metabolizing enzyme CYP2C9 have been reported to account for the majority of variations in the therapeutic response to warfarin. *Aims and Methods.* A genetic test (StripAssay) for the simultaneous detection of two VKORC1 polymorphisms (-1639G>A, 3730G>A) and the functionally defective CYP2C9 variants *2 (430C>T) and *3 (1075A>C) was developed. The protocol is based on multiplex PCR, followed by reverse-hybridization of biotin-labeled amplification products to a parallel array of allele-specific oligonucleotides immobilized on membrane teststrips. The new StripAssay is currently being used in an ongoing clinical study to classify patients into high, intermediate and low dose responders to coumarin anticoagulants. *Results.* Preliminary data based on 130 patients treated with phenprocoumon (Marcumar) indicated a considerably lower stable dosage required for therapeutic anticoagulation in carriers of a combined VKORC1 -1639A and CYP2C9 *2 or *3 genotype compared to carriers of a single variation or wildtype alleles. The VKORC1 3730G>A polymorphism seemed to have no additional predictive power for phenprocoumon dose variability. *Summary and Conclusions.* The new diagnostic assay and the results obtained during our study will assist clinicians to achieve a safer and more individualized anticoagulant therapy.

0779

SILENCING OF SURVIVIN INDUCED BY A BCR-ABL/JAK2/STAT3 PATHWAY KILLS CML CELLS AND SENSITIZES IMATINIB-RESISTANT CLONES TO THE EFFECT OF HYDROXYUREA

P. Vigneri,[1] F. Stagno,[2] S. Stella,[1] E. Tirrò,[1] E. Conte,[1] F. Stagno,[2] F. Di Raimondo,[2] A. Messina[1]

[1]Pathology Section, CATANIA; [2]Hematology Section, CATANIA, Italy

Background and Aims. The BCR-ABL oncoprotein of Chronic Myelogenous Leukemia (CML) displays strong anti-apoptotic activity that facilitates the expansion of the leukemic clone. Recent evidence has suggested that over-expression of the inhibitor of apoptosis protein Survivin may contribute to this phenomenon. *Methods and Results*. We analyzed Survivin levels in both murine and human CML cell lines and report that BCR-ABL tyrosine kinase activity induces expression of Survivin. This event requires activation of the JAK2/STAT3 pathway since silencing of either protein causes a consistent reduction of Survivin levels. In cells resistant to Imatinib Mesylate (IM), Survivin silencing fails to restore sensitivity to the drug indicating that Survivin is not directly involved in the development of IM resistance. However, down-regulation of Survivin by RNA interference strongly increases hydroxyurea-mediated killing of IM-resistant cells that have become unresponsive to the drug because of point mutations in the BCR-ABL kinase domain. Likewise, increased Survivin degradation by treatment with Shepherdin, an inhibitor of HSP90-mediated stabilization of Survivin, sensitizes both IM-sensitive and IM-resistant cells to the cytotoxic effect of hydroxyurea. *Conclusions*. These results suggest that strategies aimed at reducing Survivin expression increase the sensitivity of CML cells to apoptotic stimuli and may therefore represent a promising therapeutic option for CML patients regardless of their responsiveness to IM.

0780

A CLONAL-EVOLUTION PATHWAY OF CML BLASTS TOWARD INCREASED CLONOGENICITY AND DRUG-RESISTANCE IS ASSOCIATED WITH UPREGULATION OF BCR-ABL, AP-1 TRANSCRIPTION FACTORS AND THE ABCB1 MULTIDRUG TRANSPORTER

H. Galski, M. Simanovsky, M. Leiba, A. Nagler

Chaim Sheba Medical Center, TEL HASHOMER, Israel

Background. CML is considered as a paradigm of multi step-developing malignancies. In the advanced stage of the disease, the anti-apoptotic effect of the BCR-ABL specific tyrosine kinase activity (TK) and overexpression of multidrug transporter genes contribute to drug-resistance toward conventional cytotoxic agents. Moreover, although very effective in chronic phase CML, imatinib and second generation TK inhibitors usually have only transient effects in advanced CML (AP and BC) and their treatment inevitably fails, as drug-resistant clones shortly emerge. We observed that while most CML-BC primary blasts and cell lines grow in suspension under standard culture conditions, a small cell-fraction (2-3%) adheres to the plastic dish. As the adherence capacity of cells to plastic depends on electrostatic interactions between the plastic surface and the relative negative charges of the cell membrane, the observed differential plastic-adherence of CML-BC cells suggests that these blasts are heterogeneous, expressing differential membrane molecular signature and possibly might also have differential aggressiveness and therapeutic sensitivity. *Aims*. To test this possibility, we further investigated whether these diverse blast populations: originated from a common CML clone; have diverse malignant properties; display differential gene signature; and demonstrate different drug-sensitivity. *Methods*. We isolated plastic-adherent and non-adherent cell clones from 3 CML-BC cell lines and primary blasts from 4 CML-BC patients and compared their gene signature using cDNA-microarray and quantitative RT-PCR. Their clonogenic growth capacity and differentiation status were examined in semisolid-media assays. Protein and drug-efflux activity levels of the ABCB1 (Pgp) multidrug transporter were evaluated by Western blotting and by flow cytometry measurements. *Results*. This study revealed that the minor adherent-subsets retain repopulating ability with indications of increased clonogenicity (3.1±1.5 fold, p=0.034) and significant upregulation of the BCR-ABL, the AP-1 transcription factors genes (c-JUN and c-FOS) and the ABCB1 multidrug transporter gene (mean fold upregulation, SD<0.05: 2.5, 4.5, 2.8 and 5.4, respectively). While the gene product of ABCB1, the multidrug transporter Pgp, could not be detected on the cell membrane of the major non-adherent subsets, the plastic-adherent blasts highly expressed Pgp with further increase of its efflux activity after selection with either imatinib or various cytotoxic drugs. After selection with these drugs, the efflux of the Pgp substrates, doxorubicin and rhodamine123, increased by 2-3 folds among the various plastic-adherent subsets, and this activity was completely reversed by the Pgp modulator R-verapamil. The adherent blasts stably retained their unique properties even after elimination of the adherence selection pressure under growth on polyHEMA coated dishes. Subcloning analyses indicated that the adherent cells could be continuously evolved from any parental non-adherent clone in a unidirectional manner. *Conclusions*. The existence of a minor *pool* of blasts of greater clonogenicity and significantly higher expression level of BCR-ABL, individually or in conjunction with AP-1 transcription factors and ABCB1, might signify clonal-evolution towards both increased clonogenicity and lower therapeutic sensitivity. Moreover, this study indicates that adherence to plastic can be used as a surrogate marker to identify these aggressive CML blasts and may facilitate the development of novel therapeutic agents, targeting this distinct blast population.

0781

THE HUMAN ORGANIC CATION TRANSPORTER 1 HAS MINIMAL IMPACT IN THE UPTAKE OF DASATINIB INTO CHRONIC MYELOID LEUKAEMIA PRIMARY CELLS AND CELL LINES

A. Giannoudis, C.M. Lucas, A. Davies, R.J. Harris, M. Pirmohamed, R.E. Clark

University of Liverpool, LIVERPOOL, United Kingdom

In chronic myeloid leukaemia (CML), resistance to imatinib is an important clinical issue. It is well known that imatinib uptake into CML cells is dependent on the uptake transporter human Organic Cation Transporter 1 (hOCT1), and our lab has shown that low hOCT1 expression is an important mechanism of imatinib resistance (Wang L *et al.*, Clinical Pharmacology and Therapeutics; 2008; 83: 258-264). The new tyrosine kinase inhibitor dasatinib is effective in many imatinib-resistant patients, even those without BCR-ABL kinase domain mutations. It is possible that dasatinib is transported differently to imatinib, which might account for its favourable effects in imatinib-resistant patients. We have recently investigated its mechanism of transport into and out of CML cells. Firstly in cell lines, KCL22 cells transfected to express high levels of hOCT1, uptake of radiolabelled dasatinib (kind gift from Bristol Myers Squibb) was greater than in mock transfected cells (p=0.0197). However, prazosin and amantadine, both inhibitors of hOCT transport, did not decrease dasatinib uptake into mock transfected KCL22 cells, in sharp contrast to the inhibition of imatinib uptake seen with both these agents. The level of phosphorylated CrkL, a surrogate marker for BCR-ABL, in mock transfected (i.e. low hOCT1 expressing) KCL22 cells was decreased to 49.9%, by dasatinib, but only to 78.6% by imatinib. In addition, the efflux of dasatinib was investigated in confluent monolayers of Madin-Darby canine kidney (MDCKII) cells eon a semipermeable membrane. These cells stably express ABCB1 (MDR1) on their apical but not their basal aspect. Both dasatinib and imatinib were transported from the basal to the apical layer, indicating ABCB1 transporter-mediated efflux of both drugs (p=0.001, p<0.0001, respectively). Addition of the ABCB1 inhibitor PSC833 blocked transport of both drugs (p=0.0013, p<0.0001 respectively). Secondly, we have extended the study to newly diagnosed CML chronic phase patients. Expression levels of hOCT1 and ABCB1 were assessed by real time RT-PCR, to stratify patients as low hOCT1 (n=5) and high hOCT1 (n=3) expressors, as previously defined. All samples had superior uptake of dasatinib compared with imatinib. The addition of prazosin had minimal effect on dasatinib uptake in either low or high expressors, in contrast to its effects on imatinib uptake in low hOCT1 expressors. Overall, the data on the clinical samples so far show a similar pattern to that observed in the cell lines. This leads to the conclusion that dasatinib uptake is maintained in low hOCT1 expressing primary cells unlike the uptake of imatinib. Dasatinib, unlike imatinib, may achieve adequate intracellular levels and BCR-ABL suppression even in cells with low or blocked hOCT1 function. Efflux of dasatinib and imatinib appear similar and via ABCB1. The data suggest that dasatinib may be effective in patients with low hOCT1 expression who may be at risk of developing imatinib resistance.

0782

AN INSERTION IN THE BCR-ABL KINASE DOMAIN CONTRIBUTES TO IMATINIB MESYLATE RESISTANCE

P. Vigneri,[1] F. Stagno,[2] M. Massimino,[1] S. Berretta,[2] S. Stella,[1] V. Del Fabro,[2] A. Messina,[1] F. Di Raimondo[2]

[1]Pathology Section, CATANIA; [2]Hematology Section, CATANIA, Italy

Background and Aims. The introduction of Imatinib Mesylate (IM) has produced major advances in the treatment of Chronic Myeloid Leukemia (CML) with >85% of patients (pts) achieving complete hematologic and cytogenetic responses after 5 years of treatment. However, 17% of chronic phase pts display primary resistance to IM or acquire secondary resistance to the drug and the incidence of resistance increases in the more advanced phases of the disease. Current studies indicate that multiple mechanisms contribute to IM failure including persistence of CML quiescent stem cells, BCR-ABL amplification and BCR-ABL kinase domain mutations (KDMs). The latter phenomenon accounts for 50-90% of IM resistance. BCR-ABL KDMs have been identified in more than 48 different residues and include single nucleotide substitutions that alter the conformation of the BCR-ABL KD or abrogate the physical interaction between the KD and IM. Recently, an intron-derived insertion/truncation mutation in the BCR-ABL KD has been proposed as an alternative mechanism in three CML pts undergoing tyrosine-kinase inhibitor therapy (Laudadio *et al.* J Mol Diagn, Feb 2008). *Patients and Methods*. Here we report the same insertion/truncation mutation in four CML pts displaying resistance to IM. The four pts (3 males and 1 female, median age 55 yrs) had a long history of disease (median time since diagnosis: 122 months) and underwent long-term IM therapy (median treatment duration: 56 months). All of them eventually failed IM and, at the time of drug resistance, were on high doses IM therapy. At present they are all being treated with second generation tyrosine kinase inhibitors (two with nilotinib and two with dasatinib), with the two pts assuming nilotinib in complete hematologic and cytogenetic response. *Results and Conclusions*. Unlike the report of Laudadio *et al.*, in our pt cohort clonal sequencing revealed that the insertion was associated with both a wild-type and a mutated (m244V; F359V; H396R) BCR-ABL kinase domain. Preliminary structural studies reveal that the insertion generates a truncated BCR-ABL protein that retains most of the KD but is devoid of the large unstructured region located downstream of the

catalytic domain. Transient transfection experiments will determine the responsiveness of this truncated BCR-ABL to different tyrosine kinase inhibitors and its transforming potential in growth factor-dependent hematopoietic cell lines.

0783

NUCLEIC ACID THERAPEUTICS - A POTENTIAL SOURCE OF RESISTANCE TO NUCLEOSIDE ANALOGUES IN CANCER AND ANTIVIRAL THERAPY

R. Buhmann,[1] T. Yang,[2] M. Schifferer,[1] M. Obermeier,[3] G. Jaeger,[3] H.-J. Kolb[4]

[1]Helmholtz Center Munich, MUNICH, Germany; [2]Department of Hematology, Union Hospital, Fujian Medical University, FUZHOU, China; [3]Department of Virology, Max von Pettenkofer-Institute, Ludwig-Maximilians-Univer, MUNICH, Germany; [4]Medical Clinic III, Klinikum Grosshadern Medical Center (KGMC), Ludwig-Maximilia, MUNICH, Germany

Background. In the past years, an increasing number of nucleic acid based drugs (e.g. antisense oligonucleotides, aptamers, ribozymes, RNA interference, defibrotide) have been identified and already tested in clinical trials. But until now, no information is available whether these treatment approaches might interfere with chemically and structurally related drugs, e.g. nucleoside analogues (NA) used in cancer or in antiviral therapy. *Aims.* In the current survey we investigated, whether defibrotide (DF), a polydisperse mixture of single-stranded oligodeoxyribonucleotides (15 to 30 kD) or singular deoxynucleotides interfere with the i) cytotoxic effects of NAs (e.g. fludarabine, cytarabine) on lymphocytes or myeloid blasts or ii) the antiviral effects of acyclovir on HSV reactivation *in vitro*. *Methods.* For this purpose, T-lymphocytes or CD33 positive myeloid blasts (purity >95%) were labelled with [1μM] carboxyfluorescein diacetate, succinimidyl ester (5(6)-CFDA-SE) and incubated with different concentrations of fludarabine or cytarabine (200 μM, 20 μM und 2 μM). Cellular proliferation was induced by addition of CD3/CD28 Dynabeads for the T-cells, or a cytokine cocktail containing 50 ng/mL of SCF, 50 ng/mL of IL-3, 200 ng/mL of GM-CSF, 100 ng/mL of G-CSF, 2 U/mL of EPO, 0.47g/L of human transferrin, and 5×10-5mmol/L of 2-ME for the myeloid blasts. Defibrotide and deoxynucleotides were added at different concentrations, either immediately or with a delay of 24, 48 or 72 hours to the assay. After 5 days, quantitative CFDA distribution was measured by flow cytometry to assess the cellular proliferation of the T cells and the myeloid blasts. Apoptosis and cell cycle analysis were performed via Annexin V / PI staining. Cell viability was determined by trypan blue exclusion. Moreover standard drug resistance assays were performed using the acyclovir sensitive herpes simplex virus (HSV) strain (V0631508) in presence of 50 mM acyclovir and 4 mM of DF. *Results.* Defibrotide and singular deoxynucleotides antagonize the cytotoxic effects of NAs (fludarabine, cytarabine) in both, T-cells and myeloid blasts. Thereby, the antagonistic effects of defibrotide and deoxynucleotides were found to be concentration- and time dependent. Moreover, defibrotide restored replication of HSV in presence acyclovir. Of importance, the concentration of NAs used in these experiments, referred to concentrations applied in standard clinical treatment protocols. *Conclusions.* Treatment with DF and other nucleic-acid-based drugs might interfere with the efficacy of NAs used in cancer and antiviral therapy. Prospective clinical trials are required to confirm these *in vitro* findings.

0784

IL-7 SERUM LEVELS AND LYMPHOPENIA IN HEMODIALYSIS PATIENTS, NON-RESPONDERS TO RECOMBINANT HUMAN ERYTHROPOIETIN THERAPY

E. Costa,[1] M. Lima,[2] S. Rocha,[3] P. Rocha-Pereira,[4] E. Castro,[5] V. Miranda,[6] M. Sameiro Faria,[6] A. Loureiro,[7] A. Quintanilha,[8] L. Belo,[3] A. Santos-Silva[3]

[1]Escola Superior de Saúde, IPB. Fac Farmácia and IBMC of UP, BRAGANÇA; [2]Laboratório de Citometria, Hospital Geral Santo António, HGSA, PORTO; [3]Fac Farmácia and IBMC of UP., PORTO; [4]Universidade Beira Interior, COVILHÃ; [5]Fac Farmácia and IBMC of UP, PORTO; [6]Fresenius Medical Center, Dinefro - Diálises e Nefrologia, SA, PORTO; [7]Uninefro - Sociedade Prestadora de Cuidados Médicos e de Diálise, SA, PORTO; [8]ICBAS and IBMC of UP, PORTO, Portugal

We recently showed that hemodialysis patients present with lymphopenia (Costa *et al.* J Clin Immunol, in press), which results at least in part from a decrease in total circulating $CD3^+$ T-lymphocytes and affects both the $CD4^+$ and the $CD8^+$ T-cell subsets. We also observed that non-responders to recombinant human erythropoietin (rhEPO) therapy present with a lower number of total lymphocyte and $CD4^+$ T-cell counts, when compared with responder patients. Interleukin (IL)-7 has recently emerged as a key cytokine involved in controlling the homeostatic turnover and the survival of peripheral resting memory $CD4^+$ T cells (4), and therefore we hypothesized that the serum levels of this interleukin could be related to the decreased number of total lymphocyte and $CD4^+$ T-cell counts that we have found in hemodialysis patients, particularly in non-responders to rhEPO therapy. In order to test this hypothesis we have selected 63 hemodialysis patients (32 responders and 31 non-responders to rhEPO therapy).The rhEPO maintenance dose for responder patients was 89.65±57.62 U/Kg/week and for non-responders was 572.99±193.84 U/Kg/week. Healthy volunteers (n=26), with normal hematological and biochemical values, without any history of renal or inflammatory disease, were used as normal controls. Serum levels of IL-7 were quantified in all participants by the Quantikine high sensitivity immunoassay (R & D Systems, Minneapolic, Minnesota, USA) according to the manufacturer's recommendations. No statistically difference was found between patients and controls, concerning total white blood cell count; however, hemodialysis patients showed lymphopenia that seems to result, at least in part, from a decrease in total circulating $CD3^+$ T-lymphocytes and affects both the $CD4^+$ and the $CD8^+$ T-cell subsets. When comparing responders and non-responders to rhEPO therapy, statistically significant differences were found for total lymphocyte and $CD4^+$ T-cell counts, being lower for non-responders. Concerning to IL-7 serum levels, no statistically significant differences were found ($p>0.05$) between hemodialysis patients [10.5 (7.4-13.5 pg/mL)] and controls [11.2 (7.2-16.0 pg/mL)], suggesting that the lymphopenia found in those patients is not associated to increased serum levels of IL-7. However, among the two groups of patients, non-responders showed statistically significant higher IL-7 serum levels [12.0 (7.6-17.8 pg/mL) *vs* 9.6 (6.1-11.5 pg/mL), $p<0.05$], suggesting a relationship between the increased levels of this cytokine and decreased number of total lymphocytes and $CD4^+$ T-cell count in this group. Further studies are needed to understand the mechanism of lymphocyte loss and its involvement in the resistance to rhEPO therapy. Furthermore, the envolvment of IL-7 in this process required more attention, as a higher IL-7 concentration appears to predict a poorer response to rhEPO therapy.

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0785

DEVELOPMENT AND CHARACTERIZATION OF BORTEZOMIB RESISTANT MYELOMA CELL LINES

M. Schoester, M. Van Duin, Y. De Knegt, S.L. Corthals, A. Broyl, P. Sonneveld

Erasmus MC, ROTTERDAM, Netherlands

Background. Multiple Myeloma is a tumor of the plasma cells, which remains incurable and thus requires the development of new treatment modalities. Bortezomib, one of the novel drugs, inhibits the function of the proteasome thereby inhibiting the breakdown of e.g. IκBα leading to increasing NFκB signaling, leading to apoptosis. Bortezomib has been included in several clinical trials, such as APEX, SUMMIT and HOVON-GMMG-HD4. *Aims.* In order to investigate the mechanism of resistance to Bortezomib, we developed Bortezomib resistant cell lines from the MM1S and the U266 cell lines. *Methods.*The cell lines were cultured with increasing concentration of Bortezomib, to which the cells were exposed for one four hour period weekly. The Bortezomib concentration was increased from 1 nM to 32 nM in a period of 9 weeks, followed by weekly exposures to 16 nM for four hrs. *Results.*The MM16-32 cell line was 4 - 5 fold resistant to Bortezomib compared to the MM1S parent cell line and the U16-32 cell line was 2 fold resistant compared to the U266 cell line. Using the MTT test, no cross-resistance was seen to doxorubicin or melphalan. The Bortezomib resistant MM16-32 cell line was resistant to dexamethasone, while the parent MM1S cell line was not. The U266 cell lines and the Bortezomib resistant U16-32 cell line were both resistant to dexamethasone. Cell cycle analysis showed an accumulation of cells in the G2/M phase after addition of Bortezomib in MM1S cells, which was not seen in the Bortezomib resistant MM16-32 cells. In U266 cells addition of Bortezomib caused an accumulation of cells in the subG0 fraction, suggesting apoptosis, which was less pronounced in the Bortezomib resistant U16-32 cells. Gene Expression analysis showed 1115 significantly, differentially expressed genes in the MM16-32 cell line compared to the MM1S cell line. In this set, genes involved in cell adhe-

sion and apoptosis were found to be over represented. In the U16-32 cell line 210 genes were found to be significantly, differentially expressed compared to the U266 cell line. In this cell line the differential expression of genes related to proteasome and detoxification seems to be of importance. *Conclusions.* The Bortezomib resistant cell lines generated in this study provide a useful platform for studying the mechanism of resistance. This will in turn lead to more insight into myeloma progression.

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PBI-1402 INCREASES HEMOGLOBIN LEVEL AND RED BLOOD CELL COUNT IN CHEMOTHERAPY-INDUCED ANEMIA

L. Gagnon,[1] J. Barabé,[1] C. Penney,[1] V. Kovcin,[2] S. Bos̆njak,[3] P. Laurin[1]

[1]Prometic Biosciences, LAVAL, Canada; [2]Clinical Hospital Centre Bezanijska Kosa, BELGRADE, Serbia; [3]Institute for Oncology and Radiology of Serbia, BELGRADE, Serbia

Background. PBI-1402 is a novel orally active low molecular weight synthetic compound with erythropoiesis stimulating activity. PBI-1402, via a mechanism of action distinct from erythropoietin (EPO), promotes the differentiation of immature stem cells in CFU-GEMM and subsequent maturation to BFU-E leading to reticulocyte and erythrocyte production. A clinical phase I study demonstrated that PBI-1402 induced a significant increase (100%, $p<0.0001$, compared to placebo) of relative and absolute reticulocyte count in healthy volunteers after 21 days of oral treatment and was devoid of significant side effects. *Aims.* The objectives of this phase Ib/II trial were to study the safety and tolerability of PBI-1402 and to assess its biological efficacy on hemoglobin (Hb) level and red blood cell (RBC) count in patients with chemotherapy-induced anemia (CIA). *Methods.* Three cohorts of six patients received 8 weeks of treatment with PBI-1402 administered per os once a day, at three different doses (44, 66 and 88 mg/kg), and were monitored every two weeks for safety, tolerability, Hb level, RBC count and clinical biochemistry. Patients remained on their chemotherapy during PBI-1402 treatment. *Results.* Seventeen patients completed their 8-week PBI-1402 treatment. One patient withdrew consent at week 4. PBI-1402 was well tolerated and no severe side effects were observed. Mean increases of Hb level were highest at week 4 to 6 for the 88 mg/kg group and highest at week 8 for the 44 and 66 mg/kg groups. For all treatment groups, a statistically significant increase in mean Hb level was observed at week 8 ($p=0.0239$). Mean increases of RBC were also highest at week 4 to 6 for the 88-mg/kg group and highest at week 8 for the 44 and 66 mg/kg groups. One patient required a blood transfusion. Overall, after 8 weeks of oral PBI-1402 treatment, Hb level and RBC were increased in 75% of the patients with a significant p value of 0.032 and 0.00024 respectively. *Conclusions.* Oral treatment with PBI-1402 offers the potential for a novel therapy of CIA. In addition, PBI-1402 is safe and well tolerated.

0787

EAPB0203, A MEMBER OF THE IMIDAZOQUINOXALINE FAMILY, INHIBITS GROWTH AND INDUCES CASPASE DEPENDENT APOPTOSIS IN T CELL LYMPHOMAS AND HTLV-I ASSOCIATED ADULT T-CELL LEUKEMIA/LYMPHOMA

A. Bazarbachi,[1] G. Moarbess,[2] H. El Hajj,[3] Y. Kfoury,[3] M. El-Sabban,[3] Y. Lepelletier,[4] O. Hermine,[4] C. Deleuze-Masquéfa,[2] P. Bonnet[2]

[1]American University of Beirut Medical Center, BEIRUT, Lebanon; [2]Université Montpellier I, MONTPELLIER, France; [3]American University of Beirut, BEIRUT, Lebanon; [4]Necker Hospital, PARIS, France

Background. Imiquimod is an immune response modifier currently used as a topical treatment of genital warts, basal cell carcinoma, cutaneous metastasis of malignant melanoma, and vascular tumors. *Aims and Methods.* We developed more efficient killers from the same family of compounds, that can induce apoptosis without the prominent pro-inflammatory response associated with imiquimod. Among these new products, EAPB0203, member of the imidazo[1,2-alpha]quinoxalines exhibits an important cytotoxic activity *in vitro*. *Results.* HTLV-I associated adult T-cell leukemia (ATL) and HTLV-I-negative peripheral T cell lymphomas are associated with poor prognosis. Using potentially achievable concentrations of EAPB0203, we demonstrate inhibition of cell proliferation, G2/M cell cycle arrest, and induction of apoptosis in HTLV-I transformed and HTLV-I-negative malignant T cells and fresh ATL cells, while normal resting or activated T lymphocytes were resistant. EAPB0203 treatment significantly down-regulated the anti-apoptotic proteins c-IAP-1 and Bcl-XL, and resulted in a significant loss of mitochondrial membrane potential, cytoplasmic release of cytochrome c, and caspase dependent apoptosis. Moreover, in HTLV-I transformed cells only, EAPB0203 treatment stabilized p21 and p53 proteins but had no effect on NF-kB activation. *Conclusions.* These results support a potential therapeutic role for EAPB0203 in ATL and HTLV-I-negative T cell lymphomas, either as a systemic or topical therapy for skin lesions.

0788

MANAGEMENT OF MOLECULAR RESISTANCE IN A PATIENT WITH FIP1L1-PDGFRA POSITIVE EOSINOPHILIC LEUKEMIA

E. Lierman,[1] L. Michaux,[2] E. Beullens,[3] P. Pierre,[4] J. Cools,[3] P. Vandenberghe[5]

[1]Department of Molecular and Developmental Genetics, VIB, LEUVEN; [2]Center for Human Genetics, K.U.Leuven, LEUVEN; [3]Department of molecular and developmental genetics, VIB, LEUVEN; [4]Clinique Sud-Luxembourg, ARLON; [5]Center for human genetics, K.U.Leuven, LEUVEN, Belgium

Background. In 2003, we identified the FIP1L1-PDGFRA fusion gene as a recurrent, cytogenetically cryptic marker for chronic eosinophilic leukaemia (CEL). This fusion gene encodes a constitutively active tyrosine kinase that is highly sensitive to the kinase inhibitor imatinib. Indeed FIP1L1-PDGFRA-positive CEL shows rapid and stable responses to imatinib. Yet, a few cases of imatinib resistance have been reported, with the T674I mutation as the underlying mechanism. *Aims*. We recently identified the kinase inhibitor sorafenib (Nexavar®) as a potent inhibitor of the imatinib resistant FIP1L1-PDGFRA (T674I) mutant. We wanted to investigate the clinical potential of sorafenib for the treatment of imatinib resistant CEL. *Methods.* We treated 1 imatinib resistant CEL patient with sorafenib and performed an *in vitro* mutagenesis screen to identify sorafenib resistance mutations in FIP1L1-PDGFRA. *Results*. We report a 65-year old man who was diagnosed with FIP1L1-PDGFRA positive CEL in blastic crisis (May 2006). He achieved a complete hematological, cytogenetic and molecular remission on imatinib therapy, but relapsed in January 2007, due to acquisition of a T674I mutation, known as imatinib resistant. A therapeutic trial with sorafenib 400 mg bid was undertaken in compassionate use. Initiation of sorafenib induced a partial haematological response without achieving complete remission. FIP1L1-PDGFRA transcripts remained detectable in the peripheral blood throughout therapy. Eventually, progressive disease developed in May 2007. This time, the T674I mutation was no longer detectable, but instead a D842V mutation was found, suggesting that the D842V mutation confers resistance to sorafenib. The condition of the patient rapidly deteriorated and he succumbed in July 2007. We compared the *in vitro* sensitivity of FIP1L1-PDGFRA (D842V) and FIP1L1-PDGFRA (T674I) towards different small molecule inhibitors in Ba/F3 cells. Compared with the T674I mutant, the D842V mutant was more sensitive to imatinib but less sensitive to sorafenib, explaining the emergence of the FIP1L1-PDGFRA (D842V) mutant under sorafenib. We are presently investigating the spectrum of FIP1L1-PDGFRA kinase domain mutants selected by sorafenib, in order to improve the pharmacological approach of imatinib-resistant FIP1L1-PDGFRA CEL. Preliminary results confirm the resistance property of the FIP1L1-PDGFRA (D842V) mutant to sorafenib. *Conclusions*. This case illustrates the impact of molecular identification of resistance mechanisms on correct therapeutic choices: indeed, sorafenib was selected based on its *in vitro* activity against FIP1L1-PDGFRA (T674I) and was shown to have single agent efficacy in this patient. However, a durable remission was not reached due to the emergence of a novel, FIP1L1-PDGFRA (D842V) mutation. The rapid outgrowth of another mutant clone is reminiscent of the propensity of blast crisis CML to develop new kinase domain mutants. This study underlines the importance of identifying the spectrum of all possible kinase domain mutants, and of developing novel inhibitory compounds or combination therapies with different small molecule inhibitors in order to successfully target the potential variety of kinase domain mutants.

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ZALYPSIS® (PM00104): A VERY POTENT *IN VITRO* AND *IN VIVO* INDUCTOR OF APOPTOSIS IN MYELOMA CELLS BASED ON A P53-DEPENDENT RESPONSE

E.M. Ocio,[1] P. Maiso,[2] X. Chen,[3] M. Garayoa,[3] S. Álvarez-Hernández,[3] L. San-Segundo,[3] D. Vilanova,[3] L. López-Corral,[1] T. Hernández-García,[3] E. De-Álava,[3] M.J. Guillén,[4] P. Avilés,[4] C. Cuevas,[4] J.F. San-Miguel,[1] A. Pandiella[3]

[1]Hospital Universitario de Salamanca, SALAMANCA; [2]Centro de Investigación del Cáncer, IBMCC/CSIC. Universidad de Salamanca,, SALAMANCA; [3]1Centro de Investigación del Cáncer, IBMCC/CSIC. Universidad de Salamanca, SALAMANCA; [4]PharmaMar, MADRID, Spain

Introduction. The introduction of novel agents in the treatment armamentarium of multiple myeloma (MM) has changed the outcome of these patients; nevertheless, MM remains incurable and therefore new drugs are urgently needed. The marine environment has gained in interest in the last years as a source for drugs. Zalypsis® is a new marine-derived Jorumycin-related compound that is currently under late Phase I development in solid tumors. *Material and Methods*. The efficacy of Zalypsis® was analyzed in nine MM cell lines and in cells from BM samples from six MM patients. A human subcutaneous plasmocytoma model generated with MM1S and OPM-1 in SCID mice was used for *in vivo* experiments. *Results*. Zalypsis® decreased the viability of nine MM cell lines with IC50s at 48h of 0.2-2 nM, (*in vitro* activity 5-10 folds higher than Bortezomib). Zalypsis® was also very effective in *ex vivo* experiments on freshly isolated patients' cells. It completely overcame the proliferative effect of IL-6 and IGF-I on MM1S cells and that conferred by the coculture with BMSCs. Zalypsis® potentiated many antimyeloma agents with a synergistic effect observed in the combinations with Doxorubicin or Melphalan. Regarding the mechanism of action, Zalypsis® provoked apoptosis with a prompt induction of Annexin V, DNA laddering, and PARP, caspase-3, -8, -9 and -7 cleavage in Western-Blot. Zalypsis® also induced the loss of mitochondrial membrane potential measured by DioC6, decrease of Bcl-X and cleavage of Mcl-1. Selective inhibition of caspases with Z-VAD-FMK, Z-IETD-FMK or Z-LEHD-FMK only slightly abrogated Zalypsis®-induced apoptosis, suggesting a role for caspase-independent apoptosis that was confirmed by the mitochondrial release of AIF. Two patterns of sensitivity to Zalypsis® were observed; MM1S, MM1R and MM144 were highly sensitive (IC50s 0.2 nM) while the other cell lines (RPMI-8226, RPMI-LR5, U266, U266-LR7, OPM1 and OPM-2) were less sensitive (IC50s 1-2 nM). Interestingly the three first lines don't express p53 by Western-Blot, while the latter cell lines do express it. Moreover, treatment of the most sensitive cells lines with Zalypsis® induced the phosphorylation of H2AX, a surrogate marker of DNA damage, and an important increase in p53 protein levels. This is concordant with the results of the GEP that demonstrated the deregulation of many genes involved in DNA damage response. These results were confirmed *in vivo* in a model of human subcutaneous plasmocytoma in SCID mice. Zalypsis® (0.8 and 1 mg/Kg) decreased tumor growth and improved survival of mice implanted with either sensitive (MM1S) and less sensitive (OPM-1) plasmocytomas, though it was more evident in the first group. Immunohystochemical studies showed a decrease in BrdU uptake, cleavage of PARP and caspase-3, phosphorylation of H2AX and induction of p53 and its translocation into the nucleus in Zalypsis®-treated as compared to placebo control tumors. *Conclusions*. Zalypsis® induces a very potent antimyeloma effect *in vitro*, *ex vivo* and *in vivo*, which is, at least partially, mediated through a DNA damage response and through the induction of p53. These results provide the rationale for the use of Zalypsis® in clinical trials for patients with refractory multiple myeloma.

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EFFICACY OF 90Y-IBRITUMOMAB TIUXETAN IN EXTRANODAL MARGINAL-ZONE LYMPHOMA (EMZL)

A. Vanazzi,[1] C. Grana,[1] G. Pruneri,[1] C. Crosta,[1] S. Papi,[1] A. Pinto,[2] G. Paganelli,[1] G. Martinelli[1]

[1]European Institute of Oncology, MILANO; [2]Istituto Nazionale Tumori, NAPOLI, Italy

Background. No standard treatment exists for the management of EMZL relapsing after primary therapies. Local treatment - either surgery or radiotherapy (RT) - usually achieve excellent control. RT represents the treatment of choice for patients with EMZL and limited disease. Those patients presenting with systemic disease should be considered for systemic treatment, but few chemotherapy approaches have been evaluated and no definitive guidelines exist about the best regimen. The anti-CD20 monoclonal antibody (mAb) rituximab is effective in MZL. Radio-immunotherapy (RIT) with 90Y-Ibritumomab-Tiuxetan combines the specific anti-CD20 targeting of a mAb with the cytotoxic crossfire effect of beta-radiation. Unlike external beam radiation therapy (EBRT), RIT uses targeted radiation to simultaneously treat multiple tumor sites, while sparing most normal tissues from radiation damage. *Aims.* to evaluate efficacy of 90Y-Ibritumomab-Tiuxetan delivered at conventional activity (0,4 mCi/kg) in EMZL patients. *Methods.* All patients had histologically confirmed, CD-20 positive MZL, arisen at any extranodal site. Either the novo or relapsed/refractory disease. *Results.* From May 2004 to September 2007, 16 patients were enrolled. Median age was 57 years (36-83 ys); 9 female, 7 male. Seven out of 16 patients had Helicobacter Pylori-negative gastric MALT (mucosa-associated lymphoid tissue) NHL, 4 of them had documented HP infection at diagnosis but relapsed after eradication antibiotic therapy. Nine out of 16 patients had non gastric EMZL. At time of treatment 9 patients had disseminated disease (stage III/IV); bone marrow biopsy showed disease envolvement in 3 out of 16 patients. Median number of previous therapies received was 2 (0-4): all patients except 2 had received prior CT, 8 prior Rituximab, 3 prior RT. Toxicities were primarily haematological, as expected, and reversible. All patients are evaluable for response after a median follow-up of 6 months (range 3-35). Eleven out of 16 patients experienced a CR; PR was observed in 2 out of 16 patients. SD occurred in 3 patients. Responses are durable since 5 out of 11 CRs have been maintained at 35, 28, 24, 17 and 16 months, respectively. Consideration should be given to gastric MALT NHL, since 5 out of 6 patients with resistant-refractory gastric MALT HP negative achieved a long lasting CR. Durable responses in gastric localization could be related to the low tumor burden and consequently to higher absorbed doses to tumor. 90Y-Ibritumomab-Tiuxetan seems to be effective also in disseminated disease, since 6 out of 11 CRs occurred in patients presenting stage III/IV at time of treatment. *Conclusions.* The results support the role of RIT as an effective approach for patients with relapse-refractory MZL. 90Y-Ibritumomab-Tiuxetan, delivered as outpatient regimen, intravenously in one single injection - could offer an alternative option in the treatment of such indolent disease. Its mechanism of action mimics conventional RT already known as valid approach in MZL with localized disease. If these preliminary results can be confirmed in a larger number of patients, 90Y-Ibritumomab-Tiuxetan could represent an alternative approach to RT. An international trial will be performed in order to verify these preliminary results (ZENO trial).

0791

BIOLOGICAL PATHWAYS AND *IN VITRO* ANTI-PROLIFERATIVE ACTIVITY OF HEAT SHOCK PROTEIN 90 (HSP90) INHIBITION IN ADULT T CELL LEUKEMIA CELLS

R. Kurashina,[1] J. Ohyashiki,[1] C. Kobayashi,[1] T. Hirano,[2] K. Ohyashiki[1]

[1]Tokyo Medical University, TOKYO, Japan; [2]Tokyo University of Pharmacy and Life Science, TOKYO, Japan

Background. Heat shock protein 90 (Hsp90) is essential for the stability and the function of many client proteins, such as ERB2, C-RAF, CDK4, HIF-1 aplha and AKT. Recent reports demonstrated that inhibition of Hsp90 modulates multiple functions required for survival of human cancer, such as myeloma (Mitsiades *et al.*, Blood:107, 1092, 2006), however, the precise mechanism of anti-cancer effect of Hsp90 inhibition is still uncertain. *Aims.* The aim of this study is evaluate the effect of Hsp90 inhibition, and to identify molecular pathways responsible for anti-proliferative effect on ATL cells. *Methods.* For Hsp90 inhibition, Geldanamycin derivates, 17AAG (17-allylamino -17-demethoxygeldanamycin) and 17DMAG (17-(dimethylaminoethylamino) 17-demethoxygeldanamycin) were used in this study. Interleukin 2-independent ATL cell lines (MT-2 and MT-4) and an interleukin 2-dependent ATL cell line (TaY) were incubated, with or without Hsp90 inhibitors. Fresh ATL cells obtained from patients were also used after obtaining informed consent. Cell numbers at 48 h after incubation with or without Hsp90 inhibitors were assessed with the Cell Counting Kit-8 assay (Dojindo Molecular Technologies, Gaithersburg, MD, USA). Gene expression analysis was done using a DNA microarray (NCBI Gene expression omnibus; GPL 2531) and statistical analysis was done by a GeneSifter (VizXlabs, Seattle, WA, USA). *Results.* We found cell death induced by Hsp90 inhibitors in all the 3 ATL cell lines as well as patient specimens. Inhibitory concentration (IC50) of 17AAG in 3 ATL cell lines was 300 to 700 nM, and that of 17DMAG was 150 to 200 nM. Fresh ATL cells obtained from patients were more sensitive for either 17AAG or

17DMAG. Gene expression analysis of ATL cells revealed that up-regulation of HSPA1A encoding Hsp70, and genes related to cell cycle arrest (i.e. CDKN1A). Genes regulating cell proliferation or anti-apoptosis (i.e. MYC, BCL2 and Cyclin C), genes related to cytokine or chemokine (i.e. IL9, CCL 17, and CCL27), and notably, genes involved in Wnt/beta-catenin signaling pathway (i.e.TCF7L2 and TCF4), were remarkably repressed. Inhibition of AKT at the protein level was also evident, suggesting the possibility that AKT may down-regulates beta-catenin/TCF7L7 pathways in response to Hsp90 inhibitors in ATL cells. *Conclusions.* Our results have provided new insights into the complex molecular pharmacology of Hsp90 inhibitors, and suggest that Hsp90 inhibitors might be beneficial as anti-proliferative agent in treating ATL patients.

0792

A PHASE 1 TRIAL OF SNS-032, A POTENT AND SPECIFIC CDK 2, 7 AND 9 INHIBITOR, IN CHRONIC LYMPHOCYTIC LEUKEMIA AND MULTIPLE MYELOMA

Z. Goldberg,[1] W. Wierda,[2] R. Chen,[2] W. Plunkett,[2] S. Coutre,[3] A. Badros,[4] L. Popplewell,[5] J.A. Fox,[1] U. Hoch[1]

[1]Sunesis Pharmaceuticals, Inc., SOUTH SAN FRANCISCO; [2]MD Anderson Cancer Center, HOUSTON; [3]Stanford University, STANFORD; [4]University of Maryland Medical Center, BALTIMORE; [5]City of Hope, DUARTE, USA

Background. SNS-032 is a highly selective and potent inhibitor of cyclin-dependent kinases (CDK) 2, 7 and 9. CDK2 and CDK7 are involved in cell cycle regulation. CDK7, along with CDK9, regulate RNA polymerase II-dependent transcription. Temporary inhibition of RNA polymerase II-dependent transcription by SNS-032 has significant effects on short half-life transcripts and proteins, particularly survival factors, cell cycle regulatory proteins, and cytokines that are critical for the survival of malignant B-cells in chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). *Aims.* We are conducting a phase 1 study in patients with CLL or MM to evaluate the safety, pharmacokinetics (PK) and preliminary evidence of activity of a loading dose (LD) followed by a 6 hr infusion of SNS-032, given weekly for 3 consecutive weeks of each 28 day cycle. Dose and schedule aim to achieve and maintain for 6 hr threshold plasma concentrations equal to or greater than the *in vitro* concentration required to inhibit 90% of cell growth (IC90). The study incorporates an exploratory analysis of potential pharmacodynamic (PD) biomarkers. *Methods.* Previously treated patients with CLL or MM, measurable disease, and ECOG status 0-1 were eligible. Increasing doses of SNS-032 given as an LD followed by a 6hr infusion were evaluated. The total starting dose was 15 mg/m^2 with an LD of 5 mg/m^2 followed by 10 mg/m^2 over 6 hr. Dose escalation is by modified Fibonacci. PD studies of target modulation were performed on peripheral blood mononuclear cells (PBMC) taken pretreatment, and 2, 6 and 24 hr after the beginning of 6hr drug infusion. Immunoblotting was performed to analyse either direct target modulation or downstream effects of target inhibition: total RNA polymerase II, phospho-Ser2-RNA pol II, phospho-Ser5-RNA pol II, PARP cleavage, XIAP, Mcl-1, Bcl-2, and the loading control ,-actin. Quantitative analyses were performed with changes in phosphorylation on RNA pol II normalized to total RNA pol II protein. Changes in the other proteins were normalized to β-actin. *Results.* 12 patients have been treated to date at total doses of 15, 22 and 33 mg/m^2. Median age was 63 (range 50-82), with 5 females and 7 males. Median number of prior therapies was 3.5 (range: 1-8). No drug related AEs or DLTs have been reported. PK analyses showed that predicted and measured concentrations are similar at the doses evaluated. PD analyses showed evidence of decreased Mcl-1 or XIAP in 2 of 3 of patients with intact pretreatment samples. *Conclusions.* The mechanism of action of SNS-032 supports testing this agent in B-cell malignanices such as MM and CLL. No AEs or DLTs have been reported for the first 3 cohorts. A pharmacologically-derived dose regimen that sustains IC90 concentrations or higher for 6 hr is being studied and target levels are predicted for the next cohort. Preliminary evidence of target-specific PD modulation has been demonstrated. Enrollment in this trial is continuing.

Quality of life, ethics and economics

0793

SYMPTOMS AND THEIR ASSOCIATION WITH QUALITY OF LIFE IMPAIRMENT IN ADVANCED HEMATOLOGICAL MALIGNANCIES

A. Novik,[1] T. Ionova,[2] S. Kalyadina,[2] A. Kishtovich,[2] D. Fedorenko[1]

[1]Pirogov National Medical Surgical Center, MOSCOW; [2]Multinational Center for Quality of Life Research, ST. PETERSBURG, Russian Federation

Background. Patients with advanced hematological malignancies experience impaired quality of life (QoL) due to symptoms related to the disease itself and to treatment toxicity. Understanding the association of the number and severity of symptoms with quality of life (QoL) impairment in advanced hematological malignancies might provide the guidelines for accurate symptom management of this patients' population. The study goal was to analyze how symptom number and their severity interfere with patient's QoL. *Patients and Methods*. A total of 291 patients (mean age - 50.18 (SD 18.02); male/female - 165/126) with advanced malignancies were accrued (60 - Hodgkin's disease; 100 - non-Hodgkin's lymphoma; 38 - multiple myeloma; 31 - CLL; 31 - AML; 23 - CML; 8 - others). SF-36 and M.D. Anderson Symptom Inventory were used for patient-reported outcomes assessment. To distribute patients according to the grades of QoL impairment the Integral QoL Index was calculated for each patient by the method of Integral Profiles. The following grades of QoL impairment as compared to a population norm (PN) were used: mild (25% decrease from a PN), moderate (25-50% decrease from a PN), severe (50-75% decrease from a PN) and critical (>75% decrease from a PN). *Results*. More than a half of the patients experienced critical (29%) or severe (22.2%) QoL impairment. Mild and moderate QoL impairment was observed in 12.4% and 12.0% of patients, respectively. 24.4% of patients had no QoL impairment. Distribution of the number of patients with moderate-to-severe symptoms in the groups with no, mild, moderate, severe and critical QoL impairment was as follows: 32% *vs* 61.1% *vs* 66% *vs* 74% *vs* 96%. The differences between groups were statistically significant (Chi-square test, $p<0.001$). Symptom profiles varied depending on the type of malignancy; their severity was different across the malignancies. The dependence of the severity and number of symptoms on the type of malignancy was shown (Chi-square = 31.88, df=20, $p=0.04$). The number of patients who experienced moderate-to-severe symptoms differed significantly between the malignancies (chi-square = 15,81; df=5, $p<0.007$), e.g. only 55% of Hodgkin's disease patients experienced moderate-to-severe symptoms as compared to 81.58% in myeloma group and 83.87% in CLL group. *Conclusions*. Our findings demonstrate that each hematological malignancy in advanced stage is characterized by unique combination of symptoms differing in their severity, number and impact on QoL. Our results suggest the importance of tumor-type targeted management of multiple symptoms in advanced hematological malignancies.

0794

QUALITY-OF-LIFE DURING REMISSION MAINTENANCE IMMUNOTHERAPY IN AML: A PROSPECTIVE ASSESSMENT USING EORTC QLQ-C30 IN A RANDOMIZED TRIAL OF HISTAMINE DIHYDROCHLORIDE PLUS LOW-DOSE IL-2 (HDC/IL-2)

A. Wallhult,[1] K. Whisnant,[2] I. Nilsson,[3] D. Bhagwat,[4] K. Hellstrand,[1] L. Brune[1]

[1]Sahlgrenska Academy, University of Göteborg, GÖTEBORG, Sweden; [2]Product Development Resouces LLC, BELLE MEAD, NJ, USA; [3]Connector Medical AB, HELSINGBORG, Sweden; [4]EpiCept Corporation, TARRYTOWN, NY, USA

Background. Most patients with AML undergo induction and consolidation therapy and will achieve complete remission (CR). For many adults with AML, intensive post-consolidation strategies to extend remission duration, e.g., stem cell transplantation or chemotherapy, are associated with substantial morbidity and may not be tolerated or appropriate. A treatment suitable for long-term use by AML patients to prolong remission duration should have an acceptable safety profile and allow patients to maintain quality-of-life (QoL). HDC/IL-2 is an effective novel immunotherapy for AML patients in first CR (CR1) which was shown to prevent relapse and significantly prolong leukemia-free survival (LFS) over untreated controls ($p=0.01$; Brune *et al.* Blood, 2006). *Aims.* To compare QoL and the impact of treatment-related symptoms in AML patients in CR1 who received up to 18 months of post-consolidation HDC/IL-2 immunotherapy *vs* untreated control patients. *Meth-*

ods. AML patients in CR1 (n=261) participated in a randomized open-label study of HDC/IL-2 *vs* standard-of-care (no treatment). Twice daily subcutaneous (sc) injections of HDC (0.5 mg) and IL-2 (16,400 U/kg) were self-administered at home for ten cycles over 18 months, a period of high risk for relapse. Cycles 1-3 and 4-10 each comprised 3 weeks of HDC/IL-2 followed by 3 and 6 weeks of rest, respectively. QoL assessments were completed by treated and untreated patients at 8 pre-defined study visits (baseline; pre- and post-cycles 3, 5, and 8; and at 18 months) using the validated EORTC QLQ-C30 (v2) instrument. Fifteen between-group comparative assessments were made for global QoL, functional status, and symptom scales (fatigue, nausea/vomiting, pain), and for several single-symptom measures (e.g., dyspnea, insomnia, appetite loss, and diarrhea). Baseline QoL assessments were compared to those made at each visit. Pre- *vs* post-cycle assessments provided information about the short-term impact of HDC/IL-2 on QoL. Longitudinal QoL trends were identified by comparing baseline to last-visit QoL measures. *Results.* Over 60% of AML patients in CR1 completed baseline and at least one follow-up visit QoL questionnaire. The post-cycle *vs* pre-cycle scores in treated patients revealed mild, transient symptoms of fatigue, nausea/vomiting, pain, diarrhea, dyspnea, and appetite loss. Importantly, global health status was maintained from baseline to last evaluation both for HDC/IL-2-treated and untreated control patients (Figure 1). Physical, cognitive and role functioning, as well as symptoms of fatigue, nausea/vomiting, pain, diarrhea, and dyspnea were comparable to baseline for both treated and control patients. *Conclusions*. Remission maintenance immunotherapy using HDC/IL-2 resulted in transient fatigue and gastrointestinal symptoms compared to untreated controls. Twice daily sc injections of HDC/IL-2 for up to 10 cycles throughout 18-months had no major impact on QoL. HDC/IL-2 is a safe and well-tolerated remission maintenance therapy for AML that significantly improves LFS and therefore fulfills a critical unmet medical need.

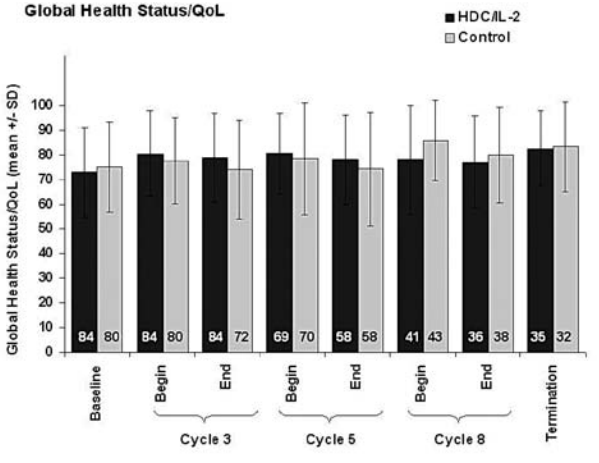

Figure 1.

0795

THE ROLE OF SERUM CYTOKINES IN THE DEVELOPMENT OF ACUTE-GVHD-RELATED SYMPTOM BURDEN AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

X. Wang, Q. Shi, A. Williams, M. Mobley, M. Reuben, B.N. Lee, S. Cleeland, S. Giralt

MD Anderson Cancer Center, HOUSTON, USA

Background. Patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) suffer from acute GVHD (aGVHD)-related or non-specific symptom burden, which necessitates intensive care during the first 100 days post-transplantation. *Aims.* To explore the role of inflammatory cytokines in sickness related to aGVHD, we studied the dynamic changes in symptoms and serum concentrations of inflammatory cytokines during the first 100 days of allo-HSCT. Our subjects were 30 patients with acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS). *Methods.* Multiple symptoms were repeatedly measured using the M. D. Anderson Symptom Inventory (MDASI), which has been validated in patients with cancer. Inflammatory cytokines were tested from serum samples taken around the same time as the symptom measurements. Mixed-effects modeling was used to analyze longitudinal data. *Results.* Twenty-six of 30 patients were diagnosed with aGVHD at Day +26, on average; 30% of these had grade 2 or 3 aGVHD. Baseline fatigue severity ($p<.05$) and interleukin (IL)-8 ($p<.001$), along with soluble receptor 1 for tumor necrosis factor (sTNF-R1) ($p<.001$), predicted the development of aGVHD. Wilcoxon rank sum test results showed that IL-1RA, and IL-12p40p70, and sTNF-R1 increased significantly from nadir of white blood cell count (around Day +8) to their peak during 100 days (Day +36) (see Figure 1; all $p<.05$, all cytokines in pg/mL). A component score (the mean of six most severe symptoms, including Pain, fatigue, sleep disturbance, dry mouth, lack of appetite, and drowsiness) over time was significantly higher in the patients who developed aGVHD than in those who did not ($p<.05$). Mixed-effect modeling showed that increasing levels of serum sTNF-R1 significantly predicted the worsening of symptom severity before ($p<.01$) and after ($p<.05$) aGVHD diagnosis. *Summary*. These results evidenced that the release of serum sTNF-R1 is associated with an increase in multiple-symptom burden in the development of aGVHD during the first 100 days of allo-HSCT in patients with AML/MDS. The results suggest a rationale for conducting randomized trials to test mechanism-driven strategies (e.g., using a TNF inhibitor) to manage severe symptom burden during aggressive cancer therapy, such as allo-HSCT.

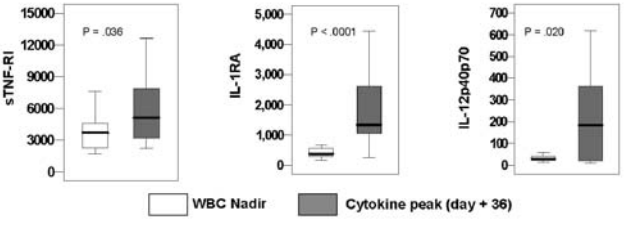

Figure 1. Changes in serum cytokine levels from WBC nadir to day +36 after allo-HSCT.

0796

LARGER STEM CELL DOSE IS ASSOCIATED WITH DECREASED SYMPTOM SEVERITY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA

L.A. Williams,[1] S.A. Giralt,[1] T.R. Mendoza,[1] K.O. Anderson,[1] G.M. Mobley,[1] R.M. Saliba,[1] M.H. Qazilbash,[1] E.L. Campagnaro,[2] C.S. Cleeland[1]

[1]The University of Texas M. D. Anderson Cancer Center, HOUSTON, TEXAS; [2]University of Michigan Comprehensive Cancer Center, ANN ARBOR, MICHIGAN, USA

Background. High-dose chemotherapy with autologous stem cell transplantation (HDC-AuSCT) has become an established procedure for treatment of multiple myeloma (MM). The symptoms associated with HDC-AuSCT are often severe and poorly controlled. Symptom burden is the combined impact of all disease- and therapy-related symptoms on the ability of persons to function as they did prior to onset of their disease and/or therapy. During HDC-AuSCT patients develop a symptom burden that substantially interferes with their daily activities. Patients could greatly benefit from improved symptom management that would enhance their quality of life during HDC-AuSCT. Minimizing the symptom burden of HDC-AuSCT may allow more patients the chance to receive its demonstrated benefits. *Aims.* The aim of this research was to explore the ability of larger numbers of $CD34^+$ cells to decrease symptom severity during HDC-AuSCT in patients with MM. *Methods.* This was a retrospective analysis of 17 patients with MM who completed the M. D. Anderson Symptom Inventory (MDASI) (0-10 measurement scale) at 6 time points from baseline to 30 days post-HDC-AuSCT. Four patients received high doses of stem cells ($9.5\text{-}15\times10^6$ $CD34^+$ cells/kg) and 13 received standard doses ($4\text{-}6\times10^6$ $CD34^+$ cells/kg). Area under the curve (AUC) methodology was used to describe the severity of the 5 most severe symptoms (fatigue, lack of appetite, difficulty sleeping, nausea, and pain) that these patients experienced during the peritransplantation period. *Results.* Means and standard deviations of symptom severity measures are in Table 1. The mean symptom severity of the 2 groups showed no difference at baseline. The difference in mean daily AUC between the 2 groups from cell infusion to 30 days post-HDC-AuSCT approached significance and, more importantly, showed a large effect size of 1.2 times the common group standard deviation. The mean symptom severity of the 5 most severe symptoms was significantly lower at nadir for the group receiving the high cell dose. *Summary and Con-*

clusions. The mean symptom severity for the 2 groups began to diverge sharply after cell infusion. The severity for patients receiving the larger cell dose declined immediately, whereas the severity for patients receiving the standard cell dose rose further before declining. The use of larger cell doses to decrease symptoms warrants prospective testing in a randomized controlled trial. The mechanism behind the ability of larger doses of stem cells to ameliorate symptom severity is unclear. The known relationship between symptom development and severity and inflammatory cytokines (e.g., IL-6) suggests a possible cytokine-based mechanism, which also should be explored.

Table 1. Symptom Severity during AuSCT

Measure	Larger Cell Dose		Standard Cell Dose		P Value (2-Tailed)
	Mean	SD	Mean	SD	
Severity at baseline of 5 most severe symptoms	1.35	1.06	1.86	1.71	0.49
Severity at nadir of 5 most severe symptoms	2.55	1.68	6.06	2.26	<0.012
Daily AUC of 5 most severe symptoms	63.55	42.02	122.95	51.24	0.053

SD – standard deviation

0797

GONADAL FUNCTION IN 248 ADULT MALE SURVIVORS OF CHILDHOOD CANCER

N.J. Van Casteren,[1] G.H.M. Van der Linden,[2] F.G.A.J. Hakvoort-Cammel,[2] K. Hahlen,[1] G.R. Dohle,[1] M.M. Van den Heuvel-Eibrink[2]

[1]Erasmus MC, ROTTERDAM; [2]Erasmus MC-Sophia, ROTTERDAM, Netherlands

Background. Pediatric cancer treatment harbors the risk of gonadal damage. *Aims.* To evaluate the role of Inhibin B as a marker for gonadal function in men who survived childhood cancer. *Methods.* We performed a cross-sectional evaluation(median follow-up time: 18 years, range 5-39 years) in a single center late-effects outpatient clinic. *Patients.* 248 adult male long-term survivors of childhood cancer. Median age at diagnosis: 5 years (range 0-18), median age at follow-up: 24 years (range 18-41). We analyzed patient characteristics, treatment modalities, testicular size, semen analysis and endocrinological parameters, i.e. luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone and Inhibin B. *Results.* The median value of Inhibin B in the cancer survivor group was 126 ng/L (range 0-393) *vs* 177 ng/L (range 60-556) in the control group($p<0.001$). In the survivors, 67% had Inhibin B levels below the normal reference value of 150 ng/L compared with 26 % in the control group($p<0.05$). Inhibin B was the most sensitive discriminator between survivors and controls. Decerased Inhibin B levels and increased FSH levels were found in men treated for Hodgkin and non-Hodgkin lymphoma, acute myeloid leukemia, neuroblastoma and sarcoma's as compared to other malignancies. Cumulative dosages of procarbazine and cyclophosphamide were the only independent chemotherapeutical-related predictors for post-chemotherapy decrease of Inhibin B levels and increase of FSH. In addition, (total body and testicular) irradiation was associated with extremely low Inhibin B values. Age at time of treatment did not influence post-treatment Inhibin B or FSH levels. *Conclusions.* Inhibin B is the most valuable serum marker to assess post-treatment gonadal function, which is severely impaired in a considerable number of childhood cancer survivors.

0798

LOW DOSE CONTINUOUS CHEMOTHERAPY (LD56): AN ACTIVE TREATMENT WITH LOW TOXICITY FOR RELAPSE/REFRACTORY LYMPHOMA

T. Intermesoli, A. Wilson, A. Rohatiner, A. Lister, S. Montoto

Dept. of Medical Oncology, Barts and the London School of Medicine and Dentistry, LONDON, UK

Background and Aims. Although a relatively high proportion of patients with lymphoma can be cured with current treatment strategies, there is still a considerable percentage with resistant disease who are unfit for intensive salvage therapies. However, in addition to symptomatic relief, sometimes durable responses can be seen with so-called palliative treatments. The aim of this study was to retrospectively analyse the results of a low dose continuous chemotherapy program (LD56) designed for resistant/relapsed lymphoma when no further curative options are available. *Methods.* Fifty patients (29 male; median age: 53 years, range: 27-84) who had previously failed one or more treatment lines (median, 4; range: 1-16, including SCT in 50%) were studied. The diagnoses were: Hodgkin's lymphoma (HL), 10 patients; DLBCL, 24; transformed FL (tFL), 16. Forty-three patients (86%) had stage III-IV, 34 (68%) extranodal involvement and 24 (48%), poor performance status (ECOG>2). LD56 consisted of vinblastine 6 mg/m^2 iv and methotrexate 100 mg/m^2 iv on days 1 and 22, lomustine 1 mg/kg po day 1, chlorambucil 5 mg po days 1-7, dexamethasone 4 mg po days 1-7 and 22-28, bleomycin 1 unit sc days 1-56. At least 2 56-day cycles were administered, upon which time treatment was continued depending on the observed response. *Results*. Median overall survival (OS) for the whole series was 8.3 months (range: 2-77). The median number of cycles administered was 3 (range 1-8). 188 cycles were assessable for toxicity. Grade 3-4 neutropenia and thrombocytopenia were observed in 19% and 8% of cases, respectively, whereas grade 3-4 non-haematological toxicity was as follows: gastrointestinal, 5%; pulmonary, 6%; infectious, 8%. Hospital admission was required in 29 (27%) cycles. Four deaths during treatment, not directly attributed to disease, were recorded (infection, 3; CNS bleeding, 1). The overall response rate amongst 46 assessable patients was 26% (CR 6, CRu 1, PR 5). Five of 10 patients (50%) with HL responded to treatment, contrasting with 7 of 40 (17%) with DLBCL (4 de novo DLBCL, 3 tFL) ($p=0.03$). Normal LDH level ($p=0.001$) and <3 treatment lines ($p=0.02$) were also associated with response to therapy. Amongst the 12 responding patients, 4 had consolidation of the response with autologous or allogeneic SCT and died due to toxicity of the procedure; 5 relapsed (4 died of disease, 1 achieved a subsequent response); and 3 patients remain alive in CR after 43, 59 and 68 months. The median duration of the response was 17 months. In 6 of 8 assessable patients the duration of remission achieved after LD56 was longer than their previous remission. After a median follow-up of 47 months, median OS was 26 months in patients responding to treatment and 4 in non-responders. *Conclusions*. Despite the palliative nature of this therapy, durable responses were seen in one quarter of the patients. Patients with HL and those having received less than 3 treatment lines benefited the most. Although some responding patients were considered fit for intensification with SCT after LD56, they died of complications of the procedure, confirming the appropriateness of a palliative approach.

0799

IMPROVED TREATMENT SATISFACTION AND CONVENIENCE WITH DEFERASIROX IN IRON-OVERLOADED PATIENTS WITH β-THALASSAEMIA: RESULTS FROM ESCALATOR TRIAL

A . Taher,[1] A. Al Jefri,[2] M.S. Elalfy,[3] K. Al Zir,[4] S. Daar,[5] G. Damanhouri,[6] J.-F. Baladi,[7] U. Kriemler-Krahn,[7] A. El-Beshlawy[8]

[1]American University of Beirut, BEIRUT, Lebanon; [2]King Faisal Specialist Hospital & Research Centre, RIYADH, Saudi Arabia; [3]Ain Shams University, CAIRO, Egypt; [4]National Thalassemia Centre, DAMASCUS, Syria; [5]Sultan Qaboos University, MUSCAT, Oman; [6]King Abdul Aziz University Hospital, JEDDAH, Saudi Arabia; [7]Novartis Pharma AG, BASEL, Switzerland; [8]Cairo University, CAIRO, Egypt

Background. Deferasirox, a once daily oral chelator, may be considered less bothersome and potentially easier for patients with iron overload to adhere to than parenteral iron chelation therapy (ICT). In addition to treatment efficacy and safety, the ESCALATOR study investigated patient-reported outcomes of deferasirox in iron-overload patients with β-thalassaemia previously receiving deferoxamine (DFO) and/or deferiprone. *Aims.* To assess the impact of deferasirox on patient satisfaction, convenience, and time lost to treatment in iron-overloaded ,-thalassaemia patients, as well as school attendance and performance in paediatric patients. *Methods.* ESCALATOR trial was a prospective open-label, 1-year, multi-centre, study conducted in the Middle East (Egypt, Lebanon, Oman, Saudi Arabia, and Syria) in iron-overloaded β-thalassaemia patients (2 years and older) who did not achieve successful iron chelation with DFO and/or deferiprone. All patients began treatment with deferasirox 20 mg/kg/d, except three paediatric patients who started on 10 mg/kg/d; doses were adjusted in response to markers of over- or under-chelation. Patients were asked at baseline and end of study (EOS, 1 year) to complete 5-point rating scales of overall convenience (very convenient to very inconvenient) and satisfaction (very satisfied to very dissatisfied) with ICT, and to record amount of time lost to treatment for normal activities. School absences and performance were assessed in paediatric patients (age 2 ≤ 16 years). *Results.* From a total of

252 patients, 85 were adults (age ≥16 years; mean age 21.6 years) and 167 were paediatric patients (age 2 ≤ 16 years; mean age 9.6 years). Prior ICT included DFO in 202 patients, deferiprone in 5 patients, and DFO/deferiprone combination in 45 patients. At EOS, 90.9% of patients reported being either *satisfied* or *very satisfied* with their chelation therapy compared to 22.6% at baseline. Similarly, 92.8% of patients considered their therapy to be either *convenient* or *very convenient* at EOS compared to 23% at baseline. Time lost to therapy for normal activities (including employment) was substantially reduced from 28.8±43.6 (mean±SD) hours per month at baseline to 3.0±8.4 hours per month at EOS. In paediatric patients, there was a decrease in school absences at EOS compared to baseline (mean±SD number of days per month -0.2±2.25), and a small change from baseline in school performance. *Conclusions.* Patients reported greater convenience and satisfaction, and reduced time lost to treatment for daily activities with deferasirox compared to previous ICT, which may help improve adherence to lifelong ICT in iron overloaded patients with β-thalassaemia.

0800

SEMEN CRYOPRESERVATION IN PUBERTAL BOYS BEFORE GONADOTOXIC TREATMENT AND THE ROLE OF ENDOCRINOLOGICAL EVALUATION IN PREDICTING SPERM YIELD

N.J. Van Casteren,[1] G.R. Dohle,[1] R. Hans,[1]
S.M.P.F. De Muinck Keizer-Schrama,[2] R.F.A. Weber,[1]
M.M. Van den Heuvel-Eibrink[2]

[1]Erasmus MC, ROTTERDAM; [2]Erasmus MC-Sophia, ROTTERDAM, Netherlands

Background. Pediatric cancer treatment harbors the risk of gonadal damage.Semencryopreservation before gonadotoxic treatment is the only method to secure fertility. *Aims.* To evaluate the feasibility of semen cryopreservation (SCP) in pubertal boys before receiving gonadotoxic therapy and to identify which pre-treatment parameters might predict successful cryopreservation. *Methods.* retrospective data analysis in a Tertiary fertility centre and an Academic Children's hospital *Patients*. Between 1995 and 2005, 80 boys (median age 16.6 year, range 13.7-18.9) consulted the outpatient clinic of Andrology for SCP before a potential gonadotoxic treatment. *Interventions*. We assessed the pre-treatment semen parameters, hormone levels and patients characteristics and the number of adolescents able to cryopreserve semen. *Results.* Thirteen boys failed to produce semen by masturbation. In 53 boys semen quality was adequate for cryopreservation. In fourteen patients semen analysis did not show motile spermatozoa and therefore SCP could not be performed. Although Inhibin B showed a strong correlation with sperm count ($p<0.01$), no significant difference was found in serum testosterone, inhibin B, LH and FSH levels in the patients with or without successful sperm yield. Moreover, median age was not different between patients with and without a successful sperm yield. *Conclusions.* Semen cryopreservation in boys is a feasible method to preserve spermatozoa before gonadotoxic therapy is started and should be offered to all pubertal boys despite their young age. Serum hormone levels do not predict sperm yield.

0801

COMPARISON OF QUALITY OF LIFE BETWEEN CHILDHOOD CANCER SURVIVORS WITH AND WITHOUT STEM CELL TRANSPLANTATION

S.Y. Kwon,[1] H.S. Kim,[1] J.W. Han,[1] M.A. Rhee,[2] U. Lee,[2] K.M. Chung,[2]
S.C. Won,[2] C.J. Lyu[2]

[1]Yonsei University College of Medicine, Department of Pediatrics, SEOUL; [2]Yonsei University, Department of Psychology, SEOUL, South-Korea

Background. Owing to the recent advances of cancer treatment, a number of childhood cancer patients are becoming long term survivors and their quality of life (QOL) with psychosocial adjustment are getting attention. Especially, intensive treatments such as hematopoietic stem cell transplantation (HSCT) are thought to be related with high risk of physical and psychosocial morbidities. In previously published reports, psychosocial adjustment and QOL among childhood cancer survivors had no significant difference from that of other children without disease. However, to our knowledge, comparison of psychosocial adjustment and QOL between children with and without HSCT has not been previously reported much. *Aims.* The purpose of this study was to compare the characteristics of psychosocial adjustment and QOL between children with and without experience of HSCT. This is a preliminary study as a part of cross sectional longitudinal study. *Methods.* 220 children who survived more than two years from off therapy with childhood cancer were included. 42 children received HSCT during their treatment and 178 children didn't. Total 7 items of psychosocial tests(PedsQL: Pediatric quality of life, CBCL: Child behavior check list, and PSI: Parenting stress index for parents, Peds QL, YSR: Youth self report, CDI: Child depression index, and RCMAS: Revised children's manifest anxiety scale for children) were performed. *Results*. Children with SCT had significantly higher total score of externalization tendency than children without SCT ($p<0.005$) from their self report. And the former group showed higher tendency of problematic behavior and low quality of life in self reports ($0<p<0.1$). Means of scores showing childhood depression and anxiety were higher in the former group; however there were no statistical significances. *Conclusions*. This study showed that there was a considerable difference of externalization tendency between childhood cancer patients with and without HSCT experience. And also, even though it didn't showed significant difference, children with HSCT showed more tendency of showing problematic behavior such as attention deficit and hyperactivity. They also had the tendency of experiencing low quality of life. Even though it is a preliminary study yet, however, we should have a close attention and give early intervention to the HSCT survivors who have problematic behaviors or low QOL.

Table 1. Comparison of psychosocial adjustment and quality of life between children with and without stem cell transplantation.

	Test	Patients with SCT (N=42)		Patients without SCT (N=178)	
		Mean of score	SD	Mean of score	SD
CBCL	Externalization*	51.0	9.47	46.2	9.97
	Internalization	53.0	11.82	48.5	9.99
	Problem Behaviors	52.0	11.49	47.1	10.67
YSR	Externalization	50.0	8.94	49.5	7.93
	Internalization	52.0	9.44	49.3	8.54
	Problem Behaviors	51.0	10.64	48.4	8.79
PedsQL self		70.5	13.84	77.6	13.87
PedsQL parent		79.6	18.56	78.3	17.04
CDI		13.0	6.36	10.9	7.45
RCMAS		15.0	6.72	12.9	5.80
PSI		67.0	26.29	63.6	27.83

SCT, stem cell transplantation; SD, standard deviation; CBCL, chil behavior check list; YRS, youth sel report; PedsQL, Pediatric quality of life; CDI, child depression index; RCMAS, revised children's manifest anxiety scale for children; PSI, parenting stress index for parents (*p<0.005).

0802

ANALYSIS OF QUALITY OF LIFE (QOL) FROM THE PHASE 3 RANDOMIZED FIRST-LINE INDOLENT TRIAL IN PATIENTS WITH ADVANCED FOLLICULAR LYMPHOMA RECEIVING CONSOLIDATION THERAPY WITH 90Y-IBRITUMOMAB TIUXETAN

F. D'Amore,[1] A. Valderrama,[2] M. Gonzalez Diaz,[3] N. O'Rourke,[4]
M. Petrini,[5] C. Sebban,[6] P.-L. Zinzani,[7] M. Gomes de Silva,[8]
N. Ketterer,[9] A. Hagenbeek[10]

[1]Århus University Hospital - THG, ÅRHUS, Denmark; [2]Bayer HealthCare Pharmaceuticals, MONTVILLE, USA; [3]Hospital Clínico Universitario de Salamanca, SALAMANCA, Spain; [4]Beatson Oncology Centre, Western Infirmary, GLASGOW, United Kingdom; [5]Azienda Ospedaliera Pisana, PISA, Italy; [6]Centre Léon Bérard, LYON, France; [7]Institute of Hematology „Seràgnoli„, University of Bologna, BOLOGNA, Italy; [8]Serviço de Hematologia, Instituto Português de Oncologia de Francisco Gentil, LISBON, Portugal; [9]Centre Hospitalier Universitaire Vaudois, LAUSANNE, Switzerland; [10]UMC Utrecht/HOVON, UTRECHT, Netherlands

Background. Treatment with the radioimmunotherapy agent 90Y-ibritumomab tiuxetan (Zevalin®; Zev) has previously been shown to improve QOL in patients with relapsed/refractory indolent lymphoma, as measured by the validated Functional Assessment of Cancer Therapy-General (FACT-G) questionnaire (Wiseman *et al.* Blood 2000;96(11):734a, abstract 3173). Recently, a phase 3 randomized First-line Indolent Trial (FIT) was conducted to evaluate the use of Zev for consolidation of remission (complete or partial responses) following first-line induction chemotherapy in patients with newly diagnosed stage III or IV follicular lymphoma. *Aims.* Health-related QOL was evaluated as one of the secondary end points of the FIT study. All patients provided informed consent. *Methods.* Health-related QOL was measured using the EORTC QLQ-C30 (version 2), which specifically assesses QOL in patients with cancer and comprises 30 items grouped into the following dimensions: functional (physical, role, cognitive, social, emotional), symptomatic (nausea, pain, fatigue), global QOL, and other (dyspnea, difficulty sleeping, anorexia, constipation, diarrhea, perceived financial difficulties). The scores for the functional dimensions and global QOL range from 0-100,

with higher scores denoting optimum level of functioning; scores for symptom-oriented dimensions also range from 0-100, with higher scores denoting greater severity in symptoms. The EuroQoL-5D (EQ-5D), which is not disease specific, was also used to assess health-related QOL. It includes both an assessment based on 5 dimensions (mobility, self-care, usual activity, pain/discomfort, and anxiety/depression) and a visual analogue scale (VAS) rating of overall state of health. The scores for the EQ-5D dimensions range from 0-1, with 1 representing an optimal state, and the scores for the VAS range from 0-100, with 100 representing the best health state. The questionnaires were administered at screening, week 14, every 6 months thereafter, and at the final follow-up visit. Descriptive statistics were used to compare scores between treatment groups. The change in scores from baseline was also assessed by gender, age, and first-line treatment. Mixed effects model was used to evaluate factors associated with the final VAS scores of the EQ-5D. *Results.* The mean EQ-5D scores at the screening and final visits were 0.83 and 0.84, respectively, for the Zev arm, and 0.84 and 0.83, respectively, for the control arm. The mean VAS scores at the screening and final visits were 77.52 and 77.64, respectively, for the Zev arm, and 76.57 and 78.60, respectively, for the control arm. An exploratory analysis of factors associated with final VAS scores showed that only the baseline VAS scores affected final VAS scores (p<0.0001). No treatment differences were observed in EORTC QLQ-C30 (all domains) scores across time points or changes from baseline. Results of subgroup analyses by baseline characteristics will be presented. *Conclusions.* In patients receiving consolidation therapy with Zev, health-related QOL parameters as measured by EORTC QLQ-C30 and EQ-5D questionnaires were similar to those of patients receiving no further treatment. Treatment with Zev consolidation is efficacious while maintaining QOL in patients with advanced-stage follicular lymphoma responsive to first-line induction treatment.

0803

DETECTION OF ANTHRACYCLINE-INDUCED CARDIOTOXICITY WITH CARDIAC TROPONINS AND ECHOCARDIOGRAPHY

J.M. Horacek,[1] L. Jebavy,[1] M. Tichy,[2] R. Pudil,[1] A. Strasova,[1] P. Zak,[1] J. Maly[1]

[1]*Charles University Hospital, HRADEC KRALOVE;* [2]*Faculty of Military Health Sciences, HRADEC KRALOVE, Czech Republic*

Background. Anthracyclines (ANT) represent the greatest risk for development of cardiotoxicity which can influence the quality of life of cancer survivors. Echocardiography (ECHO) is the most frequently adopted method for detection of cardiotoxicity. Biochemical markers, especially cardiac troponins, have been studied in this context and the results are inconsistent. *Aims.* Assessment of acute and chronic cardiotoxicity of ANT with cardiac troponins - troponin T (cTnT, Roche), troponin I (cTnI, Randox) and ECHO. *Methods.* 23 acute leukemia patients (mean age 47.0±11.1 years, 14 males) treated with 3-6 cycles of ANT-based chemotherapy (CT) were studied. Cardiac evaluation was performed at the baseline (before CT), the day after first CT (cumulative ANT dose 135.8±28.5 mg/m^2), the day after last CT (cumulative ANT dose 472.1±115.0 mg/m^2) and 6 months thereafter (6 months after CT). The cut-off value for cTnT was 0.01 µg/L, for cTnI 0.40 µg/L. Systolic left ventricular (LV) dysfunction on ECHO was defined as LVEF less than or equal to 55%, diastolic LV dysfunction as E/A inversion and E-wave deceleration time above 220 ms. *Results.* The results are summarized in Table 1.

Table 1. Abnormal cardiac findings during anthracycline treatment and floow-up (n=23).

abnormal cardiac findings	before CT	after first CT	after last CT	6 months after CT
cTnT above 0.01 µg/L	0	0	0	3 (13.0 %)
cTnI above 0.40 µg/L	0	4 (17.4 %)	4 (17.4 %)	6 (26.1 %)
systolic LV dysfunction	0	1 (4.3 %)	3 (13.0 %)	5 (21.7 %)
diastolic LV dysfunction	1 (4.3 %)	4 (17.4 %)	6 (26.1 %)	10 (43.5 %)

Positivity of CTnI correlated with systolic and diastolic LV dysfunction on ECHO - (r=0.712; p<0.00001) and (r=0.591; p<0.0001), respectively. Patients with cTnI positivity during ANT treatment had a significantly greater decrease in LVEF during the follow-up compared to cTnI-negative patients (12.2±7.4% *vs* 3.3±4.2%, p=0.003). Two patients with early cTnI positivity during ANT treatment developed cardiomyopathy with symptoms of heart failure during the follow-up. Positivity of cTnT within 6 months after CT only coincided with LV dysfunction on ECHO and cardiomyopathy. *Conclusions.* Our results suggest that evaluation of cTnI - in contrast with cTnT - during ANT treatment could identify patients at risk for development of ANT-induced cardiomyopathy in the future. CTnI seems to be superior to cTnT in the early detection of cardiac injury associated with ANT treatment. In asymptomatic patients, abnormal cardiac findings during and after ANT treatment are considered subclinical cardiac toxicity and require further follow-up.

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0804

ECONOMIC EVALUATION OF LENALIDOMIDE FOR THE TREATMENT OF MULTIPLE MYELOMA IN WALES IN PATIENTS WHO HAVE RECEIVED AT LEAST ONE PRIOR THERAPY

H.B. Deniz,[1] K.J. Ishak,[1] D.R. Edwards,[2] A. Shearer,[3] P. Dale,[1] J.J. Caro[1]

[1]*United BioSource Corporation, CONCORD, USA;* [2]*Consultant Haematologist, ALAW UNIT, YSBYTY GWYNEDD, BANGOR, United Kingdom;* [3]*Celgene Limited UK & Ireland, WINDSOR, United Kingdom*

Background. Lenalidomide in combination with high-dose dexamethasone (Len+Dex), yields improved time-to-progression (TTP) and survival compared to Dex alone in patients with multiple myeloma who have received at least one prior therapy. *Aims.* This study aimed to estimate the long-term health and cost consequences of Len+Dex *vs* Dex in patients with multiple myeloma (MM) who have received either one prior therapy or ≥ two prior therapies. *Methods.* A discrete event simulation of a patient's disease course following initiation of Len+Dex or Dex was developed. The model uses patient's response (complete, partial, stable disease or progressive disease) and estimates corresponding TTP and subsequent survival based on Weibull functions derived from pooled data from two Phase III randomized clinical trials (MM-009/010). Long-term results from the UK MRC MM IV, V, VI, and VIII trials (1980 and 1997) and outcomes from published Mayo Clinic data (between 1985 and 1998) were used to estimate Dex survival, as 47% of Dex patients crossed-over to lenalidomide treatment in MM-009/010. Time dependent adverse event rates were derived from pooled MM-009/010 data and associated management costs reflective of NHS Wales were applied. Health utilities by response level were obtained from published literature. Patients remained on treatment until disease progression. Disease management costs were reflective of clinical practice in Wales. As recommended by the UK treasury, costs and health outcomes were discounted at 3.5% per annum in order to adjust to present values. Events and costs were considered over two years reflecting trial follow-up, while survival and quality adjusted life-years (QALYs) were modeled to end of life in order to avoid truncation bias. 1,000 patients were simulated per analysis.

Table 1.

One prior therapy group	**Undiscounted**		**Discounted**	
	Len+Dex	**Dex**	**Len+Dex**	**Dex**
Life Years (projected mean)	5.62	2.20	4.54	2.00
Quality Adjusted Life Years (QALYs)	3.94	1.52	3.20	1.39
Average Cost (per patient)	£55,872	£2,184	£54,499	£2,126
Incremental cost per Life Year Gained	£15,686		£20,617	
Incremental cost per QALY Gained	£22,177		£28,943	
≥ Two prior therapies group	**Undiscounted**		**Discounted**	
	Len+Dex	**Dex**	**Len+Dex**	**Dex**
Life Years (projected mean)	4.24	1.48	3.61	1.41
Quality Adjusted Life Years (QALYs)	2.92	1.05	2.50	1.00
Average Cost (per patient)	£45,146	£1,944	£44,169	£1,896
Incremental cost per Life Year Gained	£15,673		£19,218	
Incremental cost per QALY Gained	£23,104		£28,184	

Results. The use of Len+Dex is associated with a substantial improvement in survival and QALYs. While estimated incremental costs are significant, the improvements in health outcomes yield incremental cost-effectiveness ratios (ICERs) below £30,000 per QALY. Although the health outcome gains in the ≥ two prior therapies group are lower than in the one prior therapy group, the relative incremental costs are lower and thus the cost-effectiveness estimates are similar. Undiscounted ICERs are significantly lower because survival benefits are not fully realized until end-of-life and so are subject to a higher degree of compound

discounting than the costs, which are incurred relatively early. Univariate and probabilistic sensitivity analyses showed that results remain consistent through broad changes in key parameters. *Summary and Conclusions.* Regardless of the number of prior therapies, lenalidomide in combination with high-dose dexamethasone delivers significant improvements in quality-adjusted survival in a life-limiting orphan disease and yields an estimated incremental cost per QALY which falls within a cost-effective range.

0805

COST COMPARISON OF LIPOSOMAL AMPHOTERICIN B *VS* CASPOFUNGIN FOR THE EMPIRICAL TREATMENT OF INVASIVE FUNGAL INFECTIONS

W. Malyszczak,[1] B.L. Jones,[2] B. Jackson,[3] W. Malyszczak,[1] S. Agrawal,[4] M. Jensen[5]

[1]Gilead Sciences Limited, CAMBRIDGE; [2]Glasgow Royal Infirmary, GLASGOW; [3]Royal Victoria Infirmary, NEWCASTLE UPON TYNE; [4]St. Bartholomew's Hospital, LONDON; [5]Abacus International, BICESTER, UK

Background. Successful empirical treatment of invasive fungal infections depends on the degree of broad spectrum coverage provided by anti-fungal agents. Recently, there has been an increase in the number of rarer fungal species and those resistant to a range of anti-fungal agents, highlighting the ongoing need for cost-effective treatment options in this disease area. *Aims.* The aim of this study is to compare the per patient cost of using liposomal amphotericin B *vs* caspofungin for empirical treatment of invasive fungal infections as first- and second-line treatment strategies in the UK. *Methods.* Based on licensed drug doses for the empirical treatment of invasive fungal infections, a costing model was developed comparing liposomal amphotericin B *vs* caspofungin. The base case scenario assumed a treatment duration of 10 days with liposomal amphotericin B and 14 days with caspofungin, reflecting typical treatment durations in the UK as reported by the Health Protection Agency. Length of stay in hospital was assumed to equal treatment duration, while the risk of nephrotoxicity was based on published trial data (11.5% for liposomal amphotericin B and 2.6% for caspofungin). It was assumed that patients not responding to first line anti-fungal treatment switched treatment after three to five days. Susceptibility data and epidemiology data were derived from published literature. *Results.* The average daily cost of treatment was £804 with liposomal amphotericin B and £726 with caspofungin, including cost of hospitalisations. However, allowing for treatment switches, broad spectrum profiles and the inclusion of hospitalisation costs, the average cost over the entire treatment period ranged from £7,756 to £8,338 with liposomal amphotericin B and from £9,631 to £10,406 with caspofungin depending on the fungal distribution. The results are sensitive to changes in treatment duration; however, liposomal amphotericin B remains costs neutral when the difference in treatment duration is between one and two days. *Conclusions.* Although the average daily cost of treatment with liposomal amphotericin B is higher compared with caspofungin, the reduced treatment duration and the broader spectrum coverage associated with liposomal amphotericin B results in cost-savings over the entire treatment period.

0806

THE VIRTUAL SLIDE UK NEQAS(H) DIGITAL MORPHOLOGY PILOT SCHEME FOR CONTINUING PROFESSIONAL DEVELOPMENT (CPD)

M.L. Brereton,[1] J. Burthem,[1] J. Ardern,[1] B. De la Salle,[2] L. Hickman,[1] S. Ali,[3] F. Maringanise,[3] L. Seal,[4] P. McTaggart,[2] M. West,[2] W. Gilmore,[4] D. Swirsky,[5] J. Parker-Williams,[2] K. Hyde[1]

[1]Manchester Royal Infirmary, MANCHESTER; [2]UK NEQAS(H), WATFORD; [3]Salford University, MANCHESTER; [4]Manchester Metropolitan University, MANCHESTER; [5]Leeds Hospitals, LEEDS, United Kingdom

Background. UKNEQAS(H) collaborated with Manchester Royal Infirmary and Greater Manchester Universities to develop an internet based pilot scheme for Digital Morphology(DM). The DM scheme was registered with the Institute of Biomedical Science to enable laboratory staff to collect evidence and points towards Continuing Professional Development (CPD) for haematological morphology. The aim was to promote quality standards and improve consensus of morphology by education rather than assessment. *Methods.* In April 2005 UKNEQAS(H) invited participating centres of the conventional glass slide Morphology external quality assurance (EQA) scheme to submit a named individual to register for the DM pilot. In April 2006 the number of registrants was increased from 221 to 412 individuals from 14 countries (85% UK based). For each exercise two cases were released via the internet (four releases per year, sixteen cases in total). Cases consisted of multiple digital images from smears previously released as glass slide morphology EQA. Coded report sheets were placed on the Web (www.ukneqas-haem.org.uk). This pilot scheme closed in September 2007. *Results.* On average 51% of registrants completed the exercises (range 19%-69%). The majority (72%) spent <30 minutes reviewing each case but additional time on background reading. Cases included Haemoglobinopathies, D.I.C. and both chronic and acute leukaemias. Of those who gave feedback >70% stated the exercises had improved their awareness of the haematological conditions, <20% said their knowledge had not changed (variation depended upon clinical diagnosis). With reference to education registrant feedback was positive, cases were presented with relevant additional data (cell markers, cytogenetics, immunochemistry) and expert opinion highlighting the significance of specific morphological features e.g. appearance of granulation or nuclear structure. Participants stressed the usefulness of images for teaching and education purposes, particularly for rare haematological cases and for bone marrows. Incorporating feedback the collaboration blended (or stitched) sequential high power (x60 objective) quality images to create larger composite images (virtual slides). Appropriate software developed during the Scheme, allows users to move across images creating the feel of a microscope whilst maintaining high resolution. Users see exactly the same cells which are annotated for educational purposes. *Summary*. UKNEQAS(H) and the collaboration announce the launch of a new Scheme in 2008 which includes *virtual* slides, electronic reporting, immediate annotated educational feedback and consensus data. The DM Scheme is aimed at educating laboratory professionals who wish to demonstrate evidence of CPD for morphology. With the key theme of CPD promoting improvement to the quality of morphology the new Scheme has the potential for expansion across the UK and internationally.

0807

COST-EFFECTIVENESS OF POSACONAZOLE IN THE PROPHYLAXIS OF INVASIVE FUNGAL INFECTIONS IN THE FRENCH SETTING

A.L. Lafuma,[1] M. Michallet,[2] R. Herbrecht,[3] P. Ribaud,[4] J.P. Dupont,[5] J.P. Gangneux,[3] P. Moreau,[3] P. Berger,[6] A.K. O'Sullivan[7]

[1]Cemka Eval, BOURG LA REINE, France; [2]Hospices Civils, LYON, France; [3]CHU, STRASBOURG, France; [4]Hopital Saint Louis, PARIS, France; [5]Hopital Necker, PARIS, France; [6]Institut Paoli Calmettes, MARSEILLE, France; [7]I3 Innovus, MEDFORD, USA

Background. Acute myeloblastic leukemia (AML) and high risk myelodysplastic syndrome (MDS) patients experience prolonged neutropenia during treatment with intensive chemotherapy, leading to a high risk of potentially fatal invasive fungal infections (IFI). Posaconazole recently showed that it was more efficacious than standard azoles agents in the primary prevention of IFI in this population. The higher price of posaconazole could be a limit of its use in these patients in France. *Aims.* The present study estimated the cost-effectiveness of posaconazole *vs* standard azoles for the prevention of IFI in neutropenic patients in the French setting using modeling and a dedicated survey to estimate the costs of IFI. *Methods.* A decision-tree model was developed that starts with the choice of antifungal prophylaxis: posaconazole or standard azole treatment (fluconazole or itraconazole). The decision tree was estimated using data from the recently published prospective, randomized, double blind, multi-center trial that compared both treatments in neutropenic patients receiving remission-induction chemotherapy for AML/MDS (Cornely *et al.*, 2007). Following initiation of prophylaxis, clinical events are modeled with chance nodes reflecting probabilities of IFI, IFI related death, and death from other causes. It is assumed that patients surviving the prophylactic period will have a life expectancy that reflects that of the underlying condition. This allows translation of the trial outcomes to a lifetime horizon. Data on life expectancy, were obtained from the literature. Medical resource consumption and costs were obtained from results of the clinical trial and from a dedicated survey on the costs of treating IFI. This dedicated survey used a retrospective chart review design and collected information in 6 French hematology wards. Model outcomes include incremental cost per IFI avoided and incremental cost per life years saved. *Results.* IFI treatment costs were estimated on the analysis of 50 medical files of patients with proven and probable IFI occurring during chemotherapy with an average duration of follow-up from the occurrence of the IFI of 298 days. Societal costs directly related to IFI were estimated at 51,033 € including extra costs of index hospitalization, costs of antifungal therapy (curative and

secondary prevention) and additional hospitalizations related to IFI treatment. The healthcare costs for the posaconazole strategy amounted to €5,223 (€2,697 for prophylaxis and €2,526 for IFI management) which was €859 less than the €6,083 costs with standard azoles (€469 for prophylaxis and €5614 for IFI management). Results from a probabilistic sensitivity analysis indicate that there was a 80% probability that the prophylaxis posaconazole strategy was dominant (more efficacious and less costly) and 90% of incremental cost per life year gained was below €50,000 a commonly accepted threshold for cost-effectiveness. Additional scenario analyses with different assumptions confirmed these findings. *Conclusions.* Our economic evaluation demonstrated that posaconazole prophylaxis is a dominant strategy compared to fluconazole or itraconazole treatment in neutropenic AML/MDS patients after intensive chemotherapy in France

0808

THE ECONOMIC AND SOCIAL BURDEN OF MULTIPLE MYELOMA IN ITALY. THE CO.MI.M STUDY

M.T. Petrucci,[1] E. Calabrese,[2] V. Federico,[2] M. Ceccolini,[3] P. Falco,[4] A. Gozzetti,[5] R. Rizzi,[6] R. Foà[2]

[1]University La Sapienza Roma, ROMA; [2]Haematology, University La Sapienza, ROMA; [3]Haematology Seragnoli, BOLOGNA; [4]Ospedale San Giovanni battista, TORINO; [5]Policlinico Santa Maria Alle Scotte, SIENA; [6]Haematology, Policlinico, BARI, Italy

Background. The epidemiology of multiple myeloma (MM) is well known. However, few data are available on the impact of the disease on national healthcare expenditure and on society in a broader sense. This is especially-important because the prevalence of MM continues to increase in a progressively aging population. *Aims.* Measure resource utilization associated with MM management in terms of direct and indirect costs in a societal perspective. QoL data collection are included in the protocol. *Methods.* Anonymous, subject-level data on health care utilization and costs will be obtained on 200 subjects with MM, performing a cross-sectional, retrospective, prevalence-based study involving 5 Italian hospitals. Subjects will be recruited until June 2008, according to a stratified sample procedure. Three disease phases are considered in a distribution reflecting the real clinical practice: (a) 16% asymptomatic (watch and wait); (b) 14% subjects receiving an autotransplant; (c) 70% subjects receiving drugs both for MM and for co-morbidities. A specific questionnaire is submitted to all subject-cohorts. Social, demographic and clinical data are collected, as well as data on the actual volume of the resources used to manage MM and co-morbidities. Costs are identified with regard to: (1) drugs; (2) visits; (3) laboratory tests; (4) hospital admissions; (5) support devices; (6) home assistance; (7) travel; and (8) reduced productivity of patients and caregivers. These data are analyzed for the 12 months before recruitment. Health-related quality of life (QOL) is measured using EORTC QLQ-C30 questionnaires. This abstract reports data of an interim analysis of few variables in the first 40 subjects enrolled at the Hematology Institute at *La Sapienza* University in Rome. *Results.* The sample distribution was as follows: 5% asymptomatic patients, 12.5% autotransplanted, 82.5% patients receiving drugs for MM or co-morbidities. Hospitalizations occurred in 15% of subjects enrolled and the inpatients' total cost per year was 247,708 Euros, of which 67% for the autotransplants; the average hospital cost per patient was 41,284 Euros. The estimated annual cost of drugs was on average 14,332 Euros per subjects/year, overestimated in this interim analysis by a higher proportion of patients in the phase (c) and lower in phase (a) where no costs occurred. Finally, 25% of subjects reduced or stopped working losing up to 50% of productivity. *Conclusions.* These data indicate a substantial impact of MM on the Italian health care system. With additional data it should be possible to assess the cost of each disease-phase on the society. QoL related to the disease-phases will be reported.

0809

SYMPTOMS EVALUATION IN HEMATOLOGIC PALLIATIVE CARE PATIENTS ADMITTED TO AN HOSPTITAL-BASED HOME CARE PROGRAM

B. Breccia, C. Cartoni, M. Breccia, F. Efficace, G.M. D'Elia, G. Brunetti, S.G. Morano, E Finolezzi, M. Ribersani, E. Baldacci, R. Foa', F. Mandelli

Hematology, ROME, Italy

Background. Although the main goal of palliative care is to alleviate symptoms, at present, very little evidence exists on symptom burden and symptom severity in patients with haematological diseases. Thus, the objective of this study was to investigate the feasibility and the added clinical value of patient-reported assessment in a cohort of haematological patients admitted to an hospital-based home care (HBHC) program. *Methods.* Thirty patients with a diagnosis of haematological disease were admitted to a University HBHC. The overall sample consisted of 11 patients with a diagnosis of acute myeloid leukaemia, 7 with myelodysplasia, 5 with lymphoma, 4 with chronic lymphocytic leukaemia and haemolytic anemia, 2 with multiple myeloma, and 1 with myelofibrosis. Twenty-one patients were in advanced/terminal phase of disease, whereas 9 patients were followed for chronic disease in supportive therapy. Each patient completed the Edmonton Symptom Assessment Scale (ESAS) at 3 different time points (at enrolment, after 20-30 days, at last contact). The ESAS consists of nine visual analogue scales (ranging from 0 to 10) developed for use in assessing the symptoms of patients receiving palliative care. For all scales, lower values represent higher level of functioning. We also evaluated Karnofsky performance status (KPS) and a number of clinical and laboratory parameters at each time point. *Results.* At enrolment in the HBHC program, KPS correlated with higher transfusional requirements (p=0.011) and with depression (p=0.004). Tiredness correlated with AML/MDS (p=0.013), advanced phase of disease (p=0.016) and with supportive therapy (p<0.001). After a median of 20-30 days since enrolment, the second ESAS evaluation found a significant difference in well-being (p=0.012); from a baseline median score of 5 to 8 at the second evaluation. The analysis of ESAS parameters at last contact, compared to the first evaluation, found significant differences between pain (p=0.017) (relieved in terminal patients), more tiredness -independently from treatment and supportive transfusion needs- (p=0.05) and more nausea (p<0.001). Analysis of ESAS parameters with clinical findings revealed a correlation with pain and advanced phase of disease (p=0.031) and of anxiety and nausea with disease progression (the most frequent cause of death), respectively p=0.009 and p=0.001. Evaluation of KPS at last contact also correlated with disease progression and death (p=0.012). *Conclusions.* Evaluating patient's symptoms in an HBHC program is feasible and provide valuable outcomes to further improve understanding of disease progression and patients' burden with the disease and treatment. In addition, such routine evaluation in palliative care settings of patients with haematological diseases might also in turn improve efficacy of supportive therapy.

Stem cell transplantation - Autologous/DLI /miscellaneous

0810

OUTPATIENT AUTOLOGOUS STEM CELL TRANSPLANTATION IN ELDERLY PATIENTS (≥ 60 YEARS) WITH MULTIPLE MYELOMA

M. Montanari, I. Scortechini, A. Poloni, G. Gini, M. Offidani, D. Capelli, G. Mancini, S. Trappolini, P. Leoni

Ospedali Riuniti, ANCONA, Italy

Background. Multiple myeloma (MM) is a disease of the elderly with a median age at diagnosis of 70 years. Autologous stem cell transplantation (ASCT) is an effective standard treatment for MM patients. There are several studies about ASCT in outpatient basis but this procedure is characterized by a high probability of a second hospitalization (36-43%) and there are few data about feasibility in elderly patients. *Aims and Methods.* We started in December 2001 a programm of ASCT with early discharge (day +1) with the following inclusion criteria: performance status 0-1 (WHO), absence of severe comorbidities, availability of a caregiver, estimated current time to reach the transplant centre no more than 40 minutes and good compliance to homing therapy. All patients were analysed according to the intention criteria to perform an early discharge within 24 hours post stem cells reinfusion, with clinical and laboratory surveillance 3 times a week until the complete haematological recovery. The conditioning regimen was high dose Melphalan (200 mg/m^2) with previous infusion of Amifostine 750 mg; the patients transplanted until 2004 received the combination of granulocyte colony stimulating factor (G-CSF) 5 mcg/day plus erythropoietin alfa 10000 UI/day starting from day +1 until the complete haematological reconstitution; the others received pegylated G-CSF 1 fl and darbepoietin 500 mcg 1 fl at the day +1. *Results.* Twenty-eight elderly patients underwent to 45 ASCT. The median age at the transplant was 64 (60-74) years; 48% patients were over 65 and 4 patients (8%) over 70 years. The median of CD34$^+$×10^6/kg cells reinfused was 5.8 (1.2-12.8) with a median of 10 (8-14) days observed for absolute neutrophil count >500/mcl and 12 (10-27) days for platelets > 20000/mcl. The median of transfusion requirement was 0 both for red cell and platelet units. Severe mucositis (grade 3-4 WHO) was observed in only 4 cases (8%) and neutropenic fever (>38°C) in 5 (11%); the incidence of FUO was 17%. In 8 transplants (17%) we observed a second hospitalization: 4 for infection, 2 for FUO and 2 for mucositis. Transplant-related mortality at day +90 was 0%. *Conclusions.* Our experience shows that ASCT in outpatient regimen is safe and feasible in elderly patients with MM with a negligible severe extrahaematological toxicity and a probability of a second hospitalization less than observed in previous studies.

0811

TARGETING THE POOR MOBILIZING POPULATION OF PATIENTS FOR AN AUTOLOGOUS TRANSPLANTATION PROCEDURE: A SINGLE CENTRE INSTITUTION EXPERIENCE

A. Marco,[1] A. Sureda,[1] P. Madoz,[1] G. Martín-Henao,[2] S. Brunet,[1] R. Martino,[1] J. Briones,[1] D. Valcárcel,[1] J. Delgado,[1] J. Sierra[1]

[1]Hospital de Sant Pau, BARCELONA; [2]Banc de Sang i Teixits, BARCELONA, Spain

Background. Peripheral blood (PB) has become the major source of hematopoietic stem cells for autologous stem cell transplantation (ASCT) in the last 15-20 years. Nevertheless, there is a subset of patients who do not mobilize adequate numbers of CD34+ cells. There are no clearly established guidelines about second-line mobilization protocols. *Aims.* The aim of this study has been to analyze our experience as a single centre with this population of poor mobilizers trying to a) identify clinical or biological predisposing factors for not mobilizing enough progenitor cells into PB, b) results with second line mobilization protocols and c) outcome after the ASCT of those patients who could be autografted in terms of haematological recovery. *Methods.* Poor mobilizing patients were defined as those in whom the apheresis procedure could not be started because of <10 CD34$^+$ cells/ul or those in which a number of at least 2×10^6 CD34$^+$ cells/kg could be collected in the first mobilization attempt. *Results.* From January / 2000 to January / 2008, 126 patients [70 males / 56 females, median age of 53 years (range, 20-70)] out of a total number of 450 patients mobilized for an ASCT (28%) were identified as poor mobilizers. Clinical diagnosis were: 29 multiple myeloma, 16 Hodgkin's lymphoma, 48 non-Hodgkin's lymphoma, 28 acute leukemias and 5 others. Median time from diagnosis to mobilization therapy was of 19 (range, 3-120) months and median number of therapies received before the procedure was 2 (range, 0-5). The first mobilizing protocol was G-CSF alone (5-10 ug/kg/day sc) in 72% of the patients or the combination of chemotherapy plus G-CSF 28% of the patients. A second mobilization procedure was attempted in 34 patients (28%) with high-doses of G-CSF alone (16-20 ug/kg/day sc) in 24 patients, the combination of G-CSF plus chemotherapy in 8 patients and the combination of G-CSG with stem cell factor (SCF) in 2 patients. A third mobilization attempt was performed in 6 patients (high-doses of G-CSF alone in 4 patients, G-CSF plus chemotherapy in 1 patient and G-CSF plus SCF in 1 patient). Sixty-nine patients (54%) were finally autografted. Median number of CD34$^+$ cells/kg infused were 2.15×10^6/kg (range, 1.01-4.00). Median time to neutrophil recovery was 11 days (range, 4-20). *Summary*. Patients with an inadequate mobilization constitute a significant clinical problem (25% of the whole population of patients with an indication of ASCT in our centre). Nevertheless, half of these patients can be rescued for an ASCT procedure with one or two more attempts. Neutrophil recovery after the autologous transplant in those patients undergoing the procedure seems to be similar to that of the group of patients with an adequate first mobilization attempt. New mobilizing agents should be investigated in order to increase the efficacy of the mobilization processes.

0812

PURGING AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN FOLLICULAR MALIGNANT LYMPHOMA PATIENTS: EXPERIENCE AT EUROPEAN INSTITUTE OF ONCOLOGY, MILANO

S. Bassi, C. Rabascio, E. Cocorocchio, P. Bertazzoni, F. Gigli, C. Massaro, A. Alietti, L. Calabrese, S. Steffanoni, D. Laszlo, G. Martinelli

European Institute of Oncology, MILAN, Italy

Background. In the era of monoclonal antibodies the role of autologous stem cell transplantation (ASCT) in the management of follicular lymphoma (FL) is still debated. *Aims.* To evaluate the safety and efficacy of myeloablative therapy with rescue of purged or unpurged harvests in FL pts.*Methods*. At our institution form 1997 to 2007 35 pts with FL were eligible for ASCT. Twenty-eight pts were resistant/relapsed following previous treatment and 7 were newly diagnosed. Before high dose therapy they received 2-4 cycles of CHOP-like regimen, followed by Cyclophosphamide 4g/mq to mobilize the stem cells (SC). After SC collection the pts underwent 3 cycles of subcutaneous Cladribine at a daily dose of 0,14 mg/Kg for Day 1-5 every month. The conditioning regimen was based on Mitoxantrone 60 mg/mq + Melphalan 180 mg/mq, followed by SC re-infusion 24-hours later and G-CSF starting 24 hours after re-infusion. *Results.* In 25 pts the SC underwent purging: in 15 harvests the CD34$^+$ were selected by immunomagnetic beads, while in the other 10 pts, only Rituximab was used as *purging in vivo*agent. The remaining 10 pts received unpurged SC. Before ASCT 12 pts were in complete response (CR), 16 in partial response (PR) and 2 in stable disease. Two pts were not eligible for ASCT because of progressive disease (PD). The remaining 32 pts were eligible for ASCT. The engraftment was at a median of 10 days for leucocytes and 12 days for platelets (>20.000/mmc), with a delay of one day in the pts, who received purged SC. Grade 3 mucositis was described in 9 pts. During aplasia a 40% infection rate was reported, without differences between pts with purged or unpurged SC. One patient in CR presented myelodysplastic syndrome at 18 months from ASCT. After ASCT 29 pts were in CR, one in PR and 2 pts were not valuable (both died before response assessment). Twelve pts in CR showed PD at a median time of 17 months from ASCT. With a median follow up of 5 years (range 1month -10 years), 22 pts are alive and 17 (53%) in CR. Ten pts died, 5 for progressive disease and 5 for treatment-related causes; in particular 7 of them received in-vitro purged SC. *Conclusions*. Our chemotherapy regimen, which included the purine analogue Cladribine in the induction phase, seems safe and feasible. The high rate of CR reported and the sustained freedom from progression up to now, makes such modality of treatment a valid option principally in relapsing FL patients. In our experience, the addition of a monoclonal antibody as part of treatment confirms its role *in vivo purging* without observing an increased incidence of infection.

0813

THE PREDICTIVE VALUE OF FDG-PET/CT AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

F. Sorà,[1] S. De Matteis,[2] P. Di Nardo,[2] P. Chiusolo,[2] L. Laurenti,[2] V. Rufini[2] G. Leone,[3] S. Sica[2]

[1]Policlinico A. Gemelli, Roma; [2]Policlinico a. Gemelli, ROMA; [3]Policlinico A. Gemelli, ROMA, Italy

Background. Autologous stem cell transplantation (ASCT) is a standard treatment for refractory or relapsed lymphoma. Response to treatment is usually assessed by computed tomography (CT). Residual mass may persist after treatment and CT is unable to differentiate between fibrosis and active tumour. [18F]FDG-PET, a functional imaging scanning, is a reliable method to discriminate between active lymphoma tissue and fibrosis. In order to optimize lymphoma restaging, the fusion of these two imaging methods has been tested and new data are now generated using PET/CT scanner procedure. *Aims.* The aim of this study is to evaluate the role of PET/CT to detect residual disease or early relapse after ASCT in order to design treatment intensification or complementary radiotherapy. *Methods.* We enrolled 35 patients (21 males and 14 females, median age: 48 years, range 15-66) affected by lymphoma (10 large B cell, 8 follicular, 5 mantle cell, 3 anaplastic CD30, 3 peripheral T cell, 1 marginal zone lymphoma, 1 angioimmunoblastic T cell lymphoma and 4 Hodgkin's lymphoma). Patients were homogeneously treated with salvage chemotherapy and received ASCT, respectively 15 in CR, 16 in PR, and 4 SD. PET/CT was performed prospectively on day +100 after ASCT. *Results.* FDG-PET/CT was negative in 24 patients; 22 are alive in CR (median follow-up 22.5 months, range 8-27), 2 died in CR 8 and 9 months after ASCT respectively from CMV/Pneumocystis Carinii pneumonia and from sudden death with no evidence of lymphoma. One patient had local relapse at 7 months requiring treatment and is alive at 8 months after ASCT. Eleven patients were FDG-PET/CT positive, respectively 7 in NHL group and 4 in HD group. NHL patients who had positive scans were mainly affected by DLBCL (3/7), follicular (2/7), anaplastic CD30+ (1/7) and mantle(1/7). In this group 2/7 patients went on to have local relapse/progression at 3 months after transplant and required further treatment. Both patients died from disease progression at 16 and 22 months respectively after ASCT; 2 patients in PR received radiotherapy on involved fields. These patients are alive at 11 and 27 months after ASCT in CR. Three patients did not relapse at a median of 17 months (range 10-22). Biopsy was obtained in one patient and it was consistent with inflammatory lesion. In HD group 2/4 patients relapsed/progressed at previously involved sites at a median of 3 months after ASCT and died at 5 and 10 months. One patient received local radiotherapy and is alive in CR 17 months after ASCT. One patient received haploidentical SCT and is alive in CR 9 months after the procedure.OS rates were 90.9 % 1 and 2 years after ASCT in PET-negative patients. OS rates were 83.9 % and 62.9% at 1 and 2 years respectively in PET-positive patients (p=ns); PFS rates were 100% at 1 and 2 years respectively in PET/CT-negative patients, meanwhile PFS rates were 66.7 % at 1 and 2years in PET/CT -positive patients (p=0.0037) (Figure 1). *Conclusions.* In our experience FDG-PET/CT positivity is strongly associated with lower PFS (log rank test 0.0037), but not with OS. Interestingly radiotherapy was able to induce durable CR in some of them.

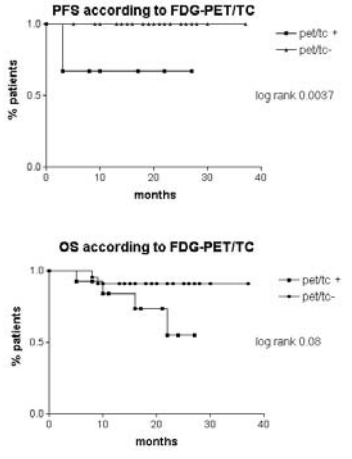

Figure 1.

0814

DIABETES MELLITUS & AUTOLOGOUS BONE MARROW DERIVED PROGENITOR CELL TRANSPLANT (ABMD-PCT). THE "CONZI'S EFFECT". COOPERATIVE STUDY URUGUAY - MEXICO

E. Novoa,[1] F. Perez Chavez,[2] R. Morales Aceves,[3] M. Soto Valdez,[2] M.A. Medina,[4] J. Ravera,[5] A. Perez Chavez,[6] R. Cazares,[7] R. Estela,[8] C. Olivet,[8] A. Ortega,[2] R. Caride[5]

[1]Ministry of Health, MONTEVIDEO, Uruguay; [2]UANL, Servicios Medicos, MONTERREY, Mexico; [3]Universidad de Guadalajara, PUERTO VALLARTA, Mexico; [4]Cellther Program, MONTEVIDEO, Uruguay; [5]Police Hospital, MONTEVIDEO, Uruguay; [6]UANL, Anestesiology Dept., MONTERREY, Mexico; [7]UANL, Patologia Clinica, MONTERREY, Mexico; [8]Hospital de San Carlos, SAN CARLOS, Uruguay

Background. diabetes mellitus accounts for more than 250 million people all around the world. It represents a pandemia, with important consequences over the health systems.*Aims.* 1) improve the quality of life in type 1 and 2 diabetic patients (methabolic control, dose reduction or suspention of hypoglucemiant drugs) and quality of life indexes, 2) evaluate other aditional therapeutic effects and 3) evaluate side effects of the procedure (ABMD-PC transplant). *Methods.* from july 2004 to january 2008, 165 diabetic patients were evaluable to be included on this protocol. 85 men and 80 women. Median age was 67 years old (8-86). 65 patients from Uruguay and 100 from Mexico. Type 1, 30 patients and type 2, 135. All the patients signed informed consent.Local anaesthesia was employed in 155/165 patients with xilocaine 2% (harvest and transplantation in the gastrocnemius muscle). Mobilization with filgrastim was employed, 5 ug/kg/ weight daily (two doses) before transplantation (48 hs). Unmanipulated autologous bone marrow derived progenitor cells were injected in one of the lower limbs in 2 mL aliquots. Mean harvest volume was 2,8 mL/kg/body weight. The mean number of transplanted mononuclear cells was $2,4\times10^9$/kg body weight.The control population was the group of transplanted patients during the 6 months before ABMD-PC transplant. Each patient was regularly (monthly) evaluated for glicemia, A1c-hemoglobin, C peptide, and body mass index (BMI). *Results.* procedure mortality rate was 0%. The only complication of this treatment was local hemathoma in the transplanted leg (4.25%). 85% of type 2 and 44% of type 1 diabetic patients discontinued the oral hipoglucemiants or insulin after 120 days post ABMD-PC transplant, for more than 6 months. *Conclusions.* Autologous bone marrow derived progenitor cell transplant, by the Conzi's effect, can be performed safely and appears to be a benefical complementary therapy for human diabetes mellitus. www.cellther.org.

0815

PREDICTIVE VALUE OF PRE-HARVEST BONE MARROW ASPIRATION IN ORDER TO ESTIMATE THE FINAL CD34+ STEM CELLS YIELD.

F. Benedetti, A. Andreini, F. Mosna, M. Sorio, S. Ledro, D. De Sabata, C. Tecchio, G. Ruggeri, R. Di Bella

Bone Marrow Transplant Unit, VERONA, Italy

Introduction. The number of bone marrow (BM) stem cells collected for allogeneic bone marrow transplantation depends on the total volume of BM collected and on the number and quality of stem cells. It depends also on the collection team skill and on bone characteristics, like fibrosis or poor cellularity. About 20mLof BM/Kg of donor weight are usually needed for a transplant, but the real number of the $CD34^+$ cells remains difficult to predict, because it is unknown the number of stem cells in the BM aspirate during the harvest. Since the count of $CD34^+$ cells in the harvested BM is not immediately available, we tried to verify if the mononucleated cells (MNCs) and $CD34^+$ cells number in a pre-harvest BM aspirate is predictive of the real stem cells yield. We know that about 1% of donors are *poor donors*. In these cases even a further peripheral stem cells collection after G-CSF is not able to reach a sufficient number of stem cells. A *poor donor* is very often a *poor mobiliser*. In most cases this means graft failure and patient death. *Methods*. From September 2004 to October 2007 fifty-three healthy volunteer donors underwent BM harvesting for bone marrow transplantation. BM samples for MNCs and $CD34^+$ cells count were collected in EDTA Vacutainers (K2E) before BM harvest in operating room. From each pre-harvest sample we evaluated the volume, the MNCs and the $CD34^+$ cells count; 100ÌL of BM (about 10^6 cells) were stained with CD45 FITC and CD34 PE; isotype matching mAbs were used as negative controls. After red blood cells lysis, samples were acquired by FACScan flow cytometer (Betcon Dickinson BD Biosciences) equipped with a 488-nm argon laser. Data

were analyzed with CellQuest software (Becton Dickinson BD Bioscences) according to ISHAGE protocol. These parameters were correlated with final MNCs and $CD34^+$ cells yield after BM harvest. The collection team was maintained the same during the study. *Results*. In pre-harvest aspirate, the number of CD34+ cells strictly correlated to aspirate cellularity (r=0,91). Besides, there was a good statistical correlation between the cellularity and $CD34^+$ cells count in the pre-harvest aspirate and the final $CD34^+$ cells yield (r=0,66). On the other hand, in harvest bag, the relationship between $CD34^+$ calls and cellularity was not so clear (r=0,53). But the most important thing, in two cases a very low count in the pre-harvest aspiration was followed by an insufficient number of cells in the collection bag ($<1\times10^8$ MNCs/Kg, $<1\times10^6$ $CD34^+$/Kg b.w.). In both cases the patients died because of graft failure. *Conclusions*. Based on this experience, a bone marrow aspirate done before BM harvest could be an easy test, recommended in order to predict the cells yield and to avoid a graft failure due to insufficient number of stem cells. From the ethical point of view, a BM aspiration may be considered a little inconvenience for the donor if it can avoid serious adverse events in a small percentage of patients.

0816

TIME-EXTENDED MULTI-AGENT CONDITIONING PRIOR TO AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR REFRACTORY OR RELAPSED HODGKIN'S LYMPHOMA - A LONG-TERM FOLLOW-UP OF A PILOT STUDY

D. Kata, D. Kata, S. Giebel, S. Grosicki, L. Kachel, M. Sadus-Wojciechowska, M. Krawczyk-Kulis, J. Wojnar, J. Holowiecki, S. Kyrcz-Krzemien

Silesian Medical University, KATOWICE, Poland

Background and Aims. Autologous peripheral blood stem cell transplantation (autoPBSCT) is widely used for the treatment of poor-risk patients with Hodgkin's lymphoma (HL), however, the choice of the most appropriate preparative regimen remains unclear. We assumed that prolonged conditioning may fit better to the biology of the disease. Therefore we modified commonly used BEAM and CBV protocols distributing the total doses to 9 days. The goal of this analysis was to evaluate safety and efficacy of those time-extended regimens. *Patients and Methods.* 32 pts. (18 males and 14 females, median age: 28 years, range 17-63) with relapsed HL were included in this study. Previous therapy consisted of 1-6 lines of treatment and up to 32 chemotherapy cycles. At the time of autoPBSCT, all patients were in CR‡2, PR or NR after relapse. 13 patients received P-BEAM (procarbazine, BCNU, etoposide, cytosine arabinoside and melphalan), whereas 19 pts. were treated with ChOPP-CBV (chlorambucil, vicristine, procarbazine, prednisone, cyclophosphamide, BCNU, etoposide) as a conditioning therapy. In the P-BEAM group the median $CD34^+$ cell dose infused was 3.9×10^6/kg b.w. (range 1.5-9.1), while the patients in the ChOPP-CBV group received the median of 4.8×10^6/kg b.w. $CD34^+$ cells (range 0.9-34.2). *Results.* 1/19 pts. died due to septic complications in ChOPP-CBV group, whereas no procedure related mortality was observed among pts. treated with P-BEAM. All remaining patients engrafted with the median time to ANC >0.5 G/L and PLT >50 G/L recovery of 15 (10-45) days and 16 (9-131) days, respectively. Severe adverse events (mucositis or infection, grade III/IV according to WHO classification) occurred in 23% of pts. after P-BEAM and 32% of pts. after ChOPP-CBV, respectively. Both groups did not differ in terms of time of hospital stay, days of intravenous antibiotics nor the demand for blood-derived products. With the median follow-up of 5.5 years, the probability of overall survival at 6 years equaled 86% for P-BEAM and 53% for ChOPP-CBV group (p=0.08). The probability of progression-free survival was 73% and 46%, respectively (p=0.11). *Conclusions.* Time-extended P-BEAM and ChOPP-CBV protocols followed by autoPBSCT are well-tolerated and effective salvage therapies for pts. with advanced HL. Prolonged administration of the therapy seem to be appropriate for this group of patients. The long-term results obtained in the P-BEAM group appear particularly encouraging.

0817

DONOR LYMPHOCYTE INFUSION USING BONE MARROW T CELLS IS SUPERIOR TO THAT USING SPLEEN T CELLS IN INDUCING CHIMERIC CONVERSION IN ALLOGENEIC MIXED CHIMERISM FOLLOWING MHC-MISMATCHED NONMYELOABLATIVE BONE MARROW TRANSPLANTATION

S.G. Cho, H.S. Park, M.J. Park, S.Y. Min, J.W. Lee, W.S. Min, J.W. Park, H.Y. Kim, C.C. Kim

Catholic University of Korea, SEOUL, South-Korea

Background. In clinical practice, donor lymphocyte infusion (DLI) has been often used to augment GVL effect in patients with posttransplantation leukemic relapse. Unfortunately, it seemed to be closely associated with GVHD, depending on the degree of major histocompatibility complex (MHC) disparity. In murine model of Allogeneic BMT, DLI in mixed chimeras have showed more potent GVL effects in spite of the absence of GVHD compared in complete chimeras. In this study we evaluated the potential of BM-T cells (Thy1.2+) for the chimeric conversion in early post-transplant period of allogeneic mixed chimerism. *Methods.* Allogeneic mixed chimerism was prepared as follows. BALB/c (H-2kd) were injected ip with anti-NK treatment on day -1. On day 0, they received TBI at a dose of 5 Gy, followed by intravenous infusion of 2×10^7 T-cell-depleted (TCD) BM cells from C57BL/6 (H-2kb). DLI using various sources such as $CD4^+$ and $CD8^+$ spleen cells, unmanipulated spleen and BM cells, isolated spleen T (SP-T) and BM-T cells, and cryopreserved BM-T cells. The degree of chimeric conversion was evaluated on peripheral blood and spleen by flow cytometry. GVHD was evaluated by clinical GVHD scores and pathologic examinations. *Results.* Donor BM-T cells facilitated conversion to full donor chimerism without GVHD, but not donor SP T cells. BM-T DLI group (1×10^6 cells) showed a higher level of donor chimerism in PB compared with SP-T LI group; 99.8% *vs* 59.7 % of H-2kb , 99.3% *vs* 32.5 % of $CD4^+$ cells, 99.3 % *vs* 48.8 % of $CD8^+$ cells, respectively. BM-T group showed complete chimeric conversion with self-limited GVHD and no pathologic changes. However, SP-T group showed persistent mixed chimerism with pathologic changes of GVHD in liver & gut. The proliferative response of donor BM-T cells to mount a proliferative response in a standard MLR was higher than that of donor SP-T cells against spleen cells of allogeneic mixed chimeric mice. *Conclusions.* This study suggested that the characteristics of BM-T cells are quite different to that of peripheral T cells (SP-T cells). BM T cells were more potent to induce chimeric conversion with small dose of DLI compared with spleen cells. Cryopreserved BM T cells obtained during TCD procedure might be effectively used to consolidate donor dominant chimerism in clinical practice without concerns of GVHD.

0818

THE ROLE OF PRE-TRANSPLANTATION SERUM IRON PARAMETERS AND ANTI-OXIDATIVE POTENTIAL IN EARLY POSTTRANSPLANT TOXICITIES

G.T. Sucak, S.Z. Aki, H. Pasaoglu, Z.N. Ozkurt, C. Demirtas, Z.A. Yegin, M. Yagci

'Gazi University Faculty of Medicine, ANKARA, Turkey

Introduction. Iron overload (IO) is a frequent condition in hematopoietic stem cell transplantation (HSCT) recipients. IO is associated with free radical generation and tissue damage which can increase toxic and infectious events early after HSCT. Free iron acts as a free radical catalyser and may aggravate toxic effects of the conditioning regimen. We retrospectively evaluated the clinical impact of pretransplantation iron status and anti- oxidative potential on early transplant related toxicities and overall survival. *Patients and Methods.* We analyzed 149 patients [52 women, 97 men; mean age 36±14,5 (range 16- 68)] who underwent allogeneic (n=97) or autologous HSCT (n=52) in our institution between September 2003 and October 2007. Serum iron parameters [transferrin saturation (TS), ferritin, nontransferrin- bound iron (NTBI), hepcidin)], interleukin- 6 (IL- 6), interleukin- 1β (IL- 1β), malondialdehyde (MDA), and antioxidant parameters [total radical antioxidant parameter (TRAP), glutathion, glutathion peroxidase (GP), superoxide dismutase (SOD)] were measured in pretransplant serum samples. *Results.* There was an inverse correlation between serum levels of NTBI *vs* SOD and ferritin *vs* GP which leads to a pro-oxidant state (p=0,019, r=-0,197 and p=0,05, r=-0,163). Increased pretransplant serum ferritin levels were associated with the higher grades of NCI toxicities including mucositis, hepatotoxicity, pulmonary toxicity and prolonged neutrophil/ platelet engraftment days (p=0,035, p=0,003, p=0,028, p=0,0001 and p=0,0001 respectively). In patients with hepatotoxicity there was a positive correlation with serum

TS, ferritin and MDA levels (p=0,0001, p=0,003 and p=0,026 respectively). NCI grade of pulmonary toxicity was positively correlated with serum ferritin and NTBI levels (p=0,028, r=0,186 and p=0,03, r=0,181) and inversely correlated with GP levels (p=0,035, r=-0,174). Duration of fever was positively correlated with ferritin levels (p=0,002, r=0,252) and inversly correlated with GP (p=0,0001, r=-0,338) and hepcidin levels (p=0,002, r=-0,256). Neither of these parameters were correlated with the development of acute graft *vs* host disease. In logistic regression analysis serum NTBI levels were an independent risk factor the development of sinusoidal obstruction syndrome (SOS) (p=0,03). Serum ferritin and hepcidin levels were associated with a decreased overall survival (OS) as an independent risk factor for the first 100 day mortality (p=0,034, p=0,035 respectively). In bivariate cox regression analysis serum NTBI, GP, hepcidin and IL- 6 were associated with a decreased OS (p= 0,084, p=0,05, p=0,025 and p= 0,034 respectively). In multivariate analysis GP and hepcidin remained significant as an independent risk factor for OS (p=0,04 and p=0,08 respectively). In our study group first 100 day mortality was 17, 4 %. With a median follow- up time of 13,04 months (range 0,03- 50,85 months) estimated OS was 59,7 5 % and progress free survival 41,36 %. *Conclusions.* IO is a frequent complication of hematological diseases requiring stem cell transplantation and plays an important role in early posttransplant toxicities. In our study IO was inversely correlated with antioxidant parameters which leads to pro-oxidant state. These patients develop early posttransplant toxicities with higher NCI grades which might be associated with early posttransplant mortality. Increased serum NTBI levels were also associated with the development of SOS and higher NCI grades of pulmonary toxicity as a potential important risk factor for early posttransplant mortality. Iron overload seems to be a potential risk factor for both the development of transplant related toxicities and early posttransplant mortality. Serum ferritin levels, transferrin saturation or NTBI levels seems to be a usefull parameter in predicting early transplant related toxicities. Iron chelation, phlebotomy + erythropoietin and antioxidant treatment might have potential benefits in modifying early post transplant complications and increasing overall survival. Prophylactic approaches in high risk patients with elevated serum iron parameters, might as well be effective in ameliorating posttransplant outcomes.

0819

BAFF LEVELS IN HAEMATOPOIETIC STEM CELL TRANSPLANTATION

L. Yáñez, L. Yáñez, M. Castañeda, M. Peña, A. Bermudez, A. Iriondo

Hospital Universitario Marqués de Valdecilla, SANTANDER, Spain

Background. B-lymphocyte activating factor (BAFF), belongs to the tumour necrosis factor (TNF) ligand super family and is expressed by macrophages, dendritic cells and neutrophils especially when there is a proinflamatory stimulation. High levels of this protein are heavily associated with B-cell function and development, but have also seen in B-cell neoplasm (Hodgkin and Non Hodgkin Lymphoma, and myeloma) and autoimmune diseases. *Aims.* Compare differences in BAFF expression between control subjects, and patients who underwent autologous (AUTO) or allogeneic (ALO) stem cell transplantation. Analyze in allogeneic patients if there is an association between high levels of BAFF expression and graft versus host disease (GVHD) development. *Patients and Methods.* BAFF plasma levels were analyzed by ELISA test in 19 subjects. Six were healthy control, 5 patients underwent autologous stem cell transplantation (4 NHL, 1 myeloma; three patients were in partial response and 2 in complete remission) and 8 patients underwent allogeneic stem cell transplantation (2 CLL, 1 myeloma, 1 ALL, 4 AML; one patient had a refractory disease, 1 had a partial response and 6 patients were in complete remission). In patients who underwent stem cell transplantation BAFF determinations were realized before conditioning, in day of stem cell infusion (day 0 of transplant), weekly during first 4 weeks, and then monthly. *Results.* 109 samples were analyzed. Patients who underwent stem cell transplantation, showed high levels before conditioning (AUTO 1142 pg/mL and ALO 655 pg/mL) compared with healthy subjects (155 pg/mL), p<0,05. We didn't find differences in plasma levels between AUTO and ALO transplants before conditioning, and during the first two months after stem cell infusion. Six patients developed GVHD, and they have had higher levels of BAFF in the day of stem cell infusion (1467 pg/mL *vs* 294 pg/mL , p=0,021), and median of two first weeks (2126 pg/mL *vs* 698 pg/mL, p=0,016) and three first weeks (2031 pg/mL *vs* 862 pg/mL, p=0,037). *Conclusions.* In our study, we have found that BAFF expression is elevated in autologous and allogeneic stem cell transplantation, compared with normal subjets. High levels of expression before conditioning in autologous transplantation can be associated with disease activity. Finally, our results suggest that a higher plasma BAFF expression during the first month may be related with GVHD development.

0820

LIMBIC ENCEPHALOPATHY AND OTHER CENTRAL NERVOUS COMPLICATIONS AFTER REDUCED INTENSITY CONDITIONING STEM CELL TRANSPLANTATION

M. Uzunov,[1] S. Lapusan,[2] T. Storme,[2] S. Wittnebel,[3] C. Boccaccio,[3] J.H. Bourhis,[3] J.P. Marie,[2] B. Rio[2]

[1]Hopital Pitié-Salpetriere, PARIS; [2]Hotel-Dieu, PARIS; [3]Institut Gustave Roussy, VILLEJUIF, France

Background. Transplantation following reduced intensity conditioning (RIC) results in lesser transplant related morbidity and mortality, due to shorter duration of neutropenia, earlier immune reconstitution and lesser regimen related toxicity. Previous clinical series in adults reported central nervous system (CNS) complications occuring in more 37% patients after standard HSCT. Reported incidence after RIC HSCT varies between 11% and 44%. *Aims.* We aimed to evaluate the nonrelapse CNS complications in patients receiving RIC HSCT for various hematologic malignancies *Methods.* We have retrospectively reviewed the medical records of 131 consecutive patients transplanted in two from November 1999 to December 2005. *Results.* Underlying disease was AML- 45patients, NHL-16 patients, myeloma-26 patients, CLL-13 patients, MDS-10 patients, HL-7 patients, ALL- 6 patients, CML- 5 patients, amylosis-1 patient, myelofibrosis -1 patient, Waldenstrom-1patient. 124 patients received a fludarabine based conditioning regimen associated to TBI2Gy, Busulfan, ATG, Endoxan, Ida, Ara-C or Melphalan. The total dose of fludarabine varied between 90 and 200 mg/m^2, according to each conditioning regimen. 91 patients (69.4%) received SCT from an identical sibling, 20 patients (15.2%) from an MUD and 17 patients (12.9%) received un unrelated cord blood. Immunosuppression consisted in CSA, MMF and/or short course MTX. Plasma cyclosporine concentration was monitored regularly. Diagnostic of CNS complications was based on clinical, neurophysiological, radiological and microbiological findings. A total of 15 patients (11.4%) developed various CNS complications with a median onset of 21 days (range 8-160). Subtypes comprised infectious, cerebrovascular , metabolic and unknown complications. Symptoms included seizures, impaired consciousness, headache, nausea, vomiting hemiparesis and coma. In all, 3 patients had cerebral toxoplasmosis, 4 patients cerebral hemorrhage, 2 patients presented hemiparesis and amyotrophy after prolonged intensive care, 1 patient presented a pyamidal syndrome of unkown etiology, 1 patient impaired consciousness due to metabolic disturbances and 4 patients developed limbic encephalitis (the clinical picture associated lethargy, cognitive dysfunction with severe memory disturbance, seizure and psychiatric symptoms - personality change, irritability). Limbic encephalopathy occurred in 2 patients receiving RIC CBT (11.7% - Minneapolis fludarabine-based reduced intensity conditioning), 1 MUD and 1 IS HSCT and was directly correlated in univariate analysis with fludarabine dose, age, renal failure or antifungal treatment during conditioning. Nine of the 15 patients (6.8%) died directly of neurologic complications. *Conclusions.* CNS complications remain a significant problem after RIC HSCT and are associated with poor survival rates.

0821

RAPID MOBILIZATION OF CD34$^+$ CELLS AND HEMATOPOIETIC ENGRAFTMENT POST AUTO HSCT FOLLOWING ADMINISTRATION OF CXCR4 ANTAGONIST AMD3100 WITH HIGH DOSE (HD) G-CSF IN PATIENTS FAILING HD G-CSF MOBILIZATION

C. D'Cunha, C. Chiriva-Internati, D. Kolb, E. Cobos

Texas Tech University, Health Science Center, LUBBOCK, USA

Background. AMD3100 (Plerixafor) is a CXCR4 antagonist that induces rapid mobilization of CD34$^+$ cells in healthy volunteers. *Aims.* Aim of this study is to see the effect of AMD3100 plus HD G-CSF with respect to CD34$^+$ cell yield and myeloid and platelet engraftment in patients who mobilize poorly with HD G-CSF alone. *Methods*. Sixty-one patients underwent hematopoietic stem cell collection for treatment of various malignancies. Six patients failed adequate collections in spite of HD G-CSF mobilization (20 µg/Kg). Fifty-five patients that mobilized well with HD G-CSF alone were used as control for comparison in terms of CD34$^+$ cells collected and myeloid/platelet engraftment. *Results.* All six patients who failed initial mobilization with HD G-CSF (20 µg/Kg) alone had adequate mobilization after using AMD3100 plus HD G-CSF to proceed to transplantation. At present, five out of six patients underwent trans-

plantation. Among patients who initially failed to mobilize adequately, the average number of $CD34^+$ cells collected using HD G-CSF alone was 0.74×10^6 $CD34^+$ cells/Kg compared to 1.7×10^6 $CD34^+$ cells/Kg after using AMD3100 plus HD G-CSF mobilization regimen on these patients. All five transplanted patients received pooled hematopoietic stem cells, consisting of cells collected prior to the AMD3100 mobilization plus cells collected after using AMD3100 plus HD G-CSF mobilization in order to assure adequate cell dose for transplantation. The difference between the average number of apheresis procedures required for stem cell collection using high dose G-CSF mobilization alone *vs* AMD3100 plus HD G-CSF was found to be insignificant (4.33 *vs* 4). However, the number of $CD34^+$ cells collected per apheresis was significantly higher with AMD3100 plus HD G-CSF mobilization (0.425×10^6 $CD34^+$cells/Kg) *vs* HD G-CSF alone (0.17×10^6 $CD34^+$ cells/Kg). Days for myeloid and platelet engraftment among patients mobilized with AMD3100 plus HD G-CSF was compared with fifty-five autologous transplanted patients who collected well with HD G-CSF alone and hence served as control. The mean days to myeloid and platelet engraftment for patients receiving AMD3100 plus HD G-CSF mobilized cells was 11.6 and 15.6 days respectively while the mean days to myeloid and platelet engraftment for control patients transplanted with HD G-CSF mobilized cells alone was 12.13 and 14.6 days respectively. The mean $CD34^+$ cell dose infused at transplantation in fifty-five control patients (mobilized with HD G-CSF alone) was 2.92×10^6 $CD34^+$ cells/Kg *vs* 2.31×10^6 $CD34^+$ cells/Kg in five patients who were mobilized using AMD3100 plus HD G-CSF. Four of six patients who received AMD3100 experienced mild local reactions. No patients discontinued AMD3100. *Conclusions*. AMD3100 appears to be effective for rapid mobilization of $CD34^+$ cells among patients who failed to mobilize using HD G-CSF alone. Patients, who were other wise not eligible for autologous transplantation due to poor mobilization, may mobilize adequately using AMD3100 plus HD G-CSF. The average number of $CD34^+$ cells collected per apheresis using AMD3100 plus HD G-CSF was better. Days to myeloid and platelet engraftment was comparable to that of control (HD G-CSF alone). Further studies in large number of patients are warranted.

0822

SAFETY AND FEASIBILITY OF POSACONAZOLE AS ORAL ANTIFUNGAL PROPHYLAXIS FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN 10 PEDIATRIC PATIENTS UNDER 12 YEARS OF AGE

M. Doering, I. Müller, P. Lang, R. Handgretinger

University Children's Hospital, TUEBINGEN, Germany

Background. Pediatric patients, who are undergoing allogeneic hematopoietic stem cell transplantation (HSCT), have an increased risk to acquire fungal infections due to immune suppression associated with HSCT. Posttransplant immune suppression, viral infections and acute graft-versus-host disease, are known risk factors for fungal infections. Thus, antifungal prophylaxis on the ward and during the early prost-transplantation period is indicated. There is only insufficient data available for pediatric patients regarding the most adequate antifungal oral prophylaxis. Patients on prophylaxis with itraconazole, voriconazole and fluconazole break-through fungal infections occurred. In consequence, we were looking for alternative treatments. Posaconazole is an oral azole with *in vitro* activity against a wide spectrum of fungi, including candida, aspergillus, fusarium and zygomycetes by inhibition of the cytochrome P450-dependent enzyme 14α-demethylase. *Aims.* Acquisition of safety and feasibility data for the use of posaconazole in pediatric patients under the age of 12 following high dose chemotherapy and stem cell transplantation. *Methods.* 10 patients (age range 1-9 years, mean 5.6 years) were treated with 2x5mg/kg b.w. per day after HSCT. The patient group consisted of pediatric patients with malignant diseases (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia and neuroblastoma) one child with myelodysplastic syndrome, one with Tay-Sachs' disease and one child with Chediak-Higashi syndrome. We analyzed the effects and side effects of posaconazole during the first hundred days after transplantation. Once per week we analyzed the serum levels of candida antigene and aspergillus galactomannan antigen. We screened evaluated the number of granulocytes, lymphocytes, and monocytes, the liver enzymes AST, ALT and GGT, bilirubin and C-reactive protein. Typical pro-inflammatory cytokines such as IL-6, IL-8 etc. were determined as well. *Results*. The number of granulocytes, lymphocytes and monocytes did not chance in connection with the treatment with posaconazole. The liver enzymes AST, ALT and GGT as well as the direct and indirect bilirubin showed no significant changes during the treatment with posaconazole. Posaconazole was well tolerated by most patients, side effects included itching and nausea in two cases. None of the 10 children acquired proven or probable fungal infection during the observation period until day +100. Serum levels of candida and galactomannan antigens remained normal. *Conclusions*. Posaconazole at 2x5mg/kg b.w. daily was a safe oral antifungal prophylaxis in pediatric patients under the age of 12 years, who underwent high dose chemotherapy and HSCT. Future analyses will include blood serum levels and tissue levels where available. This will allow for more precise dosing regimens in these select patients and furture controlled trials.

0823

CD95L-EXPRESSING ANTIGEN-PRESENTING CELLS PREVENT T CELL ACTIVATION OF NAÏVE T CELLS AND INDUCE APOPTOSIS IN ACTIVATED T CELLS

G. Strauss, H. Kasperczyk, S. Fulda, K.-M. Debatin

University Children's Hospital, ULM, Germany

Background. Death inducing ligand CD95L is predominantly expressed on activated T cells and renders T cells cytotoxic. However CD95L expression can also be detected on antigen-presenting cells (APC) and can be increased by viral infections such as CMV or HIV infections. Although APC are the most potent inducers of T cell activation, they can also function in antigen-specific tolerance induction. *Aims.* Here, we analyzed how the expression of CD95L by APC influences an immune response and whether CD95 triggering can interfere with T cell activation. *Methods.* A HLA Class I negative EBV-transformed B cell line was stably transfected with HLA-A1 and CD95L, which was constitutively expressed on the cell surface due to a mutation in the metalloprotein cleavage site. Simultaneously, a mock transfectant was established. In T cell activation assays we demonstrated the influence of CD95L on T cell activation by analyzing the expression of activation markers, proliferation, cytokine secretion, Ca-release, tyrosine-phosphorylation and activation of transcription factors. *Results.* In long-term human T cell cultures the constitutive presence of m-CD95L and the alloantigen HLA-A1 on APC prevented the expansion of $CD4^+$ and $CD8^+$ HLA-A1-specific T cells. Since human T cells develop CD95-sensitivity only after several days of stimulation and naïve T cells are constitutively CD95-resistant, we determined the effect of m-CD95L expressing APC on activated and naïve T cells. The presence of CD95L induced apoptosis in activated T cells immediately after contact. Naïve T cells, however, survived but antigenic proliferation was inhibited. Inhibition of proliferation was associated with a reduced expression of activation markers, a decrease in calcium mobilization, a reduction in tyrosine phosphorylation and NF-AT activation and a decreased cytokine secretion. Naïve T cells once silenced by CD95L expressing APC could not be induced to proliferate by repeated antigen triggering in the absence of CD95L. Non-responsiveness could be reversed by the addition of IL-2 indicating that simultaneous triggering of TCR and CD95 induces anergy. *Summary*. Thus, m-CD95L expressing APC probably exhibit a dual function; they induce apoptosis in CD95 positive, activated T cells and suppress the activation of naïve T cells and render them anergic. Since CD95L expression can be detected on several subsets of activated and virally infected APC *in vivo* it might represent an additional mechanism for immune evasion of pathogens.

0824

ALLOGENEIC BONE MARROW TRANSPLANTATION WITH CYCLOPHOSPHAMIDE AND LOW DOSE ALEMTUZUMAB FOR SEVERE APLASTIC ANEMIA

H. Bittencourt,[1] F. Lodi,[1] G. Fischer,[2] L. Fogliatto,[2] R. Lamego,[1] G. Veloso,[1] A.K. Vieira,[1] G. Magalhaes,[1] S. Magalhaes,[1] M.C. Coutinho,[1] R. Licinio,[1] M. Capra,[2] L. Borges,[1] A.F. Tiburcio,[1] A.V. Macedo[1]

[1]Hematopoietic Stem Cell Transplantation Unit, BELO HORIZONTE; [2]Hematopoietic Stem Cell Unit, PORTO ALEGRE, Brazil

Background. Allogeneic bone marrow transplantation (AlloBMT) with cyclophosphamide (Cy) and antithymocyte globulin (ATG) as conditioning regimen is the treatment of choice for young patients with severe aplastic anemia (SAA). In developing countries, and particularly in Brazil, ATG costs make its use difficult in alloBMT for SAA patients. A commonly low-cost conditioning used in Brazil, low dose busulfan (BU) with Cy, is still associated with a higher rate of rejection, especially in heavily transfused patients. (BMT 2004:33;9-13) Recently, alemtuzumab was reported as an alternative to ATG for SAA patients (BBMT 2004: 10; 867-76) with similar activity and a lower cost. *Aims.* In order to study the effect of the combination of Cy 200 mg/kg and alemtuzumab 60mg, we review alloBMT for SAA performed in the last year at a reference University Hospital in Southwest Brazil. *Methods.* Between April 2007 and February 2008, 7 patients with SAA (defined by Camitta criteria) underwent an alloBMT at Hospital das Clinicas da UFMG (n=6) and Santa Casa de Porto Alegre (n=1). Median age at transplantation was 27 (range 15-42) years. All patients have a positive CMV serology Median number of transfusion was 13 (range 11-67). All patients received an unmanipulated bone marrow graft as stem cell source and all but one patient were transplanted with an HLA-identical related sibling. Median number of nucleated cell infused was 2.67(range 1.65-3.84)$\times 10^8$/kg. Cyclosporin (CSA) alone (n=6) or in combination with methotrexate (n=1) were used as GVHD prophylaxis. *Results.* 6/7 patients presented neutrophil engraftment with a median time to >0.5$\times 10^9$ neutrophil/L of 22.5 (range 19-26) days. Platelet recovery (>20$\times 10^9$ platelets/L) also occurred in 6/7 patients with a median time of 16 (range 9-23) days. Acute GVHD was observed in just one patient (grade II). None of the 3 patients alive 100-days after alloBMT presented chronic GVHD. Four patients presented CMV reactivation. With a median follow up of 119 days, only one of the 7 transplanted patients died of alloBMT complications. This patient died 31 days after transplant of gastrointestinal infection without neutrophil engraftment (but with platelet recovery). *Summary*. In conclusion, combination of cyclophosphamide and low dose alemtuzumab is well-tolerated and effective in SAA patients undertaken alloBMT. A longer follow up is required, however, to properly evaluate late rejection and chronic GVHD incidences.

0825

MEASUREMENT OF DURABILITY OF RESPONSE IN PATIENTS WITH SKIN GVHD BEYOND SIX MONTHS TREATMENT WITH EXTR CORPOREAL PHOTOPHERESIS: EVALUATION OF LONG-TERM THERAPY ON SURVIVAL

Y. Sorour, P.C. Taylor

Rotherham General Hospital, ROTHERHAM, United Kingdom

Background. Chronic graft versus host disease (GVHD) is one of the major limitations to successful allogeneic haemopoeitic stem cell transplantation (HSCT) with a substantial impact not only on survival but also on the quality of life of otherwise cancer free patients. Chronic GVHD is a complex disease, and its diagnosis, definition, staging and therefore criteria to evaluate response are particularly challenging. Extracorporeal photopheresis (ECP) is a cell based immuno-modulatory therapy involving the separation of leucocyte-rich plasma followed by *ex vivo* administration of a photosensitiser and ultraviolet A radiation before re-infusion. *Aims*. Measurement of durability of skin response to ECP beyond 28 weeks together with overall survival (OS) in patients with chronic skin GVHD post allogeneic HSCT. *Methods*. The ECP unit at Rotherham General Hospital acts as a supra-regional referral centre, accommodating approximately one half of all UK ECP referrals for treatment of chronic GVHD. In this study we retrospectively evaluated 45 patients referred between 1996 and 2008 to our ECP unit who completed at least 28 weeks of treatment. All patients had various degrees of skin GVHD following allogeneic HSCT for various haematological disorders. Results. On completion of 28 weeks of ECP treatment 33 out of the total 45 patients were deemed to have achieved >25% skin response. Three of the 33 responders were classified as possible responders as they had a steroid reduction >50% despite a skin response <25%. All 33 responders continued to be treated with ECP beyond 28 weeks. We compared the degree of skin response of this group at 56 and 112 weeks to that achieved at 28 weeks of ECP treatment. At timescale 56 weeks 6 patients had to be discounted from evaluation,(2 having stopped, 1 having data missing and 4 still undergoing treatment but 56 weeks not reached), 2 patients had a worse response, 12 patients had no change in response and in 12 patients the response had improved. At timescale 112 weeks 27 patients had to be discounted from evaluation, (21 having stopped, and 6 still undergoing treatment but 112 week not reached), 2 patients had a worse response, 2 patients had no change in response and in 2 patients the response had improved. For the 33 responders the mean duration of further treatment post 28 weeks was 59 weeks and the mean further follow-up period was 166 weeks. Out of all 33 responders 24 had stopped ECP treatment reasons being that 15 improved not requiring further ECP treatment, 2 showed lack of response, 4 had disease relapse and 3 died. Overall survival in the group of responders was 76%, compared to 67% in the non responders. *Conclusions*. Chronic GVHD has a direct impact on survival following allogeneic HSCT and quality of life. Our study shows that ECP produces a durable GVHD response, improves OS as well as improvement of quality of life with alleviation of symptoms.

Stem cell transplantation - GvHD/graft rejection/infection

0826

CELL CYCLE AND IMMUNE-RELATED PROCESSES ARE SIGNIFICANTLY ALTERED IN CHRONIC GRAFT-VERSUS-HOST DISEASE

S. Park,[1] S.J. Oh,[2] S.B. Cho,[3] S.-H. Park,[3] C.Z. Piao,[3] S.M. Kwon,[3] I. Kim,[3] S.S. Yoon,[3] B.K. Kim,[3] E.K. Park,[4] J.J. Kang,[5] S.-J. Yang,[5] W.J. Lee,[6] C.-H. Yoo,[6] S. Hwang,[6] S.H. Kim,[6] J.H. Kim[3]

[1]Seoul National University Hospital, SEOUL; [2]Kangbuk Samsung Hospital, SEOUL; [3]Seoul National Unversity College of Medicine, SEOUL; [4]Chung-ang University College of Medicine, SEOUL; [5]Macrogen Inc., SEOUL; [6]Digital Genomics Inc., SEOUL, South-Korea

Background. The pathogenesis of chronic graft-versus-host disease (GVHD) has not been fully elucidated. In the post-genomic era, microarray technology plays a key role in whole genome analysis. *Aims*. To uncover the molecular characteristics underlying chronic GVHD, we analyzed the gene expression profiles of allogeneic hematopoietic stem cell transplantation (HSCT) recipients. *Patients and Methods*. Twenty one patients who received allogeneic HSCT were evaluated for gene expression profiles using UniSet Human 20K I Bioarray (Amersham Biosciences) and CodeLink Expression Analysis Software v4.1 (GE Healthcare Life Science). Results. Self organized map (SOM) clustering showed that the entire expression profiles of chronic GVHD samples were clearly different from those of the non-GVHD samples, and significance analysis of microarray (SAM) demonstrated that 120 genes, including PTDSS1, VAV1 and CD3D, were up-regulated, and 5 genes, including calnexin, were down-regulated in chronic GVHD patients. Gene ontology annotation revealed that these genes were related to the phosphorus metabolism and lipid biosynthesis. Quantitative real time polymerase chain reaction (qRT-PCR) experiments validated the up-regulation of PTDSS1, VAV1 and CD3D in separate samples. Pathway-wise global test revealed that differential gene expression profiles in cell cycle and T cell immune-associated pathways were significantly different between GVHD patients and non-GVHD patients. Seventeen classifier genes selected using PAM (prediction analysis of microarray) algorithm showed favorable performance (prediction accuracy=0.85) for identifying patients with chronic GVHD. Conclusions. We identified differentially expressed genes and pathways in chronic GVHD patients using microarray analysis. We also selected diagnostic genes for chronic GVHD patients.

0827

EXTRACORPOREAL PHOTOCHEMOTHERAPY (ECP) IN THE TREATMENT OF STEROID REFRACTORY ACUTE OR CHRONIC GRAFT VERSUS HOST DISEASE (GVHD); SINGLE CENTRE EXPERIENCE

E. Juvonen, L. Volin, A. Nihtinen, F. Ebeling, H. Uotinen, T. Ruutu

Helsinki University Central Hospital, HELSINKI, Finland

The mechanism by which ECP affects GVHD is not fully understood and its place in the treatment of GVHD is not established. We have treated with ECP 18 patients with steroid-refractory acute GVHD (Grade II 1, Gr III 12, and Gr IV 5) and 25 patients with chronic GVHD. All patients except one with aplastic anaemia were transplanted for a haematological malignancy. In patients with aGVHD the conditioning was myeloablative (MA) in 12 and non-myeloablative in 6 cases. The donor was a sibling for 9 and an unrelated for 9 patients including one cord blood (CB) graft. The graft was harvested from blood (PB) with the exception of the CB graft and one bone marrow (BM) graft from a sibling donor. AGVHD occurred on day 24 (median, range 6-59) post transplant. As the first line treatment all patients received high dose methylprednisolone. Second line treatments included ATG in 9 patients, infliximab in 14 patients with gut GVHD, and pentostatin in 8 patients. In addition, 4 patients were on mycophenolate mofetil. In one patient ECP was the second line treatment. ECP was performed 2 times / week but not on consecutive days. The first ECP was done on day 22 (4-104) after the dg of aGVHD. The median number of ECPs / patient was 5 (1-22). 5 patients, 4 with gut + skin and 1 with gut aGVHD treated with 4-22 ECPs, were alive with the median follow-up of 802 days (497-1554). 13 patients died in the median of 155 (50-370) days post transplant. The cause of death was transplant related in 12 patients and relapse in one patient. In 25 patients with cGVHD the median time from transplantation to the first ECP was 3.2 (0.6-7.5) years. The donor was related in 17 and an unrelated in 8 cases; graft was BM in 14 and PB in 11 cases. The conditioning was MA with one exception. In 17 patients the main indication for ECP was sclerodermal skin GVHD. In two patients the indication was lung, in one patient mouth, and in 5 patients multiorgan problems. One treatment consisted of two ECPs on consecutive days. The first six treatments were performed with 2-week intervals and thereafter once a month. The median number of treatments / patient was 15 (2-20). In six patients the response to ECP could not be evaluated. In 9 / 19 evaluable patients the response was good (CR or VGPR), in 8 patients partial, and in 2 patients minimal or there was no response. Sklerodermal skin GVHD responded best, while the response of mouth and lung symptoms was minimal. Immunosuppressive drugs could be reduced but not stopped during ECP therapy. During the follow-up all medications of two patients were discontinued successfully. *Conclusions.* ECPs were well tolerated in both patient groups. In steroid refractory aGVHD ECP should be studied as second line treatment. In the present patients chronic sclerodermal skin and acute gut GVHD showed the best response to ECP.

0828

DOWNREGULATION OF L-SELECTIN EXPRESSION ON DONOR CD4$^+$ T CELLS USING G-CSF CORRELATES WITH A LOWER INCIDENCE OF GRADES II-IV ACUTE GVHD IN PATIENTS AFTER HLA-MISMATCHED/HAPLOIDENTICAL BLOOD AND MARROW TRANSPLANTATION

Ch. Chang, X.-Y. Zhao, M.-R. Huo, X.-J. Huang

Peking University Institue of Hematology, BEIJING, China

Background. Graft-versus-host disease (GVHD) remains a significant complication of human leukocyte antigen (HLA)-matched/mismatched allogeneic hematopoietic stem cell transplantation (allo-HSCT). Cell adhesion molecules (CAMs) play a role in the migration donor T cells and regulation of graft-versus-host disease (GVHD). We, therefore, hypothesized that the expression of CAMs on donor T cells could also be modulated after *in vivo* granulocyte colony-stimulating factor (G-CSF) application, which possibly lead to a relative lower incidence of acute GVHD after HLA-mismatched/Haploidentcal blood and marrow transplantation. *Aims*. To obtain insight in which CAMs, including very late antigen 4 (VLA-4), intercellular adhesion molecule-1 (ICAM-1), L-selectin, and lymphocyte function-associated antigen-1 (LFA-1), may participate in the homing of donor T cells after HLA-mismatched/haploidentical blood and marrow transplantation. *Methods*. The expression of VLA-4, ICAM-1, L-selectin, and LFA-1 was measured on donor T cells using multi-color flow cytometry either in bone marrow grafts or in peripheral blood stem cell grafts before and after G-CSF treatment. The number of T cells expressing these CAMs present in G-CSF-primed bone marrow grafts (G-BM) and G-CSF mobilized peripheral blood stem cell grafts (G-PB) was quantified and correlated with incidence of aute GVHD in 28 patients after transplantation. *Results*. Compared to patients with grades 0-I acute GVHD, the numbers of CD4$^+$CD62L$^+$ cells, and CD4$^+$CD45RA$^+$CD62L$^+$ cells infused/kg recipient weight were significantly higher in patients with grades II-IV acute GVHD (p=0.042, and 0.025, respectively). The counts of CD4$^+$CD45RO$^+$CD62L$^+$ cells infused/kg recipient weight was also higher in patients with grades II-IV acute GVHD (p=0.053). When L-selectin expressing CD4$^+$, naïve and memory CD4+ T cells were used as parameters, the thresholds were cacluated to be 29.69×10^6 CD4$^+$CD62L$^+$ cells/kg, 20.90×10^6 CD4$^+$ CD45RA$^+$CD62L$^+$ cells/kg, and 16.12×10^6 CD4$^+$CD45RO$^+$CD62L$^+$ cells/kg, respectively. The expression of VLA-4, ICAM-1, and L-selectin on CD4$^+$ and CD8$^+$ T cells, including naïve and memory T cells, in bone marrow grafts was significantly lower after G-CSF treatment than before. LFA-1 was expressed at a significantly lower percentage on CD4$^+$ T cells in G-BM than that in steady-state bone marrow grafts. Mobilization with G-CSF decreased the median expression of L-selectin on CD4$^+$ T cells, naïve and memory CD4$^+$ T cells with marginal significance compared with those in steady-state peripheral blood. Compared with G-PB, the expression of ICAM-1 and LFA-1 on both type of CD4$^+$ T cells in G-BM was significantly lower. The expression of L-selectin on naïve and memory CD8$^+$ T cells as well as the expression of LFA-1 on both type of CD8$^+$ T cells were also significantly lower than those in G-PB. *Conclusions*. The results suggest that G-CSF treatment of healthy donors decreases the expression of L-selectin on CD4$^+$ T cells, which may be related to the lower incidence of grades II-IV acute GVHD in patients after HLA-mismatched/Haploidentical blood and marrow transplantation.

0829

A DUAL ROLE FOR HOST B CELLS AFTER REDUCED INTENSITY CONDITIONING TRANSPLANTATION: ATTENUATION DURING ACUTE GVHD AND INITIATION OF CHRONIC GVHD?

J.H.E. Kuball,[1] S. van Dorp,[2] F. Pietersma,[2] M. Wölfl,[3] H.M. Lokhorst,[2] E. Petersen,[2] L. Verdonck,[2] M. Theobald,[1] E. Meijer[4]

[1]UMC Utrecht, UTRECHT, Netherlands; [2]University Medical Centre, UTRECHT, Netherlands; [3]Children's Hospital, University of Würzburg, WÜRZBURG, Germany; [4]Erasmus Medical Centre, ROTTERDAM, Netherlands

Background. Chronic graft-versus-host-disease (cGVHD) is the major cause of late morbidity and mortality after allogeneic stem cell transplantation. B-cells have been reported to be involved in mediating cGVHD. *Aims and Methods.* To assess whether pre-emptive host B-cell-depletion prevents extensive cGVHD after allogeneic reduced intensity-conditioning-transplantation (RICT), 166 patients treated with RICT for various haematological diseases, who have or have not received host B-cell-depletion within 6 month prior to RICT, were analyzed retrospectively. *Results.* Host B-cell-depletion selectively reduced extensive cGVHD significantly from 46.4% to 18.3%. However, once acute GVHD (aGVHD) grade III-IV occurred in B-cell-depleted patients, it occurred earlier and was associated with a higher aGVHD-related mortality. *Conclusions.* Host B-cells influence both, aGVHD and cGVHD. We hypothesize that host B-cells protect the host during aGVHD from early onset and death, but also initiate cGVHD. Thus, timing of B-cell-depletion in RICT in order to reduce cGVHD needs to be carefully considered.

0830

RECIPIENT CHIMERISM IN THE T-CELL LINEAGE IS INDICATIVE OF IMPENDING GRAFT REJECTION IN PEDIATRIC PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

T. Lion,[1] S. Breuer,[2] H. Daxberger,[1] M. König,[1] G. Fritsch,[1] U. Pötschger,[1] A. Lawitschka,[3] C. Peters,[3] S. Matthes[3]

[1]Children´s Cancer Research Institute, VIENNA; [2]St. Anna Children´s Hospital, VIENNA; [3]St.Anna Children´s Hospital, VIENNA, Austria

Early diagnosis of impending graft rejection after allogeneic hematopoietic stem cell transplantation (HSCT) is crucial for timely onset of therapeutic measures to prevent graft loss. We have investigated whether close surveillance of lineage-specific chimerism in children undergoing HSCT permits assessment of the risk of graft rejection. A total of 228 pediatric patients with malignant and non-malignant diseases who underwent HSCT at our center between January 1997 and December 2006 were monitored for cell subset-specific chimerism at short intervals during the post-transplant period. Different fractions of peripheral blood leukocytes including CD33, CD15, CD56, CD4, CD8, and CD19 positive cells were isolated by FACS and lineage-specific chimerism patterns were evaluated by STR-PCR or FISH analysis. Of 191 patients eligible for analysis, a total of 19 individuals experienced graft rejection between days +20 and +157 after transplantation (median +63). All patients showing either persistent complete recipient chimerism or increasing recipient chimerism with ≥90% cells of recipient origin in the T-cell compartment rejected the graft, unless sustained mixed chimerism or donor chimerism could be induced by donor leukocyte infusions (DLI). By contrast, no rejections have occurred in patients exhibiting pure donor chimerism or stable mixed chimerism within the T-cell population. Our data suggest that close monitoring of chimerism within the T-cell lineage permits timely risk assessment of impending graft rejection in children undergoing allogeneic stem cell transplantation and may therefore provide a basis for appropriate therapeutic interventions.

0831

REDUCED-INTENSITY CONDITIONING IS ASSOCIATED WITH SHORTER DURATION OF CHRONIC GVHD THAN MYELOABLATIVE CONDITIONING AND PROVIDES VERY GOOD QUALITY OF LIFE FOR LONG-TERM SURVIVORS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

A. Shimoni, I. Hardan, N. Shem-Tov, A. Rand, E. Ribakovski, R. Yerushalmi, A. Nagler

Chaim Sheba Medical Center, TEL-HASHOMER, Israel

Background. Reduced-intensity conditioning (RIC) has been increasingly used over the last decade as a curative approach for patients not eligible for myeloablative conditioning (MAC). RIC allows consistent engraftment and reduces toxicity of allogeneic stem-cell transplantation (SCT). However, the long-term effects are less defined. *Aims.* This analysis was designed to determine the long-term outcome after RIC, and in particular the duration of immunosuppressive therapy (IST) needed and quality of life of long-term survivors. *Methods.* We analyzed the results of 61 patients given RIC from 1/2000 to 1/2003, such that survivors have at least 5 year follow-up, and compared them to 50 patients given MAC during the same period. *Results.* The RIC group included older patients than the MAC group, median age 49 (range, 16-65) and 36 (range, 18-65), respectively (p=0.001). The MAC group included more patients with acute myeloid leukemia/MDS (54% *vs* 25%, p=0.005) while patients with myeloma were given RIC exclusively (28% of the RIC group, p=0.001). 48% of patients in the RIC group and none in the MAC group had a prior autologous SCT (p=0.001). There was no difference in donor type and stem-cell source. After a median follow-up of 6.2 years (range, 5.0-7.9) 48 patients are alive, 25 after RIC and 23 after MAC with estimated survival of 44% (95CI, 31-57) and 45% (95CI, 30-59), respectively (p=NS). Long-term survival with RIC was achieved across all diagnoses. Chronic GVHD occurred in 30 patients after RIC and 32 after MAC with cumulative incidence of 51% (39-66) and 66% (53-81), respectively (p=0.08). 16 of 30 patients with chronic GVHD after RIC were eventually able to stop IST, 12 died on IST (relapse-8, non-relapse mortality (NRM)-4) and only 2 of 25 long-term survivors were still on IST at last follow-up. The median duration of IST was 18 months and the cumulative probability of stopping IST after 5 years (with relapse been competing risk) was 72%. In the MAC group 11 of 32 patients with chronic GVHD were able to stop IST, 10 died on IST (relapse-8, NRM-2) and 11 of 23 long-term survivors were still on IST at last follow-up. The median duration of IST was 45 months (p=0.01) and the cumulative probability of stopping IST after 5 years was 40% (p=0.007). Two women gave birth in the RIC group while 2 men in the MAC group fathered children spontaneously. There was one secondary malignancy in the MAC group and none in the RIC group. Two patients in the MAC group sustained myocardial infarction (one fatal) compared to none in the RIC group. One patient in the RIC group had reversible nephrotic syndrome. *Conclusions.* Long-term survival is similar after both RIC and MAC, however IST is significantly shorter after RIC and quality of life seems better. Overall, all 25 long-term surviving (>5 years) after RIC sustained excellent quality of life and only two still required IST. The mechanism of more rapid achievement of transplantation tolerance with RIC requires further investigation. These observations merit further confirmation in larger scale registry studies.

0832

VIDEO-CAPSULE ENDOSCOPY IN THE MANAGEMENT OF ACUTE GASTRO-INTESTINAL GRAFT-VERSUS-HOST DISEASE: FIVE YEARS OF EXPERIENCE

J.-B. Micol,[1] V. Maunoury,[2] F. Jourdain,[1] V. Coiteux,[1] L. Terriou,[1] J.-F. Colombel,[2] J.-P. Jouet,[1] I. Yakoub-Agha[1]

[1]Maladies du Sang, LILLE; [2]Maladies de l'appareil digestif, LILLE, France

Background. Acute gastro-intestinal graft-versus-host disease (GI-GVHD) is a major complication following allogeneic stem cell transplantation (allo-SCT) and results in high morbidity and mortality. Diagnosis of GI-GVHD is problematic due a lack of specific symptoms and confounding variables in allo-SCT patients. Although diarrhea is the most common (but non-specific) presenting symptom in acute GI-GVHD, diagnosis is especially difficult when the diarrheal disorder is atypical (i.e. when there is no or limited skin involvement). In a previous study, we reported the positive impact of wireless video-capsule endoscopy (VCE) in the diagnosis of post-transplant diarrhea. *Aims.* Here, we report our experience over the last 5 years with an overall diagnostic approach (including VCE) to the management of allo-SCT patients with suspected acute GI-GVHD. *Methods*. In addition to wireless VCE, patients with atypical post-transplant diarrhea underwent bacterial and viral investigations and upper and/or lower GI-tract endoscopy (plus biopsies, as appropriate). VCE images were scored according to standard endoscopic classification. The final diagnosis took account of the results of the investigation as a whole and the response to therapy. *Results.* Between August 2002 and October 2007, 240 patients underwent allo-SCT. Thirty-two underwent extensive investigation, with VCE being performed in the following situations: febrile and/or hemorrhagic diarrhea (n=17), isolated diarrhea (n=10), persistent diarrhea or relapse despite appropriate adjustment of immunosuppressive (IS) treatment (n=5). Median time between allo-SCT and VCE was 37 days (range: 19-197). The diagnosis was acute GVHD (n=14), viral infection (n=8, with

5 CMVs and 3 HHV6s) and a combination of both in 2 cases. The result of our approach was negative in 8 patients (all with a normal GI tract in VCE) who were ultimately diagnosed as having functional diarrhea and recovered without any specific treatment. We observed 5 (15%) VCE failures, either due to an absence of intestinal passage (n=3) or major GI hemorrhage (n=2). In the other cases, VCE results were concordant with the final diagnosis. It was noteworthy that VCE was superior to biopsies in some cases. Thus, while VCE demonstrated typical GI-GVHD lesions in 7 patients with histological proven GI-GVHD, VCE showed a normal GI tract (n=4) or GI-GVHD features in 7 other cases where the biopsies were uncertain (n=5) or non-contributive (n=2). The response to appropriate treatment was favorable in 20 cases but was unfavorable and required further therapeutic adjustment in 8 cases (7 GVHDs, 1 CMV). Four patients died of GVHD (n=3) or HHV6 infection (n=1). *Conclusions.* this study confirms that VCE is a more sensitive investigative method than GI-endoscopy. This approach enhanced our ability to modulate IS treatments in patients suffering from atypical post-transplant diarrhea. Further investigation of VCE's apparently high predictive value will be of great interest, particularly with a view to avoiding unnecessary digestive biopsies.

0833

CELLULAR THERAPY FOR CYTOMEGALOVIRUS OR EPSTEIN BARR VIRUS REACTIVATION FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION USING VIRUS-SPECIFIC CYTOTOXIC T LYMPHOCYTES

X. Cao,[1] T. Wu,[1] J.B. Wang,[1] L.J. Chang,[2] J.L. Tang,[3] Y. Liang,[4] L. Kuo,[4] C.R. Tong,[1] Y.M. Yin,[1] Y.L. Zhao,[1] D.P. Lu[1]

[1]Beijing Daopei Hospital, BEIJING, China; [2]Molecular Genetics and Microbiology, University of Florida,, GAINESVILLE, USA, [3]National Taiwan University Hospital, TAIPEI, Taiwan, China, [4]Vectorite Biomedica Inc, TAIPEI, Taiwan, China

Background. Cytomegalovirus(CMV) and Epstein-Barr virus (EBV) reactivation after allogeneic hematopoietic stem cell transplantation (HSCT) can result in life-threatening complications such as interstitial pneumonia and post-transplant lymphoproliferative disease. Adoptive transfer of CMV or EBV-specific cytotoxic T lymphocytes (CTL) offers the potential for accelerating reconstitution of virus-specific immunity and reducing the morbidity and mortality of severe viral diseases post HSCT. *Aims.* In present clinical study, the safety and efficacy of CMV-CTL and EBV-CTL were examined in allogeneic-HSCT setting. *Methods.* Total 23 patients with refractory CMV viramia (9 cases)/disease (8 cases) or EBV viramia (1 case)/disease (5 cases) were enrolled. They either responded poorly to antiviral agents or could not tolerate to antiviral medicines due to neutropenia or thrombocytopenia. The consent forms were signed by all patients or their guardians. Primary diagnosis included ALL (n=8), AML (n=7), CML (n=4), MDS (n=1), NHL (n=2) and SAA (n=1). Eight patients underwent unrelated HSCT, and 15 patients received haploidentical-HSCT. Dendritic cells from either donor's or patient's peripheral blood (PB) were primed with a mixture of 138 CMV pp65 pentadecamer peptides and co-cultured with T cells. Using an innovative interferon-gamma-responding T cell selection approach, we primed the donor's lymphocytes in PB with a mixture of EBV late membrane protein 2 (LMP2) pentadecamer peptides. CMV and EBV DNA in plasma or tissue from biopsy were measured by real-time PCR. Patients received 1 to 7 CMV-CTL infusions at median day 63 (39 to 210) after HSCT. The median CMV-CTLs infused were 1×10^6 (1.5×10^5-1.6×10^7). Patients received 1 to 3 EBV-CTL infusions with the median cell dose as 1.85×10^6 (4×10^5-9.5×10^6). *Results.* None or mild reactions were seen in a few patients which relevant to CMV-CTL or EBV-CTL infusion, such as temporary fever, chill, and transient skin rash. No pre-existed GVHD deteriorated. With CMV-CTL therapy, nine of 17 patients (53%) achieved complete response (CR); six of 17 patients (35%) had partial response (PR). Two patients had no response to CMV-CTL therapy and died from CMV disease. Patients with CMV viramia (6/9 in CR) had better response than that with CMV disease (3/8 in CR). With EBV-CTL therapy, four of 6 patients (67%) achieved complete response (CR); another two patients (33%) had partial response (PR). Two patients in PR died from EBV disease. *Conclusions.* Our findings indicate that under current protocol, application of CMV-CTL or EBV-CTL following allogeneic-HSCT is safe and effective even for CMV/EBV disease.

0834

TWO-WEEK SCHEDULE PEGYLATED G-CSF IS EQUIVALENT TO DAILY G-CSF IN SUPPORTING NEUTROPHIL RECOVERY AFTER UNRELATED CORD BLOOD TRANSPLANTATION USING THE INTRABONE TECHNIQUE

V. Pinto, A. Ibatici, A.M. Raiola, F. Gualandi, B. Bruno, N. Sessarego, M. Podestà, A. Bacigalupo, F. Frassoni

San Martino Hospital, GENOA, Italy

Background. We have recently developed a novel technique of unrelated cord blood transplantation (UCBT) in adults by direct intrabone marrow injection (IBM) of cells to overcome the issue of the cell-dose barrier. The use of post-transplant Granulocyte Colony-Stimulating Factor (G-CSF) on a daily schedule in CBT accelerates neutrophil recovery and is associated with a higher probability of engraftment. *Aims.* In this study, we have been investigating whether pegylated G-CSF (PegG-CSF) can be used as an alternative supportive therapy to recombinant human G-CSF after IBM-CBT. *Methods.* Twenty nine patients with advanced haematological malignancies received single-unit graft IBM-UCBT after myeloablative conditioning. Median age was 33 ys (18-63) and weight was 65 kg (49-102). Currently, 18 pts are alive, all in CR with a median F-U of 12 months (1-22). Median F-U of pts who received G-CSF and PegG-CSF was 8 (1-22) and 3 (1-12) months ($p<0.05$). Donor/recipient HLA matching was 5/6 in 6 pts, 4/6 in 20 pts and 3/6 in 3 pts. GVHD prophylaxis consisted of CSA and MMF, and ATG according to our institutional protocols. G-CSF at a dose of 30 MU/day (n=19 pts) and PegG-CSF at a total dose of 6mg every 2 weeks (n=10 pts) were administered SQ since day+5 until neutrophil recovery. Median nucleated cells infused was 2.6 (1.6-3.5) and 3.2 (2.4-4)$\times10^7$/kg in the G-CSF and PegG-CSF pts ($p<0.05$). *Results*. There were no graft failures/rejections. All 29 pts evaluable had 100% donor engraftment. Estimated OS was 48% at 22 months. Grade I-II GVHD was seen in 8 pts. No growth factor-related adverse events were seen. Median number of G-CSF and PegG-CSF injection was 29 (13-49) and 2 (2-3). Median time to neutrophil recovery >0.5 and $>1\times10^9$/L was 27 (15-37) and 37 (17-47) days for the G-CSF group, and 23 (19-29) and 25 (21-34) days for the PegG-CSF group (p=NS between the 2 groups). Median time to platelet recovery (20×10^9/L) was 37 days (29-75) in G-CSF and 35 days (28-41) in PegG-CSF group (p=NS). There was no difference in the median time of hospitalisation of G-CSF and PegG-CSF pts, with 50 days (34-96) and 46 days (38-167), respectively (p=NS). The median cost of G-CSF was 1979 €/patient *vs* 1500 €/patient for PegG-CSF. *Conclusions*. In this preliminary analysis, G-CSF and PegG-CSF were compared as supportive treatment for donor engraftment. PegG-CSF was found to be not inferior to daily G-SCF as: 1) safety profile; 2) time to neutrophil and platelet engraftment; 3) patient compliance. The 2 groups differ in the median number of CB cells injected, thus limiting any speculation in terms of efficacy. In addition PegG-CSF may be cost-effective compared with G-CSF.

0835

TETRAMER-BASED QUANTIFICATION OF CYTOMEGALOVIRUS (CMV)-SPECIFIC CD4$^+$ AND CD8$^+$ T LYMPHOCYTES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION MAY IDENTIFY PATIENTS AT RISK FOR CMV INFECTION DISEASE

D. Pastore, A. Mestice, M. Delia, M. Leo, P. Carluccio, A. Ricco, A. Mazzone, P. Mongelli, M. Casanova, V. Liso, G. Specchia

Hematology, University of Bari, BARI, Italy

Recovery of cytomegalovirus (CMV)-specific T-cells after allogeneic stem cell (SCT) is critical for protection against CMV disease; in humans, both the CMV-specific CD4$^+$ and CD8$^+$ arms of T-cell immune response must be regenerated after SCT in order to obtain long-term protection against CMV reactivation and disease. In our study we used fluorochrome-conjugated tetrameric complexes of HLA-A101, HLA-A201, HLA-B702, HLA-B801, HLA B3501 to monitor recovery of CD4 and CD8 CMV-specific T-cells (according to the patient's HLA) in 47 patients after SCT; the patients were transplanted with unmanipulated peripheral blood stem cells from an HLA identical related donor (n=45) an HLA identical unrelated donor (n=2). To prevent CMV disease all patients received acyclovir 10 mg/Kg intravenously every 8 h until engraftment and then 1200 mg/day p.o. until day +180. Patients were monitored for CMV infection and disease using peripheral blood mononuclear cells obtained weekly until 1 year. Median age was 36 years (range 18-61); diagnoses were acute myeloid leukaemia (n=37), acute lymphoblastic leukaemia (n=5), chronic myeloid leukaemia (n=3), lymphoma (n=1), myelofibrosis (n=1). Five patients (R-) were CMV seronegative and 3 of them received grafts from a CMV-seropositive donor; forty patients (R$^+$)

were CMV seropositive. The median absolute number of CMV-specific CD4+ T-cells detected at 1, 3, 6, 12 months was 1 μL (range 0-6), 3 μL (range 0-12), 4 μL (range 0- 19) and 8 μL (range 0-48), respectively. The median absolute number of CMV-specific CD8+ T-cells detected at 1, 3, 6, 12 months was 2 μL (range 0-15), 16 μL (range 0-22), 21 μL (range 0-47), 22 μL (0-62), respectively. Tetramer analysis showed that 27/47 (57%) patients reconstituted CMV-specific CD4+ and CD8+ T-cells at 3 months; in this group only 3/27 (11 %) patient developed CMV infection. CMV infections were observed in 18/20 (90 %) who failed to generate CMV-specific CD4+ and CD8+ T-cells response. In our experience no CMV infection/disease was observed with CMV-specific CD4+ T-cells> 2 μL and CMV-specific CD8+ T-cells> 5 μL. Recovery of both CMV-specific CD4+ and CD8+ T-cell immunity occurred in 40/42 (95%) R+ patients within 6 months and 3/5 (60%) R- patients within 12 months. The cumulative incidence of CMV infection was 21/47 or 44% at 1 year, with a median reactivation time of 45 days (range 28-96); one patient, without CMV-specific CD4+ and CD8+ T-cells recovery, developed CMV disease (colitis-pancreatitis) and died. In conclusion we suggest that failure to recover CMV-specific CD4+ and CD8+ T-cells after SCT is associated with the development of CMV infection/disease and we envision that this strategy may enable us to identify those patients who may benefit from preemptive therapy, in particular the adoptive transfer of CMV-specific T lymphocytes for the prevention of CMV disease.

0836

HIGH RATE OF SEVERE OPPORTUNISTIC INFECTIONS AFTER UNRELATED CORD BLOOD TRANSPLANTATION (UCBT)

C. Touzeau, F. Rialland, P. Chevallier, T. Guillaume, J. Delaunay, S. Ayari, N. Blin, T. Gastinne, S. Le Gouil, B. Mahe, V. Dubruille, P. Moreau, J.L. Harousseau, M. Mohty

University Hospital of Nantes, NANTES, France

The use of UCBT in adult patients with hematological malignancies has significantly increased over the last 5 years, since it can represent an attractive treatment modality for those high risk patients who lack a suitable HLA-matched related or unrelated donor. The aim of this analysis was to assess the outcome of 18 patients who received UCBT in a single centre, with a special focus on infectious complications. All 18 patients had high risk disease features (AML, n=6; ALL, n=5; NHL, n=4; CLL, n=1; MDS, n=1 and myelofibrosis, n=1). 12 patients (67%) were in CR (CR1, n=10; CR2, n=2), whereas 6 had a more advanced disease at time of UCBT. Of note, 4 patients (22%) had already received and failed prior autologous transplantation. The median age and weight of recipients were 47 (range, 26-62) years and 62 (range, 50-100) kg respectively. 10 patients received a reduced intensity conditioning regimen including fludarabine 200 mg/m^2, cyclophosphamide 50 mg/kg and low dose TBI (2 Gy) in 7 patients, and fludarabine 150 mg/m^2, cyclophosphamide 100 mg/kg and antithymocyte globulins 5 mg/kg in 3 patients. 8 patients received a standard myeloablative regimen (cyclophosphamide 120 mg/kg and TBI, 12Gy). All patients received CsA and MMF for GVHD prophylaxis. Three patients (17%) received a single CB unit, whereas 15 (83%) received 2 CB units in order to achieve a minimum cryopreserved cell dose of 3.0×10^7 TNC/kg. For the entire group, the median cryopreserved and infused cell doses were 3.8×10^7 TNC/Kg (range, 2,60-5,00) and 0.14×10^6 CD34+ cells/Kg (range, 0.02-0.35) respectively. For the 3 patients who received one CB unit, CB where 4/6 (n=2) and 5/6 (n=1) HLA matched. For the 16 patients who received two CB units, CB where 4/6 (n=3), 5/6 (n=8) and 4/6-5/6 (n=4) HLA matched. Neutrophil recovery (ANC>500/μL) occurred in 16 patients (89%) at a median of 28 (range, 8-60) days after UCBT. A sustained platelets recovery (>50000/μL) was observed in 13 patients (72%) at a median of 94 (range, 7-277) days after UCBT. However, based on donor-recipient chimerism analysis, autologous neutrophil recovery (<50% cells of donor origin) was observed in 7 patients (39%). In patients evaluable for acute GVHD, the overall incidence of grade II-IV acute GVHD was 54% (2 grades II, 4 grades III and 0 grade IV). 12 patients were evaluable for chronic GVHD for an overall incidence of 5% (1 patient). In terms of opportunistic infections, 7 patients (39%) experienced at least one episode of a *severe* or *life-threatening* infectious complication (virus other than CMV reactivation, n=3 (1 EBV-PTLD, 1 VZV pneumonia, 1 adenovirus related multi organ failure); invasive aspergillosis, n=3; cerebral toxoplasmosis, n=1; other, n=1), requiring long-term hospitalization, and of whom 1 patient was in severe acute GVHD. Moreover, 11 patients (61%) experienced HHV6 reactivation requiring systemic therapy. Of note, 4 patients among the 7 experiencing a severe opportunistic infection required transfer to ICU. With a median follow-up of 268 (range, 44-725) days after UCBT, 3 patients (16%) had relapsed, and 8 patients died (infection, n=6; GVHD, n=1; relapse, n=1; TRM=39%). In all, we conclude that UCBT is a feasible therapeutic approach for high risk hematological malignancies in adult patients. However, delayed engraftment and delayed immune recovery with its corollary of serious opportunistic infections, are still a matter of concern warranting prospective efforts to define optimal prophylactic approaches.

0837

FIVE YEARS OF EXPERIENCE OF EARLY ENTERAL FEEDING IN PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION FOLLOWING MYELOABLATIVE CONDITIONING

I. Yakoub-Agha,[1] M. Ben Rejeb,[2] H. Baudelle,[2] C. Dendoncker,[2] V. Coiteux,[1] L. Terriou,[1] J.P. Jouet,[1] D. Seguy[2]

[1]Maladies du Sang, LILLE; [2]Unité de Nutrition, LILLE, France

Background. Since the encouraging preliminary results of our pivotal study in early 2003 (Seguy *et al.*, transplantation), all patients referred to our unit for myeloablative allogenic stem cell transplantation (allo-CST) were immediately given enteral feeding via a naso-gastric tube (NGT). *Aims.* The objective of the present work was to investigate our changing practice in nutritional support over the last five years and assess its impact on early outcomes. *Methods.* All patients were provided with comprehensive information regarding NGT feeding during the systematic, individual pre-transplantation interview and then on an ongoing basis from a multidisciplinary care team. Between Jan 2001 and Dec 2005, 121 patients having undergone myeloablative allo-SCT were offered EN via an NGT. Of these, 94 (78%) agreed to receive EN (the EN group) and 27 (22%) refused the NGT (non-EN group) and received either intravenous parenteral nutrition (PN) (n=22) or oral feeding only (n=5). The NGT was inserted shortly after transplantation. A bacteriologically controlled oral diet was encouraged for as long as the patient was able to sustain it. The daily oral intake was scheduled to provide 100% of estimated energy requirements (30-35 kcal/kg/day). Depending on the patient's tolerance, overnight NGT feeding was gradually increased with a view to reaching 50-70% of energy requirements within 5 days. If EN was poorly tolerated, additional or total PN was given. In the non-EN group, patients received PN when the total oral intake was less than two-thirds of the average energy requirement over 5 days. *Results.* Except for the patients' age (EN-group, 38y *vs* non-EN 28y, p=0.038), the two groups were comparable in terms of initial characteristics, disease status and transplantation modalities. The median duration of EN was 14 days (range: 1-59) and 61 patients received no additional PN while the median duration of PN was 12 days (2-70). There was no significant difference between the two groups regarding duration of hospitalization, nutritional status at discharge and mucositis duration and grade. Significant differences were observed, however: engraftment, 100% *vs* 93%, p=0.05; duration of neutropenia, 20d (10-64) *vs* 25d (18-100), p=0.0001; duration of thrombopenia 27d (6-100) *vs* 56d (50-100), p=0.014; serum albumin level at discharge < 35g/L, 45% *vs* 76%, p=0.005 for the EN *vs* the non-EN-group, respectively. A lower proportion of patients with EN developed acute grade III/IV GVHD (9% *vs* 37%; p=0.001) and non-bacterial infections (9% *vs* 41%; p=0.0002). Furthermore, patients with enteral feeding had better 100-day survival (92% *vs* 67%, p=0.001) and less infection-related deaths. In a multivariate analysis, the absence of enteral nutrition was the only factor adversely influencing 100-day survival (95% CI: 1.55-14.9; p=0.007). In order to evaluate the change over time in our unit's nutritional support practice, we compared the initial period (2001-2002, when patients (n=41) had the choice between EN and PN) with the second period (2003-2005, when EN was offered systematically (n=80)). In the second period, patients received PN less often (73% *vs* 31%, p<0.0001) and received EN more often (49% *vs* 93%, p<0.0001) and for longer (10d *vs* 15d; p=0.001). In addition, EN started earlier after transplantation (5d *vs* 2d, p=0.004). *Conclusions.* EN has been well tolerated and dramatically reduced the proportion of patients requiring PN. This study confirms the positive impact of EN on early outcomes in patients undergoing myeloablative allo-CST. When possible, EN should be preferred to PN.

0838

IS GVATLL REACTION FOR OR AGAINST ATLL? -RELAPSE AND PROGRESSION CASES AFTER STEM CELL TRANSPLANTATION (SCT) FOR ADULT T-CELL LEUKEMIA/LYMPHOMA (ATLL)-

N. Nakano

Imamura Bun-in hospital, KAGOSHIMA, Japan

Background. Adult T-cell leukemia/lymphoma (ATLL) has a poor prognosis because of its chemo-resistance. Many chemotherapeutic regimens have been created but none of them have shown sufficient results. We proposed allogeneic stem cell transplantation (allo-SCT) for ATLL patients and showed an improved survival rate. However, relapse or progression of ATLL is one of the major limiting factors of survival in post SCT patients. *Aims.* In order to establish a better treatment strategy for poor responders after SCT for ATLL, we analyzed the outcome of relapse or progression cases after allo-SCT. We paid special attention to the graft versus ATLL (GvATLL) effect. *Methods.* There were 33 ATLL patients in which allo-SCT was performed in Imamura Bun-in Hospital (IBH) from June 1998 to November 2007. Twenty seven cases survived over 90 days after SCT. Sixteen of the 27 patients relapsed. Using data in medical records of IBH, we analyzed transplant characteristics and the outcome of these 16 patients retrospectively. *Results*. Disease status at SCT was CR in 2 patients, 2 PR, 5 SD, and 7 PD. Eight patients received conventional stem cell transplantation (CST) and the other eight patients received reduced-intensity stem cell transplantation (RIST). Fourteen patients in 16 obtained remission (9 CR and 5 PR), but the remaining 2 did not (1 SD and 1 PD) after SCT. The sites of relapse or progression in 16 were skin in 10 patients, 7 peripheral blood, 6 lymph node, 3 central nervous system, and 1 bone. All patients discontinued immunosuppressants after relapse or progression. Eleven patients obtained remission. Especially, in 6 out of 11 patients, remission was obtained only by discontinuation of immunosuppressants (graft-versus-ATLL effect), and the time to remission after discontinuation of immunosuppressants was between 1 to 14 days. Twelve patients were complicated with acute GVHD (grade I-IV). Twelve patients died after SCT. The causes of death were disease progression of ATLL in 5 patients, 3 acute GvHD, 3 infectious complications, and 1 interstitial pneumonia. Four patients who were complicated with acute GvHD survived over 24 months. *Summary and conclusions.* Skin was a major site of relapse or progression after SCT in ATLL patients. A certain number of patients obtained remission only by the discontinuation of immunosuppressants. Four patients survived more than 2 years with their complication of acute GVHD. These results suggest that the GvATLL effect after SCT exists and plays an important role in longer survival for poor responders of post allo-SCT in ATLL patients.

0839

FEASIBILITY OF NIH CONSENSUS CRITERIA FOR CHRONIC GRAFT VERSUS HOST DISEASE

C. Byung-Sik, K.S. Eom, Y.J. Kim, H.J. Kim, S. Lee, C.K. Min, S.G. Cho, D.W. Kim, J.H. Lee, W.S. Min, J.W. Park, C.C. Kim

Catholic Hematopoietc Stem Cell Transplantation Center, SEOUL, South-Korea

Background. Chronic graft-versus host disease (cGVHD) is classically defined as occurring more than 100 days after allogeneic hematopoietic stem cell transplantation (HSCT). But, newer donor types and transplant strategies are changing the presentation of the syndrome. The Diagnosis and Staging Working Group of the NIH Consensus Development Project on Criteria for Clinical Trials in cGVHD proposed new criteria for diagnosis and assessment of overall cGVHD severity. *Aims and Methods*. We retrospectively reviewed 463 patients who underwent allogeneic HSCT and surviving until day 100 after transplant between January 2002 to December 2005 to assess the applicability of the new criteria in predicting survival and transplant related mortality (TRM). To study a homogenous cohort, patients with the following criteria were excluded: received 2 allogeneic HSCT, received donor lymphocyte infusion, received chemotherapy for a relapse subsequent to allogeneic SCT, and received additional stem cells with or without prior standard dose chemotherapy. The final study cohort was comprised of 437 patients. *Results*. Two hundred eleven patients were diagnosed with cGVHD (cumulative incidence 54.6%). Of these, 42 patients (19.9%) were reclassified as late acute GVHD; 144 patients (68.2%) had classic cGVHD and 25 patients (11.8%) had overlap syndrome. Patients with classic cGVHD and overlap syndrome (n=169) were graded. Twenty three patients (13.9%) had mild, 81 (47.9%) moderate and 64 (37.9%) severe cGVHD. After a median follow up of 46 months (range 5-71 months), 4 year probability of overall survival (OS) was significantly worse in overlap syndrome (59.7±9.9%) as compared to late acute GVHD (78.6%±6.3%) and classic cGVHD (72.7±4.7%), p=0.049, while there was no difference in TRM (late acute 12.5±5.2%, classic 17.6±3.8%, Overlap 26.8±9.4%, p=0.202). Among 132 patients who experienced acute manifestations, there were significant differences in outcome (OS, p=0.001 and TRM, p=0.004) according to the pattern of onset of acute GVHD (late onset *vs* persistent *vs* recurrent). Among patients with overlap syndrome and classic cGVHD (n=169), OS and TRM were significantly different as to new grading system (OS: mild 87%±7.0%, moderate 74.7±6.1%, severe 59.8±7.5%, p=0.042, TRM : mild 4.8±4.6%, moderate 11.6±4.4%, severe 33.2±7.0%, p=0.001). On multivariate analysis, new grading system was not only a more reliable factor associated with TRM than modified Seattle criteria (limited *vs* extensive), but also one of factors which can predict the resolution of cGVHD. *Conclusions*. This analysis indicates that the consensus guidelines are applicable to allogeneic HSCT recipients and the new grading system more reliably predicts TRM and the resolution of cGVHD than older classification of cGVHD. Prospective validation is still needed before widespread acceptance.

0840

TTV VIREMIA IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS: A NEW SURROGATE MARKER OF IMMUNE DYSFUNCTION?

DF Focosi, M. Maggi, V. Ricci, M. Albani, J. Rocchi, L. Matera, L. Lanini, E. Andreoli, M.L. Vatteroni, M. Pistello, M. Bendinelli, L. Ceccherini-Nelli, M. Petrini

University of Pisa, PISA, Italy

Background. We currently lack markers of functional immune dysfunction which could drive rational discontinuation of antimicrobials after high-dose chemotherapy. The size of expansion of $CD8^+CD57^+$ T lymphocytes has been proposed as one of the most reliable markers, being a common denominator with other immunedeficiency settings (e.g. cancer, AIDS, autoimmune diseases, and ageing). Torquetenovirus (TTV; from torques and tenuis, Latin for necklace and thin, respectively) is a non-enveloped virus with a small single-stranded circular DNA genome, classified into the newly established floating genus Anellovirus. *Aims.* In order to support the hypothesis that the size of expasion of $CD8^+CD57^+$ T lymphocytes correlates with susceptibility to viral infection/reactivation, we investigated the kinetics of TTV viremia during high-dose chemotherapy and correlated changes with $CD8^+CD57^+$ T lymphocytes. *Methods.* Peripheral blood samples from 19 consecutive multiple myeloma patients undergoing high-dose melphalan supported by autologous hematopoietic stem cell transplantation (HSCT) were collected before the conditioning regimen, and at days 0, +10, +40, +70 and +100 after HSCT. Specimens were used to achieve complete blood counts, TTV serum titres by quantitative PCR, and lymphocyte immunophenotyping. All patients received at least 1 irradiated and leucodepleted platelet concentrate before day +10. *Results*. We observed significant changes in plasma TTV loads over a 100 days follow-up. More interestingly, TTV levels were found to correlate with the percentage of $CD8^+57^+$ T lymphocytes, a recognized surrogate marker of immune dysfunction, as shown in Figure 1. *Conclusions*. The importance of such findings and the potential role of TTV as new functional marker of the immune status in HSCT recipients will be discussed. The orphan status of this virus and the lack of drugs modifying serum titres make TTV an ideal tool to investigate immune reconstitution after HSCT.

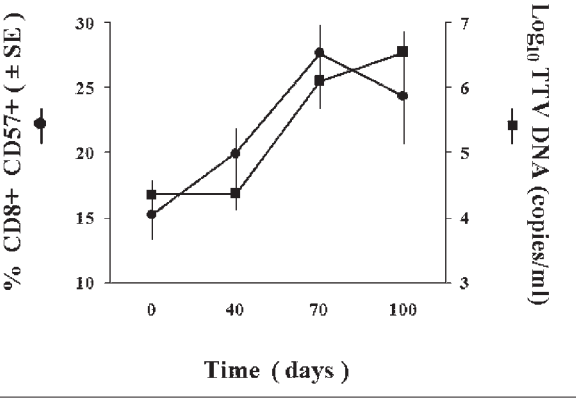

Figure 1.

0841

A STANDARDIZED MONITORING STRATEGY FOR EARLY DIAGNOSIS OF PULMONARY CHRONIC GRAFT-VERSUS-HOST DISEASE: IMPROVEMENT OF OUTCOME?

M. Stadler, B. Hertenstein, R. Ahlborn, E. Dammann, J. Gottlieb, T. Welte, A. Ganser, M. Eder

Hannover Medical School, HANNOVER, Germany

Background. Involvement of the lung is a most devastating form of chronic graft-versus-host disease (cGvHD) after allogeneic hematopoietic cell transplantation (alloHCT). Its clinical manifestations, bronchiolitis obliterans syndrome (BOS, with predominant small airways obstruction) and bronchiolitis obliterans organizing pneumonia (BOOP, with predominant restrictive changes) carry a high morbidity and mortality. Treatment options are limited. *Aims.* We hypothesized that earlier diagnosis of pulmonary cGvHD might improve outcome. *Methods.* In August 2004, we implemented a prospective monitoring approach at our institution aiming at early diagnosis of pulmonary cGvHD: all patients were scheduled for pulmonary function tests (spirometry, body plethysmograpy, and diffusion capacity) at three, six and twelve months post alloHCT. If pulmonary values were found deteriorated by at least 10% compared to baseline before alloHCT, further work-up with computed tomography of the lung and fibre optic bronchoscopy was performed. Patients with confirmed pulmonary cGvHD received appropriate escalating therapy, including steroids, calcineurin inhibitors, MMF, betamimetics, acithromycin, leukotriene inhibitor, imatinib, extracorporeal photopheresis, mesenchymal stem cells, or even lung transplantation.

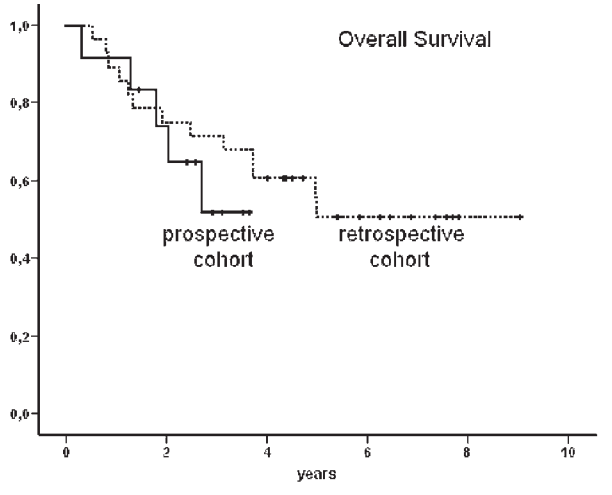

Figure 1.

Results. Here we report the 12 patients diagnosed with pulmonary cGvHD after implementation of our prospective algorithm, compared to the 28 consecutive retrospective cases identified between 1998 and 2004. Median age was 42 years (range: 20 to 59); 11 patients were female and 29 male. HLA-matched peripheral blood stem cell (n=36) or bone marrow (n=4) transplantation from family (n=23) or unrelated (n=17) donors had been performed after myeloablative (n=26) or reduced intensity (n=14) conditioning, for AML (n=15), ALL (n=4), MDS/secondary AML (n=7), CML (n=6), myelofibrosis (n=3) or lymphoma (n=5). Both groups were comparable regarding diagnoses, disease status, donor type, stem cell source, conditioning, and cGvHD risk factors (e.g., donor lymphocyte infusions). Patients in the prospective group were younger (median 34 *vs* 43 years); in the retrospective group, fewer male patients had a female donor (25% *vs* 42%). Malignant relapse occurred in a third of patients with pulmonary cGvHD (3/12 in the prospective, and 10/28 in the retrospective group). Initial diagnosis of pulmonary cGvHD was made in the prospective cohort after a median of 9 months (range: 3 to 24) *vs* 18 months (range: 3 to 59) in retrospective patients. 5 and 7 patients were diagnosed with BOS and BOOP in the prospective, 14 and 14 in the retrospective group, respectively. Currently, 7/12 from the prospective cohort (58%) *vs* 15/28 of retrospective patients (54%) are alive; probability of overall survival is 52% after 3 years *vs* 51% after 5 years, respectively. *Conclusions.* Following a standardized monitoring strategy, pulmonary cGvHD could indeed be diagnosed earlier: this approach should be implemented in clinical care after alloHCT. However, earlier diagnosis did not translate into improved survival in our preliminary analysis. Whether more frequent pulmonary monitoring or more aggressive (preemptive) therapy might enhance outcome remains to be evaluated. In addition, new agents for effective therapy of pulmonary cGvHD are urgently needed.

0842

PRE-EMPTIVE THERAPY OF ACUTE GVHD BASE ON PROTEOMICS PATTERN: SINGLE CENTER EXPIRIENCE

M Weissinger,[1] A. Hahn,[1] M. Stadler,[1] H. Diedrich,[1] J. Kontsendorn,[1] S. Buchholz,[1] E. Dammann,[1] A. Krons,[2] A. Ganser[1]

[1]Hannover Medical School, HANNOVER; [2]Mosaiques-Diagnostics GmbH, HANNOVER, Germany

Background. Allogeneic hematopoietic stem cell transplantation (alloHSCT) is the only curative treatment for many hematologic malignancies or hematopoietic dysfunction syndromes, but the application is still limited due to major complications, such as severe graft-versus-host disease (GvHD). The diagnosis of GvHD is based on clinical features and biopsies; currently, a proteomic screening test for early recognition of aGvHD is evaluated. To date the proteomic pattern specific for aGvHD was evaluated blindly on 1230 samples collected from 218 patients undergoing allo-HSCT at MHH between 2005 and June 2007. The majority of the patients included were transplanted for hematological malignancies (n=210), 8 for non malignant hematopoietic failure syndromes. Eighty-seven patients were treated with dose-reduced conditioning regimens; GvHD-prophylaxis consisted of cyclosporin (CsA) plus methotrexate (MTX) or mycophenolic acid (MMF), and antibodies (n=178) respectively. Most patients were transplanted from matched unrelated donors (MUD, n=135), while 75 received stem cells from matched related sibling donors (MRD, 2 syngeneic), 4 from haplo-identical related, and 4 from mismatched donors. An aGVHD-specific pattern of 31 peptides either absent/decreased (15) or present /increased (16) was previously evaluated prospectively in a multicenter study (1). A pilot trial of pre-emptive therapy, with treatment of patients with 1mg prednisolone/kg BW upon positivity of the aGvHD-specific proteomic pattern, was started and the outcome of the pre-emptively treated group was compared to patients conventionally treated with 2mg prednisolone/kg BW upon clinical manifestation of aGvHD. In 2005, 90 transplanted patients were screened with the aGvHD proteomics pattern, but not pre-emptively treated. Between April 2006 and August 2007, 91 patients were transplanted. In 30 patients the aGvHD proteomic analysis showed a clear pattern indicative for aGvHD >II. 41 (45%) of 91 transplanted patients had aGvHD (grade I-IV). Twelve received pre-emptive therapy with 1mg prednisolone /kg BW upon pattern positivity, while 11 were treated upon clinical manifestation of aGvHD> II according to standard treatment protocols (2mg/kg BW). In the pre-emptive therapy group 3 patients (3 of 12 =25 %) developed aGvHD II or more, 2 had aGvHD II, only 1 developed IV, 2 died (2/12: 16%) by day +100. Of the 11 (all aGvHD >II) standard therapy patients, 5 developed aGvHD grade III or IV and to date 3 of those have died (5/11 standard treatment group$p$=45%) by day +100. Of 33 (33+3; 3= pre-emptive, but developing signs of aGvHD >II or more) patients, who developed any signs of aGvHD, 22 patients had GvHD grade 1. Of those patients (33) 26 were dead by day +160 (78 %), while only 4 of 12 (33%) had died in the pre-emptive group. Thus, taken together our results indicate that pre-emptive treatment may decrease the severity of aGvHD and may lead to a better overall survival.

0843

FOLLOW-UP OF BEDSIDE TESTING FOR SERUM C-REACTIVE PROTEIN (CRP) AND CELL BLOOD COUNTS (CBC) HAS POTENTIAL CLINICAL AND ECONOMIC IMPACTS ON THE MANAGEMENT OF PATIENTS HAVING HIGH DOSE THERAPY (HDT)

J.F. Rossi,[1] T. Kanouni,[1] S. Bouyha,[1] V. Rouillé,[1] J.F. Schved,[1] R. Borba,[2] J.P. Daures,[3] P. Milian[4]

[1]University Hospital Montpellier, CHU Lapeyronie, MONTPELLIER; [2]Horba ABX, MONTPELLIER; [3]Unité statistiques, Université Médecine, MONTPELLIER; [4]Horiba ABX, MONTPELLIER, France

Background. CBC and CRP are major biological tests in aplasia, with clinical and therapeutic implications, particularly for transfusion, antibiotics, haematopoietic growth factors and discharge of the patients. In addition, CRP affects tumor cell growth and survival in myeloma (Yang J *et al.* Cancer Cell 2007, 12:252-65), meaning that CRP follow-up may have clinical and therapeutic interest. *Aims.* In a prospective study, we analyze 1/the kinetic of CRP after high dose therapy (HDT), particularly in myeloma, to optimize the use of anti-IL-6 monoclonal antibody, following a previous study we made with a murine MoAb (BE-8) (Rossi JF *et al.* BMT 2005, 36:771-9), 2/the medical interest (i.e. infection, mucositis, clinical follow-up) for testing both markers, in addition to evaluate the medico-economic impact of bedside testing compared to standard biological testing. *Methods.* 40 patients having autologous transplantation (myeloma: melphalan 200mg/m^2 or lymphoma: BEAM conditioning regimen) or induction therapy for acute leukaemia were included in this prospective study. A daily comparison of CBC and CRP was made between bedside testing and central laboratories, till haematopoïetic recovery and patients' discharge. Bedside testing was performed on an ABX MICROS CRP200 (combining CBC and CRP analysis on the same blood sample). Results from this analysis were compared with the following routine analyzer's results at hospital laboratories, results which were only used by MDs for deciding the different treatment modifications, particularly for antibiotics, transfusion and patients' discharge. *Results.* We observed a correlation between these two types of analysis (rÇ=0.9964-0.9194) for all the markers analyzed (CRP, haemoglobin, WBC, platelets). Similar curves were observed between temperature (mean daily temperature after measurement twice a day) and daily C-RP serum levels. CRP curve eliminates the variations of the temperature due sometimes to antipyretic drugs. In addition, there was a strong correlation between severe infection and grade ≥2 mucositis with dynamic CRP, particularly the slope of the CRP, but with not prediction of the severity of the mucositis by comparing grade 3-4 and AUC of the CRP (ARIMA methodology). Highest levels of CRP were correlated to the most severe infections. CRP peaks at day 11 post-HDT, with variations due to mucositis and infections. In addition, bedside testing reduced time for medical decision making by a median of 6h 45, which impacts on the fees lowering it by 8-10%, particularly on transfusion, antibiotics, hematopoietic growth factor and hospitalization fee. *Summary and Conclusions.* Daily follow-up of CRP in patients having HDT for myeloma is a usefull marker, peaking at day 11, which allows to optimize the use of anti-IL-6 and to monitor it. Daily and bedside CRP and CBC has clinical and medico-economic impacts in this context of aplasia.

Thalassemia and iron overload

0844

MRI T2* DEMONSTRATES REDUCED CARDIAC IRON BURDEN FOLLOWING MODERATE- TO HIGH-DOSE DEFERASIROX TREATMENT IN CHRONICALLY TRANSFUSED BETA-THALASSEMIA PATIENTS

J. Wood,[1] A.A. Thompson,[2] C. Paley,[3] B. Kang,[3] P. Giardina,[4] P. Harmatz,[5] J. Virkus,[3] T. Coates[1]

[1]Childrens Hospital Los Angeles, LOS ANGELES; [2]Children's Memorial Hospital, CHICAGO; [3]Novartis Pharmaceuticals Corporation, EAST HANOVER; [4]Weill Cornell Medical Center, NEW TORK; [5]Children's Hospital Oakland, OAKLAND, USA

Background. Despite the availability of iron chelation therapy, accumulation of excess iron in the heart following red blood cell transfusions results in cardiomyopathy, congestive heart failure and death in approximately 71% of patients with β-thalassemia. Deferasirox (ICL670) is a once-daily, oral iron chelator with demonstrated efficacy in reducing liver iron concentration (LIC); furthermore, preclinical and single-institution clinical studies have demonstrated its efficacy in removing cardiac iron. *Aims.* To evaluate the effects of deferasirox on cardiac iron in patients with β-thalassemia major in a prospective, single-arm, multi-center trial using cardiac MRI T2*. *Methods.* In this ongoing study, deferasirox is administered at 30 mg/kg/day for 18 months, with the option to escalate to a maximum of 40 mg/kg/day if there is <25% improvement in cardiac T2* compared with baseline values, provided LIC is ≥3 mg Fe/g dry weight (dw). Entry criteria include MRI evidence of cardiac iron (T2* <20 ms) and normal left ventricular ejection fraction (LVEF ≥56%). Serum ferritin (SF) is assessed monthly and MRI assessments for LIC, cardiac T2* and LVEF are assessed every 6 months. Labile plasma iron (LPI), serum creatinine, and biochemical and hematological status are also being monitored. All results are reported as mean±SEM (range). *Baseline results.* Preliminary results are reported from 18 of 20 evaluable patients (three male, 15 female) with β-thalassemia major and mean age 21.9 years (range 10-45) who completed 6 months' treatment with deferasirox. Baseline SF was 4927±987 µg/L (range 395-10751; n=12), cardiac T2* was 9.5±1.0 ms (range 4.6-16.1), LIC was 20.5±4.2 mg Fe/g dw (range 3.6-62.3) and LVEF was 61.7±1.0%. LPI was abnormal (≥0.5 µmol/L) in 33% of patients, with a mean value of 0.73±0.28 µmol/L (n=11). One of the two excluded patients was found to be ineligible post-enrollment (LVEF <56%), and the other developed cardiac failure prior to 6 months and was switched to continuous deferoxamine. This patient had markedly elevated cardiac iron (T2*=1.8 ms) at enrollment. *Efficacy results.* During the first 6 months of treatment, all 18 patients were dosed at 30 mg/kg/day. The mean decrease in SF over 6 months was 516 µg/L; 14/18 (78%) and 16/18 (89%) patients had decreases in cardiac and hepatic iron, respectively, with mean reductions of 14.2% (p=0.020) and 22.9% (p=0.002), respectively (Figure 1).

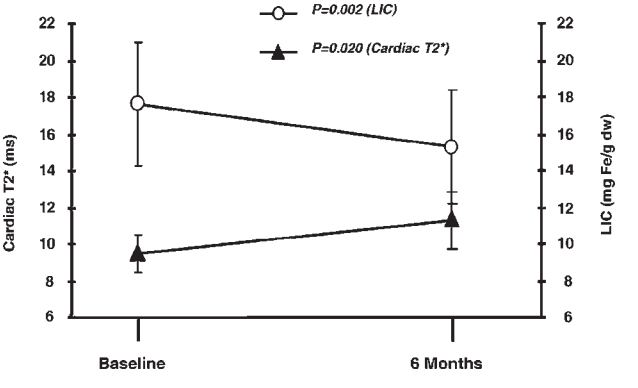

Figure 1. Changes in cardiac T2* and LIC (mean ± SEM)

There was no change in LVEF as measured by MRI. All patients had normal LPI at 6 months; in those with abnormal LPI at baseline (n=5), mean LPI fell from 1.6±0.3 to 0.26±0.1 µmol/L (p=0.003). No patients developed creatinine >upper limit of normal. Four patients had abnormal transaminases on ≥2 occasions; all were abnormal at baseline. *Conclusions.* These data demonstrate the efficacy of deferasirox 30 mg/kg/day

in patients with β-thalassemia and severe cardiac and hepatic iron burdens, producing statistically significant improvements in cardiac T2*, LIC and LPI in 78%, 89% and 100% of patients, respectively, over 6 months. Ongoing assessments over 12 and 18 months will elucidate if deferasirox continues to improve cardiac iron burden and maintains/improves cardiac function in severely iron-overloaded patients.

0845

EFFICACY AND TOLERABILITY OF DEFERASIROX DOSES >30 MG/KG/DAY IN PATIENTS WITH TRANSFUSION-DEPENDENT ANAEMIA AND IRON OVERLOAD

M.D. Cappellini,[1] A. Taher,[2] E. Vichinsky,[3] R. Galanello,[4] A. Piga,[5] T. Lawniczek,[6] V. Jehl,[6] L. Rojkjaer,[6] J.B. Porter[7]

[1]Università di Milano, MILAN, Italy; [2]American University of Beirut, BEIRUT, Lebanon; [3]Children's Hospital and Research Center, OAKLAND, USA; [4]Università di Cagliari, CAGLIARI, Italy; [5]Università di Turin, TURIN, Italy; [6]Novartis Pharma AG, BASEL, Switzerland; [7]University College London, LONDON, UK

Background. Effective iron chelation for patients with transfusion-dependent anaemias should be able to either maintain iron balance or decrease total body iron stores, depending on the goal of therapy. Deferasirox is a tridentate iron chelator that has been approved for the treatment of iron overload due to blood transfusions. Clinical evaluation has shown that the efficacy of deferasirox is dose dependent and influenced by transfusional iron intake. The highest dose of deferasirox currently approved by health authorities is 30 mg/kg/day. During the 1-year core trials, doses up to 30 mg/kg/day generally decreased body iron stores in patients with β-thalassaemia major (TM), sickle cell disease (SCD) and other rare anaemias, as measured by serum ferritin (SF), but some patients required further dose escalation to achieve therapeutic goals. *Aims.* The aim of this analysis was to investigate efficacy (based on change in SF levels) and safety (based on laboratory parameters and adverse event [AE] reports) of deferasirox doses >30 mg/kg/day in patients with transfusion-dependent anaemias. *Methods.* This is a retrospective analysis (up to September 28, 2007) of patients receiving deferasirox in studies 107E, 108E, 109E and ESCALATOR. Dose adjustments were permitted based upon SF. To evaluate patients planned for doses >30 mg/kg/day and avoid variability based on tablet strength, only those patients with an actual daily dose ≥32.5 mg/kg/day were analyzed. *Results.* In total, 228 patients (198 TM, 24 SCD, 6 rare anaemia) received doses >30 mg/kg/day; 28 received >30 to <32.5 mg/kg/day, 89 received ≥32.5 to <37.5 mg/kg/day, 109 received ≥37.5 to <42.5 mg/kg/day and 2 received ≥42.5 mg/kg/day. 225 patients met requirements for the efficacy analysis (SF assessment at baseline and at least once after initiating deferasirox >30 mg/kg/day), while 226 met requirements for the safety analysis (at least one safety assessment after receiving deferasirox >30 mg/kg/day). Overall median exposure from the first to last administration of deferasirox >30 mg/kg/day was 36.1 weeks. In total, 34, 176 and 18 patients received <7, 7-14 and >14 mL/kg/month of blood, respectively. There was a statistically significant median decrease in SF of 370 ng/mL (p<0.001) from pre-dose-escalation (>30 mg/kg/day) to the time-of-analysis. In total, 137 patients (60.6%) experienced an AE after starting treatment with deferasirox >30 mg/kg/day, of which 35 (15.5%) were assessed as possibly drug related. The most common drug-related AEs were gastrointestinal events such as vomiting (n=7, 3.1%), abdominal pain and nausea (n=4, 1.8% for both). After starting treatment with deferasirox >30 mg/kg/day serum creatinine level remained close to pre-high dose level (median relative change ranging from -2.5% to 4.0% within the first 24 months after starting high-dose treatment). *Summary and Conclusions.* Despite a limited exposure, deferasirox doses >30 mg/kg/day appear to effectively reduce SF levels in patients with transfusion-dependent anaemias, with a safety profile consistent with previously published data. This has important implications for patients who are heavily transfused and may require higher doses to reduce body iron burden. Regular monitoring of compliance, transfusional iron intake, SF levels, renal and hepatic function are important for optimizing therapy.

0846

IMPROVEMENT IN CARDIAC T2* WITH DEFERASIROX IN TRANSFUSED PATIENTS WITH CARDIAC IRON OVERLOAD

Y. Reyal,[1] O. Chowdhury,[1] P. Kirk,[2] E. Prescott,[1] B. Davis,[1] F. Shah[1]

[1]Whittington Hospital, LONDON; [2]Cardiovascular Magnetic Resonance Unit, Royal Brompton Hospital, LONDON, United Kingdom

Background. Cardiac iron overload remains the leading cause of death in thalassaemia major (TM). Early data suggests that deferasirox can improve myocardial iron overload. *Aims.* This study evaluates the impact of deferasirox on myocardial iron using cardiac MRI as a non-invasive means of assessing iron burden. A cardiac T2* less than 20ms is indicative of myocardial iron loading. *Methods.* Fifteen patients started deferasirox as part of the Novartis 2203 study and 4 patients were treated outside of the trial. Patients underwent a cardiac MRI (cMRI) prior to starting deferasirox treatment and a follow-up scan at one to two years after initiation as part of routine monitoring. All patients received a dose of deferasirox 20-35 mg/kg/day and serum ferritin levels were regularly recorded. The dose of deferasirox was adjusted according to the trend in ferritin measurements. *Results*. In 18 patients with TM and 1with sideroblastic anaemia the change in cardiac T2*, liver iron concentration and serum ferritin was recorded over a mean treatment period of 488 days (range 233-680 days). The average daily dose of deferasirox prescribed was 21.1mg/kg/day, with a range of 20-35 mg/kg/day. Median adherence to the prescribed dose of deferasirox was 95% (range 73-100%). Patients were subdivided into two groups according to whether the baseline cardiac T2* was greater or less than 20ms. Cardiac T2*< 20ms at initiation of therapy (n=9): there was a significant improvement in cardiac T2* by a mean of 3.6±2.6 ms (range -0.8-+9ms) SEM 0.89 (p=0.002) from a baseline of 10.6±4.2 ms (6.3-20ms) to 14.1±6.0ms (9-29ms). Liver iron concentration fell by a mean of 1.9±2.4 mg/g dry weight (dw) (range +0.8 to -6.1) SEM 0.78, from a baseline of 6.2±3.8mg/g dw (range 1.4 -10.8) to 4.3±3.1 mg/g dw (range 0.9-10.8) (p=0.03). This improvement in cardiac and liver iron loading was not reflected in the serum ferritin, which showed a slight rise from 2002±752 µg/L to 2694±1971 µg/L µg/L (p=ns) Cardiac T2*> 20ms at initiation of therapy (n=10): the mean cardiac T2* was 31.8±8.3 ms at start of study and 28.5±9.5 ms at end of study (p=ns). In two patients the cardiac T2* fell below 20ms; both patients had a documented history of previous cardiac iron loading and were non-adherent to treatment. There was an overall improvement in liver iron concentration from a baseline of 7.4±3.3 mg/g dw to 5.1± 3.3 mg/g dw (p=ns). A relationship between adherence to treatment and improvement in cardiac T2* was seen, with an overall improvement in cardiac T2* of 2.6± 1.5ms in patients with ≥95% adherence and an overall fall in T2* of 4.5± 1.85ms in patients with <95% adherence (p= 0.01). *Summary and conclusions.* A significant improvement in cardiac iron status was demonstrated in patients with cardiac iron overload despite a low dose treatment regime and a rising ferritin.

0847

LIVER FIBROSIS IN ADULT THALASSEMIA PATIENTS ASSESSED BY TRANSIENT ELASTOGRAPHY

E. Cassinerio,[1] M. Fraquelli,[2] M. Fasulo,[3] C. Cesaretti,[3] P. Pattoneri,[1] C. Rigamonti,[4] D. Conte,[2] M.D. Cappellini[3]

[1]Fondazione Ospedale Maggiore Policlinico, Milano, MILANO; [2]I Division of Gastroenterology, Fondazione Policlinico Ospedale Maggiore Milano, MILANO; [3]Hereditary Anemia Centre, Dept Internal Medicine, Ospedale Policlinico Milano, MILANO; [4]II Division of Gastroenterology, Fondazione Policlinico Ospedale Maggiore Milano, MILANO, Italy

Background and Aims. Transient elastography (TE) is a reliable and reproducible tool for staging hepatic fibrosis in patients with chronic liver disease (CLD), but its role in patients with b-thalassemia has not been extensively investigated. *Methods*. 115 consecutive adults with b-thalassemia were studied in a single Thalassemia Care Centre in Italy. Fifty-nine (Group I: 20M, 33±6.6 yrs) had b-thalassemia major and 56 intermedia (Group II: 26M, 40±12 yrs). Twenty-nine patients (49%) in group I and nine (11%) in group II were hepatitis C virus (HCV-RNA)-positive. All patients were examined by TE (Fibroscan®; Echosens, Paris, France). The cut off TE values for diagnosing different stages of hepatic fibrosis were defined as > 7.9 kPa for F≥2; > 10.3 for F≥3 and > 11.9 for F=4. In 14 patients (all in group I) liver biopsy were also performed. Necroinflammation and fibrosis were scored by METAVIR classification. Fourty-seven patients with TM and 26 patients with TI underwent liver iron

determination (LIC) by T2* Magnetic Imaging (T2*MRI) assessment. *Results*. A significant positive correlation between TE and fibrosis stage at liver biopsy was detected (r=0.73, p=0.003) but no significant correlation was found with necroinflammatory activity and LIC measured by T2*. Group I had significantly higher mean TE and serum ferritin values (SF) than group II (9.7±9.1 *vs* 6.6±3.2 kPa and 1525±1337 *vs* 722±638 ng/mL, respectively p=0.01 and p<0.001).Group I showed a significant positive correlation between TE and ALT (p=0.007), GGT (p=0.01) and bilirubin (p=0.04). In Group II there were a significant positive correlation between TE and ALT (p=0.01), GGT (p=0.001), bilirubin (p=0.01) and HCV-RNA positivity (p=0.03). *Conclusions*. TE is a reliable technique to stage liver fibrosis in patients with b-thalassemia major, including those with concomitant HCV infection who had a significant or severe fibrosis in about one third of the cases. In beta thalassemia patients TE results were independently associated with biochemical activity and were not influenced by iron overload parameters measured with ferritin levels and T2* MRI. A TE cut off >10.3 and > 12 kPa allowed to confirm the presence of severe fibrosis or cirrhosis in thalassemic patients.

0848

FOUR YAERS FOLLOW-UP OF HYDROXYUREA TREATMENT IN ALGERIAN WITH BETA-THALASSAEMIA MAJOR AND INTERMEDIA

M. Bradai,[1] F. Talbi,[2] F. Lamraoui,[1] F.Z. Ardjoun[3]

[1]Institute of Medicin of Blida, BLIDA; [2]Istitute of Medicin of Algiers, ALGIERS; [3]Institute of Medicin of Algiers, ALGIERS, Angola

Background. Hydroxyurea (HU) treatment can eliminate transfusion requirement in patients with thalassaemia (THL).Good response has been reported chiefly in patients with THL intermedia (TI), effects in THL major (TM) are controversial. Nevertheless benefits have been reported in Iranian patients (Yavarian M and *et al.*, Heamatologica 2004; 9:1172-8), Algerian patients (Bradai M *et al.* Transfusion 2007;47:1830-36) with severe beta-THL. *Aims.* We reported the outcome over 4 years period of HU treatment in Algerian patients with TI an TM with good response to HU, by assessment of haemoglobin(Hb) level, ferritin level, cardiac function, the influence on growth velocity and sexual maturation. We defined good response when a decrease in annual transfusion (TS) requirement greater 70%, with sustained Hb above 7 g/dL. *Patients and Methods.* Since 2001 HU treatment was initiated in 77 patients with THL. 30 patients with good response were valuables, 7 TI and 23TM. At the initiation of treatment median age were 11±5 (range3- 21 years), only 4 patients aged more than 16years, 14 male and 16 female. Blood counts were monitored monthly (Sysmex SF 3000), the serum ferritin (determined withElecsys2010) was evaluated every 3 to 6 months, Growth and sexual development evaluated by measurement height, weight and Tanner stage. Short height was seen in 18 patients (Standard Deviation DS<2σ) and low weight in 11 patients (DS<2σ). Hypogonadism: absence of any pubertal sign was indicated by age of 16.Cardiac function and Left Ventricular Ejection Fraction (LVEF) has been monitored every year. The management of hemochromatosis was based on Deferoxamine (mean dose 30 mg/kg/day) and phlebotomy performed in 9 patients when Hb level >8 g/dL, the amount blood removed ranged between 3 and 8 ml/kg every 2-4weeks. *Results.* The median follow-up of this study was 49±20 months (range: 19-85), the mean daily dose of HU was 16,9±1,8 mg/kg/d. 16 patients(7TI and 9TM)became completely TS free and 14 received 1 to 4 blood units per year throughout the study. The mean Hb level increase was 3,7g/dL in TI and 1,5 g/dL in TM group. The mean Hb level was 7,8±0,7 g/dL Serum ferritin values fell from pretherapy mean of 3295±2609(range:730-11020) to 1395±1214 (335-5696 ng/mL) (p<0,002) and was below 1000ng/mL in 16 patients. LVEF improved in 1 patient (54% to 65%), and remains preserved in all others (69±4%), any arrhythmia was noted. The growth velocity was improved: short height was observed only 7 patients, however any weight gain was observed in 13 patients. Sexual maturation was obtained in 13/15 patients older than 16 years (median age 18.9yrs). In addition facial deformities have regressed in the majority of patients. No serious haematopoietic complication was observed. All patients are alive and leading normal lives, they also felt significantly better. Compliance with treatment was good. *Conclusions.* The threshold of 7g/dL of Hb obtained, HU seems effective to allow normal activity of life, to promote linear growth, prevent cardiac damage and leads also improving in the management of iron overload, by reducing TS, allowing phlebotomy in some patients. HU appears to be safe and effective when administrated in thalassemic patients.

0849

MOLECULAR BASIS OF β-THALASSEMIA MUTATIONS IN AN URBAN AREA OF GAZIANTEP TURKEY

S. Pehlivan,[1] V. Okan,[2] E. Guler,[3] M. Yilmaz,[2] T. Sever,[4] E. Dikensoy,[5] C. Kilincarslan,[4] O. Balat,[5] M. Pehlivan[2]

[1]Gaziantep Uviversity Medical School, GAZIANTEP; [2]Gaziantep University School of Medicine, Department of Hematology, GAZIANTEP; [3]Gaziantep University Medical School Pediatric Oncology, GAZIANTEP; [4]Gaziantep University School of Medicine, Department of Medical Genetics, GAZIANTEP; [5]Gaziantep University School of Medicine, Department of Obstetrics and Gynecology, GAZIANTEP, Turkey

Background. β-Thalassemia is a genetic disorder, in which the mutations on the 11th chromosome of β-globulin gene cause a decrease or lack in β- globulin chain synthesis. It is estimated that in 3% of the world population thalassemia gene was inherited. This disease is commonly seen in Mediterranean countries, Africa, India, South Asia, Iran, and Turkey. The city of Gaziantep located just adjacent to the Mediterranean area of Turkey. Until recently more than 200 thalassemia mutations have been identified. *Aims.* To determine frequency of and types of β-thalassemia mutations in Gaziantep city. *Methods.* Blood taken from the patients were immediately transferred into EDTA-including tubes. From these samples DNA isolation was performed. PCR reaction was conducted at 25 µL consisting of the amplification mixture of 15µL relating to β-thalassemia kit, 1 unite Taq polymerases, 4 µL Taq dilution buffer and 5µL DNA. Initial denaturation was performed with 35 rotations at 2 minutes at 94°C, then 10 seconds at 94°C, 15 seconds at 54°C and 45 seconds at 72°C. The amplification process was completed after waiting for 3 minutes at 72°C. PCR products (206bp, 236 bp, 295 bp, 318 bp) were displayed under UV on horizontal electrophoresis using 3% agarose gel. Then, strips were placed into different wells for each patient to study in Auto-Lipa device. Steps for this process were performed and in 108 β- thalassemia patients and in amnion fluid in three of these patients, mutation analysis were performed in our molecular diagnosis department by the probes including 22 different mutations by reverse hybridization method. *Results.* 108 patients were included in the study (58 male, 50 female) between the ages of 1 and 52 (mean: 15.1±36.1). Totally 168 chromosomes were examined (48 heterozygote, 49 homozygote and 11 compound). The most detected mutations and their frequencies were: IVS 1.110 (G'A)%33.3(56/168), IVS 2.1 (G'A)%14.3(24/168), IVS 1.1 (G'A) %8.9(15/168), Codon 8(-AA)%6.5(11/168), -30(T'A) %6.0(10/168), IVS 1.6 (T'C) %5.4(9/168), Codon 39 (C'T)%4.8(8/168), Codon 44(-C) %3.6(6/168), Codon 8/9(+G) %2.4(4/168), IVS 1.5 (G'C) %2.4(4/168), Codon 36/37 (-T) %2.4(4/168), IVS 2.745 (C'G) %1.8(3/168), 22(7bp del)) %0.6(1/168), Codon 5(-CT)) %0.6(1/168). In three families that we made prenatal diagnosis the mutations were following: In the first family in both mother and father Codon 39 (C'T) heterozygote, in fetus Codon 39 (C'T) heterozygote; in the second family in both parents IVS 1.110 (G'A) heterozygote, in the baby no mutation was identified; in the third family in mother codon 36/37(-T), in father codon 44(-C) and in fetus Codon 39 (C'T) heterozygote. The mutation of the parents were included in 168 chromosomes, however the mutations of the fetuses were included. We did not identified any mutation in 12 chromosomes. *Conclusions.* Obtained data from this study were generally similar to the general population in Turkey; however, we found some regional differences. Currently, since the treatment of thalassemia is unavailable after birth, prenatal diagnosis is the best way of dealing with thalassemia disorder. In conclusion, after we determine the most frequent mutations, we believe that prenatal diagnosis frequency will promptly increase. Therefore, this will provide beneficial effects to both health population and to the country economy.

0850

HIGH LEVELS OF HUMAN GAMMA-GLOBIN ARE EXPRESSED IN ADULT MICE CARRYING A TRANSGENE OF THE BRAZILIAN TYPE OF HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN

A. Cunha,[1] A.F. Brugnerotto,[1] M.B. de Melo,[1] M.A.F. Corat,[1] A.P. Gimenes,[1] L.A.C. Passos,[1] E.E. Devlin,[2] D. Bodine,[2] S.T.O. Saad,[1] F.F. Costa[1]

[1]State University of Campinas, CAMPINAS, Brazil; [2]Hematopoiesis Section, NHGRI, BETHESDA, MD, USA

Hereditary persistence of fetal hemoglobin (HPFH), describes a hereditary benign disease, characterized by an increase in fetal hemoglobin (HbF) during adult life. Non-deletional forms of HPFH are characterized

by single-base mutations in the promoter region (most of them between -114 and -202 from the cap site) of either the gamma-G or gamma-A -globin gene, resulting in an increase of HbF ranging from 3 to 20% in heterozygotes. Many point mutations in this region have been described, including the gamma-A -195 C -G mutation that causes the Brazilian type of HPFH (HPFH-B). A previous study showed that the -195 mutations alone was not able to increase gene expression *in vitro* and a modest increase was observed when a LCR element fragment (HS2) was introduced in the construction. This study showed too, that the the mechanism of HbF elevation by the -195 mutation was neither mediated by the Sp-1 transcription factor nor by the creation of a CACCC box, as described for the -198 mutation. Thus, other proteins may be involved in the over-expression of the gamma-globin chain and/or may depend on DNA structure and these mechanisms remain to be clarified. To better understand this mechanism we have developed HPFH-B transgenic mice. The promoter with mutation was amplified using the genomic DNA of a HPFH-B patient and cloned in a µLCRA cosmid. This construct, containing the micro-LCR and other essential elements of human beta-globin gene cluster was microinjected into single cell mouse embryos. To detect the differences in developmental regulation of the human gamma-globin gene expression in the transgenic mice, we analyzed the yolk sac derived embryonic blood at embryonic day 10.5 (E10.5), the fetal liver of mouse embryos at E13.5, the newborn and adult (13 weeks) blood an the adult bone marrow of both mutated and non-mutated transgenic mice by RNAse Protection Assay (RPA) and Real time PCR (RT-PCR). Levels of expression of murine alpha-globin mRNA were used as internal controls in the RPA experiment and levels of murine-GAPDH were used in RT-PCR. mRNA levels of human gamma-globin of transgenic mice containing mutation were clearly higher, as compared with control transgenic mice bearing cosmid construct with wild a type sequence gamma promoter. Thus, our data indicate that the - 195 mutation is the unique cause of elevation of HbF in Brazilian HPFH, but the exact mechanism needs to be clarified. These results could provide the opportunity to study the modifying effects of the HbF in the phenotype of sickle cell disease and beta thalassemia.

0851

THE SAFETY AND TOLERABILITY OF A NEW FORMULATION OF DEFERIPRONE IN CHILDREN WITH TRANSFUSIONAL IRON OVERLOAD

M. El-Alfy,[1] F.T. Tricta,[2] A. El-Beshlawy,[1] M. El-Tagui,[1] I. Youssry,[1] M. Hamdy,[1] M. Ghamrawy[1]

[1]Pediatric Hospital, Cairo University, CAIRO, Egypt; [2]ApoPharma Inc., TORONTO, Canada

Limited data are available on the use of deferiprone in young children. The current multi-national, open label study evaluated the tolerability of a new liquid formulation of deferiprone in iron-overloaded pediatric patients with transfusion-dependent anemias. The study also evaluated the absolute neutrophil count (ANC) of children who were maintained on deferiprone therapy during episodes of mild neutropenia [ANC < 1.5×10^9/L but > 1.0×10^9/L]. Main inclusion criteria for enrollment were 1) ≤ 10 years of age; 2) transfusion-dependent anemia; 3) have received ≥8 transfusions/ year for a minimum of 1 year; 4) serum ferritin > 1000 µg/L. The study was approved by the relevant regulatory authorities and ethics review boards. Informed consent was obtained from the patients' legal representatives. One hundred children [Thal major=91, HbE=8, DCF=1; 45 female and 55 male; 76 Caucasian (Egyptian), 24 Asian (9 Chinese, 13 Indonesian, 2 Malaysian)] ranging from 1.5 to 10 years of age (median age=5.1 years) were enrolled. At enrollment, 52 children were being treated with deferoxamine (mean duration = 1.9 ± 2.1 years; range from 0.1-7.3 years), 20 with deferiprone (mean duration = 0.5 ± 0.6 years; range from 0.1-2 years), 8 patients with deferasirox (mean duration = 0.4 ± 0.5 years) and 20 patients were naïve to chelation therapy. Mean ± SD serum ferritin at time of enrollment was 2521.9 ±1458.9 µg/L (range 1002-7480 µg/L). Deferiprone therapy was initiated at a total daily dose of 50 mg/kg, divided in 3 doses, for the first 2 weeks and then increased to a total daily dose of 75 mg/kg. The dose could be further increased to 100 mg/kg/day for those patients with ferritin > 2500 µg/L at baseline. The oral solution was tolerated well by all children and there were no unexpected adverse reactions. The data suggest that there was lower incidence of gastrointestinal adverse reactions (vomiting; 6% of patients; abdominal pain= 3% and no reports of nausea) than what has been reported with the tablet formulation of deferiprone (nausea= 16% of patients; vomiting=13%; abdominal pain=14%). One case of arthralgia and one of agranulocytosis (ANC < 0.5×10^9/L) was observed. Deferiprone was discontinued at onset of agranulocytosis, G-CSF was prescribed and the event resolved in 9 days. Mild neutropenia was observed in 3 children. Deferiprone therapy was not discontinued during those episodes and the ANC was monitored daily. No G-CSF was used. One episode of mild neutropenia resolved in 3 days, whereas the other two episodes resolved in 5 days and there were no subsequent recurrence of neutropenia. In summary, 3 patients experiencing mild neutropenia (1.0×10^9/L≤ ANC < 1.5×10^9/L) remained on deferiprone and none progressed to agranulocytosis. Treatment with the oral solution of deferiprone was well tolerated by children, the frequency of adverse reactions was lower than what has been observed with the tablet formulation of deferiprone, and its use was not associated with new safety concerns.

0852

MAGNETIC RESONANCE MUTISLICE MULTIECHO T2* TECHNIQUE FOR SEGMENTAL AND GLOBAL QUANTIFICATION OF MYOCARDIAL IRON: MULTI-CENTRE VALIDATION OF THE TRANSFERABILITY IN THE MIOT (MYOCARDIAL IRON OVERLOAD IN THALASSEMIA) NETWORK

A. Pepe,[1] A. Ramazzotti,[1] A. Maggio,[2] P. Cianciulli,[3] M. Centra,[4] V. Caruso,[5] D. Maddaloni,[6] V. De Sanctis,[7] E. Grassedonio,[8] M. Brizi,[9] G. Valeri,[10] G. Restaino,[11] A. Luciani,[12] D. De Marchi,[1] G. Rossi,[1] V. Positano,[1] M. Lombardi[1]

[1]Institute of Clinical Physiology, CNR, PISA; [2]Ematologia II con Talassemia, "V. Cervello" Hospital, PALERMO; [3]Centro Talassemie, Sant'Eugenio Hospital, ROMA; [4]Centro Microcitemia, Casa del Sollievo IRCCS Hospital, SAN GIOVANNI ROTONDO (FOGGIA); [5]Centro Microcitemia Garibaldi Hospital, CATANIA; [6]Department Materno/Infantile, 'Engles Profili' Hospital,, FABRIANO (ANCONA); [7]Department Reproduction and Growth, Pediatric Adolescent Unit, St Anna Hospital, FERRARA; [8]Department of Radiology, University of Palermo, PALERMO; [9]Department of Radiology, Catholic University, ROMA; [10]Department of Radiology, University of Ancona, ANCONA; [11]Radiology Department, "John Paul II" Catholic University, CAMPOBASSO; [12]Institute of Radiology, Garibaldi Hospital,, CATANIA, Italy

Background. Iron induced cardiomiopathy remains the main cause of mortality in thalassimia major population. For this reason, strategies to reduce heart disease by improving chelation regimens have of the highest priority in this phase. These strategies include validated and available evaluation of cardiac iron status, and careful epidemiological assessment of thalassemia patients living in industrialized countries with high health standards. T2* magnetic resonance imaging (MRI) is the unique technique to quantify myocardial iron burden to tailor the chelation therapy. Assessment of myocardial iron loading using the multislice, multiecho T2* approach accounting for a segmental and global myocardial iron distribution, has only been performed at the MRI centre in Pisa. *Aims.* To assess the transferability of the multislice, multiecho T2* technique in 6 different Italian MRI sites setting-up a reliable MIOT (Myocardial Iron Overload in Thalassemia) network where a substantial number of thalassemia patients can be scanned with homogeneous standard procedures. *Methods.* Heart mutislice multiecho T2* and liver T2* MR acquisition sequences were installed on MR scanners (GE Healthcare 1.5 T) at 6 different sites in Italy. First, 5 healthy subjects (n=30) were scanned in each centre to verify the homogeneity of normal T2* ranges. Then, T2* was assessed locally in 5 thalassemia major patients (n=25), and subjects were rescanned at the standardization centre in Pisa within two months. Written informed consent was obtained from all subjects. Among the 25 enrolled patients, abnormal global heart T2*, T2* in the mid-ventricular septum and liver T2* values were present in the 45%, 40% and 20%, respectively. Continuous groups data on healthy subjects were analyzed by one-way analysis of variance (ANOVA), using a post hoc analysis (Sheffé). Coefficients of variation (Cv) were calculated as the ratio of the standard deviation of the mean square of the differences between the repeated values, to the general mean. Interclass correlation coefficients (ICC) were obtained choosing a two-ways mixed model for reliability analysis. Statistical analysis was performed by SPSS software *Results*. On healthy subjects we did not find significant differences among the centres about all 16 segmental and global heart, and liver T2* values. The Cv calculated for each MR site for global heart T2*, T2* in the mid-ventricular septum and liver T2* values ranged from 0.04 to 0.12 (mean 0.09), from 0.07 to 0.22 (mean 0.12) and from 0.10 to 0.18 (mean 0.13), respectively, with mean absolute differences in T2* of 0.9, 0.8 and 0.7 ms, respectively. The ICC for global heart T2*, T2* in the mid-ventricular septum and liver T2* values were 0.96, 0.94 and 0.96, respectively. *Summary and Conclusions*. The MR mutislice multiecho T2* technique is transferable among scanners with good agreement, reproducibility and homogeneity, although the majority of enrolled patients

showed normal T2* values. In fact, the T2* sequence was designed to have the higher accuracy in the abnormal range. The myocardial iron T2* estimations by segmental and global approach and the liver T2* assessment were consistent among the centres validating the MIOT network as a reliable system where a substantial number of thalassemia patients (more than 1000 per year) can be scanned with homogeneous standard procedures.

0853

SPINAL CORD COMPRESSION AND EXTRAMEDULLARY HEMATOPOIESIS IN YOUNG β -THALASSEMIA PATIENTS

A.A.G. Tantawy, A.A.M. Adly, S.A.R. Mahdy, G.Z. Kamel

Faculty of Medicine,Ain Shams University, CAIRO, Egypt

Background. B-thalassemia is a significant public health problem in Egypt where over 1000 of the annual 1.5 million newborns are expected to be affected with this disorder. Extramedullary hematopoiesis (EMH) is a compensatory phenomenon that occurs in patients with chronic hemolytic anemias when bone marrow function is not sufficient to maintain the circulatory demand. It is a common manifestation in thalassemia in response to long standing anemia. Spinal cord compression as a consequence of extramedullary hematopoiesis in the intraspinal epidural space is a rare complication, though reported in thalassemia. Aim of the study: to assess the problem of spinal cord compression related to extramedullary hematopoiesis in young β-thalassemia patients, both clinically and radiologically and its correlation with the laboratory parameters of anemia and hemosiderosis. Design:The present study was conducted in the Hematology Unit, Children's Hospital, Ain Shams University, Cairo, Egypt. It included 60 patients with B-thalassemia divided into two groups; Group I:40 patients with B-thalassemia major (TM) aged 7-30 years with a mean of 15±5.3 years, group II: 20 patients with B-thalassemia intermedia (TI) aged 6-20 years with a mean of 13±4.6 years. They were subjected to full history taking, thorough clinical examination especially neurological examination. Laboratory and radiological investigations included: complete blood count, hemoglobin electrophoresis ,serum ferritin ,thoracic and lumbosacral CT scan for all patients as a screening technique for EMH, MRI for suspected patients having spinal EMH to confirm the diagnosis. *Results.* Spinal EMH was found in 13.3% of thalassemic patients (8 out of 60) with increasing incidence in thalassemia intermedia (20%) compared to thalassemia major patients (10%) (p= 0.03). EMH was more frequent in the older age group; 40% in TI patients aged > 15 years and 13% in TM patient aged > 15 years. Patients with radiological evidence of spinal EMH had significantly higher mean serum ferritin compared to patients without EMH (2625±474 ngm/mL *vs* 1503±1013 ng/mL, respectively;p<0.0001), had lower mean pretransfusion hemoglobin (5.8±2.4 gm/dL *vs* 7.2±0.8 gm/dL; p<0.002) ,and had lower transfusion index (83±20 ml/kg/y *vs* 120±31 ml/kg/y respectively; p<0.0001). Patients with spinal EMH complained of vague neurological symptoms (back pain, numbness of the lower limbs). 3% of thalassemic patients in this study had neurological symptoms without radiological evidence of EMH, so other causes of neurological insult have to be considered. *Conclusions.* EMH in thalassemia is not uncommon as it was thought. It is more evident in TI patients, starting from the second decade of life and is mainly related to inadequate transfusion therapy and inadequate chelation.

0854

IRON OVERLOAD AND THE ROLE OF ORAL CHELATORS IN THE MANAGEMENT OF A PAEDIATRIC POPULATION ON CHRONIC TRANSFUSION PROGRAMMES: EFFICACY AND TOLERABILITY

R. Desmond, H. Conway, H. Elwan, R. Geoghegan, C. McMahon

Our Lady's Children's Hospital, DUBLIN 12, Ireland

Chronic transfusion programmes have significantly altered life expectancy in individuals with Beta Thalassaemia Major and those with life-threatening complications of Sickle Cell Disease. Iron overload is an inevitable complication of long-term red cell transfusions and myocardial siderosis is invariably fatal without iron chelation. Desferioxamine administered subcutaneously or intravenously has been standard iron chelation therapy for many years but poor compliance has inevitably led to treatment failures. Deferasirox (Exjade), an oral iron chelator, has now become available in Europe. Phase 3 studies have demonstrated that it is effective in the management of severe iron overload and improves long-term compliance. However, Deferasirox does have the potential for serious toxicity. Patients need regular monitoring of kidney and liver function. Recent post marketing surveillance has identified irreversible renal failure and cytopenias as potential life-threatening toxicities. The underlying mechanism behind the serum creatinine increases is unknown, the increases may be related to an overchelation effect in patients who are infrequently transfused. Our patient cohort of sixteen children (10M: 6F) aged 4-17 years (mean 8.5 yrs) have been treated with Exjade for 3-15 months (median 9 months). Thirteen children had sickle cell disease, 2 ,-Thalassaemia Major and 1 congenital hypoplastic anaemia. All patients were monitored with monthly serum ferritin and 2 to 4 weekly renal and liver function tests. Fifteen had previously been treated with Desferioximine for 6-73 months (mean 32) and the majority (13/16) were commenced on Exjade because of poor compliance and increasing Ferritin levels. The first 2 children commenced on Exjade at a dose of 30mg/kg/day and developed intractable puritus which resolved only when the drug was temporarily discontinued. Thereafter all children were commenced on Exjade at between 6 and 15mg/kg/day and increased without further episodes of puritus. The mean tolerated dose was 22mg/kg/day (range 11.3-29.2). Thirteen patients required dose reductions; 10 because of rising transaminase levels and 3 because of decreasing creatinine clearance. Treatment was discontinued in 6 patients (1 with deteriorating renal function, 4 with deterioration of liver function tests and 1 whose transfusion programme was discontinued). All patients with liver toxicity had sickle cell disease and were of African ethnicity. No haematological side effects were noted. The median change in serum ferritin was -548mg/L. Exjade is a new oral iron chelator which is clearly efficacious. It is a significant breakthrough for the management of iron overload but it is not without side effects. In our experience Exjade therapy should be commenced at a lower dose than suggested. While this drug is a welcome development in the management of children on transfusion programmes there are significant toxicity issues.

0855

ISOVOLEMIC ERYTHROCYTAPHERESIS TECHNIQUE AS AN ALTERNATIVE FOR CONVENTIONAL PHLEBOTOMY IN PATIENTS WITH POLYCYTHEMIA AND HEMOCHROMATOSIS.

P. Wijermans, L. Van Egmond, P. Ypma, J.L. Kerkhoffs, M. Schipperus, L. Bohmer, E. Agteresch

Haga Hospital, THE HAGUE, Netherlands

Introduction. Patients with a primary or secondary erythrocytosis or iron overload (hemachromatosis, alpha thallasemia) are often treated with phlebotomy to reduce the erythrocyte volume and/or to reduce the iron stores. Frequently, repeated phlebotomies are necessary to achieve the treatment target. However this procedure not seldom causes side effects and the desired effect is not always reached. We developed an erythrocytapheresis technique based upon an isovolemic principle that can be used to remove larger amounts of erythrocytes and is experienced as more convenient by the patient. *Technique*. The erythrocytapheresis is performed according to the standard procedures using a Cobe Spectra. In this study we compared a standard fixed volume apheresis of 500 mLwith a patient tailored erythrocyte depletion with a lowering of the Ht as indicated by the physician. The removed erythrocyte volume was replaced with an equal volume of albumin and ACD. *Patients*. Twenty-three hemochromatosis (HC) patients with a median age of 52 years (range 20-78y) underwent 217 procedures and 27 polycythemia vera patients (PV) (median age 58y range 37-98y) were treated with 115 erythrocytaphereses. One patient with iron overload due to alpha thalassaemia (alpha Thal) was treated with 10 procedures and 15 patients with sec. polyglobulinaemia (sec PG) (median age 59y range 47-79y) underwent 53 procedures. *Results*. A total number of 395 procedures were performed in 66 patients. The oldest patient who underwent this treatment was 89 years old. Only one patient with hemochromatosis complained because of suffering of symptoms of anaemia (Ht after the procedure 0.35). All patients who could compare this treatment with the conventional phlebotomies experienced this treatment as much more convenient. In 4% of the patients the apheresis was a failure i.e. insufficient compared with the calculated removal of erythrocytes with phlebotomy. Success as defined by a removal of more then 1.5 x the erythrocyte volume that would be removed with a phlebotomy was observed in 85%, 48%, 58% and 80% of the procedures in the HC, PV, sec PG patients and alpha Thal patient respectively. In 17% of the procedures the apheresis was > 2.5 times more effective in lowering the erythrocyte volume. Standard fixed volume apheresis was as effective as the procedures based on calculated desired Ht changes. With the apheresis we were able to remove a median of 19% of the erythrocyte volume in the hemachromatosis patients. This was an improvement of 99% (twice as effective) as compared with phlebotomy. In the PV patients the erythrocyte volume was decreased by 15%, an improvement of 69%. In the

patients with secondary polyglobulinemia this procedure was less effective (only 5%) with a median improvement of 54%. The treatment goal, lowering the iron overload in HC and the thalassemia patient was seen in all but one patient. In the PV patients the Ht was sufficiently lowered in all patients. However in three patients this procedure was less effective then conventional phlebotomy. *Conclusions*. Isovolemic erythrocytapheresis is an efficient and save alternative for phlebotomy. It is experienced as convenient by the patients

0856

CORRELATION OF RET-Y WITH SOLUBLE TRANSFERRIN RECEPTOR, HEMOGLOBIN A2 AND HEMOGLOBIN F LEVELS IN β-THALASSEMIA TRAIT

A. Agorasti, N. Stylianidou, D. Konstantinidou

General hospital of Xanthi, XANTHI, Greece

Background. RET-Y (the mean value of the forward-scattered light histogram within the reticulocyte population, generated by Sysmex analyzer) and CHr (the reticulocyte hemoglobin content, generated by Bayer) measure the same phenomenon: the reticulocyte hemoglobinization (Thomas, Clin Chem Lab Med, 2005). CHr and RET-Y can both be used as a gold standard for iron-deficient erythropoiesis. CHr values correlate negatively with hemoglobin A2 values in iron sufficient β-thalassemia heterozygotes (Skarmoutsou, Haematologica, 2003). *Aims.* The aim of this study is to investigate the correlation of RET-Y, expressed in arbitrary units (AU), with soluble transferrin receptor (sTfR), hemoglobin A2 (HbA2) and hemoglobin F (HbF) values in β-thalassemia trait. *Patients and Methods.* One hundred five patients with β-thalassemia trait (29 men, 76 women, aged 18-62 years) assigned as group A were selected to enter the study. Forty healthy individuals (15 men, 25 women, aged 18-59 years), hematologically normal, constituted the control group designated as group B. Specimens were analyzed on a Sysmex XT-2000i instrument in the reticulocyte channel for complete blood cell count (RET-Y is provided by service data). The separation of hemoglobin fractions was performed by automated high-performance liquid chromatography (G7 β-Thalassemia Mode, HLC 723G7, TOSOH). Ferritin was determined on an ADVIA Centaur® CP Immunoassay System (Chemiluminescence, Bayer) and sTfR was measured using the Dade Behring BN ProSpec® Nephelometer. Statistical analysis. the data were expressed as the mean ± SD and analyzed with Student test, Pearson correlation and regression analysis. Statistical significance was set at P value <0.05. *Results.* The hematological profile of the two groups and the comparison between the groups are presented in Table 1.

Table 1. Data are presented as mean±SD, *p=0.000, "p=0.656.

Group, n	Hb, g/dL	Ferritin, ng/mL	sTfR, mg/L	RET-Y, AU	HbA2, %	HbF,%
A, 105	11.4±1.5 *	142.0±50.6†	2.34±0.68*	1291±112*	5.4±0.8*	1.7±0.6*
B, 40	13.5±1.1	64.9±24.1	1.22±0.26	1760±61	3.0±0.3	1.1±0.3

In group A, sTfR levels present a statistically significant positive correlation with both HbA2 (r=0.391, p=0.017) and HbF (r=0.401, p=0.014) levels. In the same group RET-Y levels correlate negatively in a statistically significant degree with sTfR (r=-0.624, linear regression equation: y=-107.32x+1546.2, p=0.000), HbA2 (r=-0.282, linear regression equation: y=-38.474x+1502.2, p=0.004) and HbF (r=-0.246, linear regression equation: y=-45.613x+1370.3, p=0.011). In group B the same parameters did not show statistically significant correlation (sTfR *vs* HbA2 and HbF, p=0.906 and p=0.791, respectively; RET-Y *vs* sTfR, HbA2 and HbF, p=0.231, p=0.717, p=0.983, respectively). *Conclusions.* The results from the patients with β-thalassemia trait showed a good negative correlation between RET-Y and sTfR values (r=-0.624) and a weak but statistically significant negative correlation between RET-Y, HbA2 and HbF levels (r=-0.282 and r=-0.246, respectively). The regression lines for both comparisons (RET-Y *vs* HbA2 and RET-Y *vs* HbF) showed low slopes (38.474 and 45.613, respectively) demonstrating that a relatively high decrease of HbA2 and HbF levels (found in patients with mild genotypes) implies a relatively low raise of reticulocyte hemoglobinization.

0857

MYOCARDIAL IRON OVERLOAD AND LEFT VENTRICULAR DIASTOLIC FUNCTION IN MULTITRANSFUSED PATIENTS WITH THALASSEMIA AND ACQUIRED ANEMIAS: CORRELATION OF TISSUE DOPPLER ECHOCARDIOGRAPHY WITH MAGNETIC RESONANCE IMAGING (MRI)T2*

A. Fragasso,[1] A. Ciancio,[2] C . Mannarella,[2] G. Centonze,[2] S. De Santis,[2] C. Ottonello,[2] C. Turchetti[2]

[1]*Dipartimento di Medicina Interna, Unità semplice di Ematologia, MATERA;* [2]*Ospedale di Matera, Hematolgy Unit, MATERA, Italy*

Iron-induced cardiomyopathy is the commonest cause of death for patients suffering from thalassemic disease, as well a recurrent request in the case of multitransfused patients with acquired anemias. MRI T2* is, at the moment, the gold standard in assessing myocardial iron. Diastolic dysfunction is one of the first signs of myocardial dysfunction in cardiac hemosiderosis. We measured the following left ventricular (LV) diastolic function indexes: early mitral valve flow velocity (E), late mitral valve flow velocity (A), early tissue Doppler (TD) lengthening velocity (E'), late TD lengthening velocity (A'), and the ratios E/A, E/E', E'/A'. The aim of our study was to find Echo measurements that might be correlated with MRI T2*. We compared the Echo parameters E/A, E/E', E'/A' with MRI T2* found in 2 subsets of patients: 1) 30 with thalassemia major (15 males, 15 females), 30 years median age (range 17-45); 2) 8 multitransfused patients (5 males, 3 females) with acquired anemias (7 with myelodysplastic syndromes and 1 with aplastic anemia), 74 years median age (range 70-84). The latter group of patients received a median number of 52 (range 12-139) packed red blood cell units; all were negative for HFE mutations and 5 of out 8 patients were on iron chelation. Median serum ferritin values were 1659±670ng/mL in thalassemic patients and 2029±1309ng/mL in the other ones. In the group 1) cardiac MRI T2* median values were 26±7msec; pathologic value (<20msec) was found in 10 (33%) patients. None had impaired systolic function (LV shortening fraction<30%). Echo results are the following: E/A=2±0,3 (n.v.<2); E/E'=5±0,3 (n.v.<8); E'/A'=2±0,2 (n.v.<2). Serum ferritin was negatively associated with T* values (R=-0,40, p<0,05), but 3 patients with serum ferritin<1000ng/mL had a T2* value<20msec. Echo parameter E/A attained a statistically significant correlation to T2* values (p=0,005), while E/E' and E'/A' didn't (p=0,5, p=0,16, respectively). We divided the patients according to their T2* values into subgroup 1a (T2*<20msec), and 1b (T2*>20msec). In the subgroup 1a we found: median T2* values 13±3, E/A=2,05±0,6, E/E'=5±0,4, E'/A'=2,2±0,7. In the subgroup1b the findings were: median T2* values 32±5, E/A=1,5±0,3, E/E'=5,5±1,4, E'/A'=1,8±0,6. Comparing echo data between subgroups 1a and 1b, we discovered a significant difference only for E/A ratio (p<0,05), although this index was pathologic in 3 patients with T2* values>20msc. In the group 2) cardiac MRI T2* median values were 37±10 msec; pathologic value (<20msec) was found in none. Echo results (E/A=0,73±0,09, E/E'=7,5±2,2, E'/A'=0,61±0,1) were in the normal range for the age. *Conclusions.* 1) our experience in thalassemic patients the E/A ratio, measured with conventional echocardiography, showed a better correlation with MRI T2* values than the parameters E/E' and E'/A', measured with Doppler TDE did; 2) in patients with acquired anemias more transfusions are likely required to induce cardiac hemosiderosis.

0858

HEREDITARY HEMOCHROMATOSIS: EARLY PHENOTYPIC EXPRESSION IN YOUNG C282Y HOMOZYGOUS WOMEN

P. Aguilar-Martinez,[1] S. Cunat,[1] C. Rose,[2] M. Giansily Blaizot,[1] J.F. Schved[1]

[1]*CHRU de Montpellier, MONTPELLIER;* [2]*University of Lille, Saint-Vincent-de-Paul, hospital, LILLE, France*

Background and Aims. The homozygous HFE-C282Y genotype is responsible for the common adult form of Hereditary Hemochromatosis (HH), an apparently simple recessively inherited monogenic disorder. Since the description of this genotype in 1996, extensive epidemiological studies have progressively demonstrated that as few as 1% of the C282Y homozygotes should express the disease. This incomplete penetrance seems particularly relevant in women who have non-genetic factors of attenuation such as menstruation bleedings and pregnancies. Unexpectedly, a few number of young C282Y homozygous women present with early clinical or biological expression of iron overload. *Methods.* We have reviewed the cases of C282Y/C282Y young women (<30 years) diagnosed with early and/or severe manifestations of iron

overload in our laboratory. We identified 5 such patients among 5500 individuals of both genders referred to us for HFE genotyping since 1996. For all of them, an intensive search of acquired or genetic causes of iron overload has been performed. *Results*. All 5 young females, age ranging from 13 to 28, presented with raised serum ferritin levels and high transferrin saturation. A high hepatic iron concentration was shown in 3 of them by MRI, and one needed more than 26 phlebotomies to decrease ferritin levels under 50 µg/L. Two, aged 13 and 18 were found to carry an additional iron gene mutation. They were heterozygous for an HJV (hemojuvelin) c.[-89-4dupT] and a TFR2 p.V183V mutation respectively. Although the causal implication of these additional sequence alterations needs further investigations, such associations have already been described in the literature and referred to as digenism. Two other girls, aged 14 and 28 at diagnosis, had an associated globin gene disorder. The first one was diagnosed with heterozygous beta thalassemia at the age of 7 and developed progressive iron overload during the adolescence, whereas the second had a beta globin gene variant at the heterozygous state. For the fifth girl, who had hypogonadism of unknown cause at 18, no additional genetic abnormality has been found until now. *Discussion and Conclusions*. The HFE gene is not considered the first candidate when dealing with young women or female adolescents with a phenotypic expression of primitive iron overload. They should primarily be screened for abnormalities of genes implicated in juvenile hemochromatosis, such as HAMP (hepcidin) or HJV. Furthermore, when a homozygous C282Y genotype is found, it must not be considered a sufficient explanation for an early phenotypic expression, and additional acquired or genetic factors must be investigated. Among these, defects of iron genes, including HJV, HAMP and TFR2 mutations, as well as globin genes abnormalities should be the main targets.

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0859

RFLP LINKED MARKERS USED TO INFER THE ORIGIN OF β-THALASSEMIA MUTATIONS IN ROMANIAN POPULATION

L Cherry,[1] P. Perrin,[2] M. Currat,[3] C. Calo,[4] R. Talmaci,[5] L. Dan,[1] D. Coriu,[5] L. Gavrila[1]

[1]Genetics Institute of Bucharest University, BUCHAREST, Romania; [2]Université de Montpellier 2, MONTPELLIER, France; [3]AGB Lab, Department of Anthropology and Ecology, University of Geneva, GENEVA, Switzerland; [4]University of Cagliari, MONSERRATO, Italy; [5]Hematology Department, University of Medicine and Pharmacy Carol Davila, BUCHAREST, Romania

To determine the origin and the spread of beta-thalassemia mutations among Romanian and Mediterranean populations we have investigated the RFLP haplotypes associated with the most preponderant β-thalassemia mutations IVSI-110(G 'A), cd39(C'T), IVSII-745(C'G), IVSI-6(T'C), IVSI-1(G'A) and cd8(-AA) in the Romanian population. Furthermore to investigate the migration events between Romania and some other European and Asian countries we have studied beta-globin haplotypes associated with normal Romanian chromosomes and compare our results with those obtained by other laboratories from the study of other different populations performed on the same topics and published elsewhere. This study comprises 104 and 87 normal and thalassemia chromosomes respectively. A clinical analysis based on the haematological parameters followed by a molecular analysis using PCR based methods; DGGE, Light Cycler, ARMS-PCR and RFLP-PCR were done. The haplotype analysis was realized studying seven restriction sites including: Hinc II 5' to ε, Hind III sites in the Aγ and Gγ genes, Hinc II sites in the Ψβ locus, an Ava II site in the IVS II of β gene, and finally a Bam HI site 3' to the β-globin gene. Finally, to accomplish this study we have calculated the haplotype frequency estimation using an expectation-maximization algorithm (Arlequin 2000 software) and R-Matrix (Harpending *et al.*, 1973). The obtained results are as following: IVSI-110 mutation was found to be associated with three different RFLP-haplotypes I (+----++), IX (-+-++++) and (-+-++--), mutation cd39 with haplotypes I (+----++), II (-++-+++) and (----++-), while mutation IVSI-6 with haplotypes V (+----+-), V (-++---+) and I (+-----++). On the other hand, mutations IVSII-745, IVSI-1, and cd8 are associated with haplotypes VII (+------+), V (+----+-) and VII (+-----+), respectively. The new association of IVSI-110, cd39 and IVSI-6 mutations with haplotypes (-+-++--), (----++-) and V (+----+-), respectively, could be the result of possible recombination events, and suggests the old origin of these mutations in Romania. R-Matrix result comparing RFLP haplotypes associated with βA chromosomes in different populations including Romanian has shown a relative low degree of genetic affinity between Romanian population and its neighboring European and Mediterranean people. All these results reflect the migration and settlement events which occurred in the past in the Carpathian area.

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0860

ELEVATION OF CYSTATIN-C AND REDUCTION OF TNF-ALPHA IN PATIENTS WITH THALASSEMIA AFTER 12 MONTHS OF DEFERASIROX ADMINISTRATION

E. Voskaridou,[1] E. Plata,[1] D. Christoulas,[2] C. Xirakia,[1] P. Tsaftaridis,[1] E. Stoupa,[2] A. Papatheodorou,[2] E. Terpos[2]

[1]Thalassemia Center, Laikon General Hospital, ATHENS; [2]Department of Medical Research, 251 General Air Force Hospital, ATHENS, Greece

Background and Aims. Iron overload is a sever problem in beta-thalassemia major (TM). Deferasirox is an oral iron chelator approved for the management of iron overload in TM. However, there are some concerns for its effect on renal function. Cystatin C (Cys-C) is a cysteine protease inhibitor, which is considered as an accurate endogenous marker of GFR. It is generally accepted that Cys-C is superior over serum creatinine (Cr) in terms of diagnostic sensitivity for reduced GFR. Inflammation process has been recently implicated in TM pathophysiology. The aim of this study was to evaluate the effect of deferasirox on renal function and inflammatory cytokines in TM. *Patients and Methods.* Fifty-two TM patients were evaluated (22M/30F, median age 39.5 years). Deferasirox was administered at a dose between 10-30 mg/kg/day for a period of 12 months. Serum Cys-C, Cr, clearance of creatinine (Ccr), albuminuria and inflammatory cytokines including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), IL-1alpha, IL-1beta, IL-4, IL-10 and transforming growth factor (TGF)-beta1 and beta2, were measured at baseline and then after 6 and 12 months post-deferasirox therapy. Standard hematology and biochemistry was evaluated monthly. Serum Cys-C was measured using a latex particle-enhanced nephelometric immunoassay (Dade Behring, Liederbach, Germany). Serum levels of the above cytokines were determined using ELISA methodology (R&D Systems, Minneapolis, MN, USA, for ILs, and Diaclone, Bensancon, France for TNF-alpha, TGF-beta1 and TGF-beta2). Ten healthy blood donors of similar age and gender were also evaluated as control group. *Results.* At baseline, TM patients had elevated values of Cys-C ($p<0.0001$) compared with controls. Specifically, 21/52 patients (40%) had higher Cys-C values than the upper normal limit according to manufacturer (0.95 mg/L), while no patient had increased levels of serum Cr (>1.4 mg/dL) and only 6 (11.5%) had low Ccr (<80 ml/min). Before deferasirox administration, TM patients had also increased levels of IL-6 ($p=0.008$), IL-1apha ($p=0.015$), TGF-beta2 ($p=0.017$), IL-10 (0.021), IL-4 ($p=0.039$), and a borderline increase of TNF-alpha ($p=0.05$) compared with controls. Serum levels of Cys-C correlated strongly with Cr (r=0.657, $p<0.0001$), and Ccr (r=-0.625, $p<0.0001$) but also with IL-6 (r=0.441, r<0.001) and proteinuria (r=0.261, $p=0.037$). Il-1alpha correlated with Hb (r=-0.417, $p<0.001$) and IL-4 (r=0.474, $p<0.001$), while IL-6 correlated with TNF-alpha (r=0.37, $p<0.01$). After 6 and 12 months of therapy, deferasirox produced a dramatic reduction of ferritin, SGOT and SGPT compared with baseline values ($p<0.0001$), but concomitantly we observed an increase of Cys-C and Ccr during the same period ($p<0.0001$). In particular at the end of the study 32/52 patients (61.5%) had increased Cys-C values, while 10 (19.2%) had low Ccr and only one high serum Cr. Interestingly serum levels of TNF-alpha reduced post-deferasirox administration ($p=0.01$), while the levels of all other cytokines remained unchanged during therapy. *Summary and Conclusions.* Our study suggests that deferasirox is an effective chelator in TM. However, its effect on renal function is not insignificant and needs further investigation. Inflammatory cytokines seem to have a role in the pathogenesis of TM but further studies are needed to fully elucidate this role as well as the effect of deferasirox, if any, on inflammation.

0861

CORRELATIONS OF NON TRANSFERRIN BOUND IRON LEVELS IN 74 PATIENTS WITH THALASSEMIA INTERMEDIA

F. El Rassi,[1] A. Taher,[1] A. Inati,[2] S. Koussa,[3] M.D. Cappellini[4]

[1]American University of Beirut Medical Center, BEIRUT, Lebanon; [2]Rafic Hariri University Hopsital, BEIRUT, Lebanon; [3]Chronic Care Center, HAZMIEH, Lebanon; [4]University of Milano, MILANO, Italy

Background. Unlike patients with thalassemia major (TM), patients with thalassemia intermedia (TI) do not require regular blood transfusion therapy but are still susceptible to iron overload due to increased intestinal iron uptake triggered by ineffective erythropoiesis. Effective monitoring of iron burden is therefore an important element of patient management. Assessment of serum ferritin (SF) levels is a convenient and widely used method. In addition, newer and more precise methods have been devised to assess the iron status in such patients; non transferrin bound iron (NTBI) determination is one of them. Plasma non-transferrin-bound-iron (NTBI) is believed to be responsible for catalyzing the formation of reactive radicals in the circulation of iron overloaded subjects, resulting in accumulation of oxidation products. NTBI has been studied in thalassaemias but results have been conflicting. In this study of patients with TI, evaluation of the correlation of NTBI and other variables including SF, liver iron content and complications is attempted. *Methods.* This was a cross-sectional study of randomly selected TI patients treated at a chronic care center in Hazmieh, Lebanon. Patient charts were reviewed and a medical history, including previous blood transfusion therapy, was compiled. Blood samples were taken for NTBI, SF assessment and LIC was determined by R2* MRI. *Results.* Data from 74 patients were included in this analysis (33 male, 41 female; mean age 26.5±11.5 years). Of this group, 59 (79.7%) of patients were splenectomized. Overall mean NTBI values were 2.92±3.43, mean SF values were 1023±780 ng/mL (range 15±4140) and mean LIC levels were 9.0±7.4 mg Fe/g dry weight [dw] (range 0.5±32.1). A significant positive correlation between mean NTBI and SF values was seen (Pearson correlation 0.421; p=0.000). The comparison of NTBI data to LIC was significant (Pearson correlation 0.36; p=0.002). As far as complications are concerned, comparison of NTBI with the respective complications of pulmonary hypertension, thrombosis, leg ulcers, and endocrine dysfunction was not significant. *Conclusions.* NTBI is a good modality for determining iron content in thalassemia. Significant correlations were determined between NTBI and SF in turn and NTBI and LIC adding strength to the above statement. In this study, TI patients had significant levels of NTBI. This strengthens the fact that thalassemia intermedia patients tend to be iron overloaded. NTBI will be the essential in following up the iron status of TI patients when iron chelation therapy is employed.

Thrombosis II

0862

THE 408-427 REGION OF THE FIBRINOGEN ELONGATED G-CHAIN INHIBITS THROMBIN-INDUCED PLATELET ACTIVATION, HINDERING THE INTERACTION WITH DIFFERENT RECEPTORS

R. De Cristofaro,[1] S. Lancellotti,[1] S. Rutella,[1] V. De Filippis,[2] B. Rocca[3]

[1]Catholic University School of Medicine, ROMA; [2]3Department of Pharmaceutical Sciences, University of Padua, PADOVA; [3]4Center of Excellence on Aging, G. d'Annunzio University Foundation, Chieti, CHIETI, Italy

Background. The expression of the elongated fibrinogen γ chain, termed γ', derived from alternative splicing of mRNA, is inversely correlated with the risk of venous thromboembolism. The inserted sequence of 20 amino acids interacts with the anion binding exosite (ABE)-II of thrombin. *Aims.* This study investigated whether and how γ' binding to ABE-II affects thrombin interaction with the platelet receptors, i.e. Glycoprotein Ibα (GpIbα), protease-activated receptor (PAR)-1 and -4. *Methods.* Thrombin-induced platelet aggregation was investigated by Born's aggregometry. The effect of both synthetic fibrinogen 408-427 γ' peptide and a purified fibrinogen fragment D containing γ' sequence (D*) was studied by steady-state enzymatic assays using RP-HPLC/spectroscopic and cytofluorimetric methods, whereas interactions of thrombin were studied by solid-phase binding methods. *Results.* both synthetic γ' peptide and a purified fibrinogen fragment D* inhibited thrombin-induced platelet aggregation, up to 70%, with IC50 values of 42±3.5 μM and 0.47±0.03 μM, respectively. Likewise, D*-fragment and the synthetic γ' peptide, competitively inhibited the thrombin binding to GpIbα with a Ki ≈ 40 μM and ≈ 0.5 μM, respectively. Both these γ' chain-containing ligands non-competitively inhibited the thrombin cleavage of a synthetic PAR-1 peptide and native PAR-1 molecules on intact platelets, as well as the synthetic chromogenic peptide D-Phe-Pip-Arg-pNA. PAR-4 cleavage was unaffected by both γ'- peptide and fibrinogen fragment D*. *Conclusions.* These results indicate that an enhanced ratio of γ'/ γA chain in circulating fibrinogen could act as a *buffering system* for active thrombin, inhibiting platelet activation by thrombin. On the contrary, when the γ'/ γA chain ratio is decreased, thrombin-induced platelet may be promoted. This effect could possibly enhance the risk for arterial thrombosis, where platelet activation plays a major role.

0863

IMPROVEMENT OF THE PERFORMANCE OF D-DIMER ASSAYS IN THE ELDERLY

F.J.L.M. Haas,[1] R.E.G. Schutgens,[2] D.H. Biesma[2]

[1]St. Antonius Hospital, NIEUWEGEIN; [2]UMC Utrecht, UTRECHT, Netherlands

Background. The use of the D-dimer assay in outpatients suspected for deep venous thrombosis (DVT) in combination with the preclinical prediction (PCP) rules of Wells to exclude DVT is well accepted. Using this algorithm the clinicians are often confronted with false positive D-dimer results in the elderly with the consequence of additional radiological investigations for a safe exclusion of DVT. *Aims*. The evaluation of the clinical accuracy of a new D-dimer assay and comparison with two other frequent used D-dimer assays with a special emphasis on the performance in the elderly. *Methods.* In this study 466 symptomatic outpatients were included who were participants in a multicentre management study investigating the use of the D-dimer assay in combination with the rules of Wells in the exclusion of DVT (ref). The new Innovance D-DIMER assay (Dade Behring Marburg GmBH, a Siemens Company, Germany) was compared with Tina Quant (Roche, Mannheim, Germany) and STALia (Diagnostica Stago, Asnière, France). The cohort of patients was divided in age quartiles and the results of the whole cohort were compared with the fourth quartile. Sensitivity, specificity, negative (NPV) and positive predictive values (PPV) and the area under the curve (AUC) of the receiver operation characteristics (ROC) as measure of the clinical accuracy were calculated. *Results*. Using the recommended cut-off values of 500 μg/L, all assays had a sensitivity of 100% in the fourth age quartile. Specificity for Innovance was 10.0%, Tina Quant 23.8% and STALia 15.0%. Combined with a PCP score < 2, these values increased to 18.8%, 31.2% and 18.8% resp. The AUC for the assays in the fourth age quartile were 0.955 for Innovance, 0.944 for Tina Quant and 0.920 for STALia, indicating an excellent clinical performance. Therefore we recalculated sensitivity, specificity and NPV for the cut-off

values of 750 and 1000 µg/L. Combined with a PCP score <2, all assays had a sensitivity of 100%. Specificity for Innovance was 38.7/41.9%, for Tina Quant 54.8/67.7% and STALIa 32.3/45.2% for the two different cut-off values. *Conclusions*. Using a higher D-dimer cut-off value in the elderly resulted in an increase of specificity with excellent sensitivity combined with a low PCP.

0864

THE POTENTIAL BIOMARKERS FOR THROMBOEMBOLISM DETECTED BY SELDI-TOF-MS

Y. Hu, X. Zhang, T. Guo, H.F. Wang, M. Hong, W.J. He, H. Mei

Institute of Hematology of Union Hospital, WUHAN, China

Background. Few studies were concerned about searching for specific biomarkers for thromboembolism by the use of Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF-MS). *Aims.* The purpose of our study was to determine whether there were certain biomarkers in plasma useful for accurate diagnosis of thromboembolism. *Methods.* We screened for potential biomarkers in 69 plasma samples, including samples from 20 patients with idiopathetic deep venous thrombosis (DVT), 20 patients with acute myocardial infarction (AMI), and 29 healthy controls without a history of thromboembolism. Pretreated plasma samples were analyzed on the Protein Biology System IIc plus SELDI-TOF-MS (Ciphergen Biosystems, Fremont, CA). Proteomic spectra of mass to charge ratio (m/z) were generated by the application of plasma to immobilized metal affinity capture (IMAC-3) ProteinChip arrays activated with copper. *Results*. A pattern of three biomarkers (m/z: 2 667, 5 914, and 6 890 Da, respectively) with a total accuracy of 100% was selected based on their collective contribution to the optimal separation between patients with AMI and healthy controls. Another pattern consisting of only one biomarker (m/z: 5 914 Da) could totally discriminate patients with DVT and control subjects. For further analysis between patients with AMI and those with DVT, a pattern of four biomarkers (m/z: 3 418, 5 271, 33 378, and 68 125 Da, respectively) was selected with a total accuracy of 82.5%. *Conclusions*. Plasma proteomic profiling with SELDI-TOF-MS and ProteinChip technologies shows some potential in discriminating patients with thromboembolism and healthy subjects. The discovered biomarkers might show great potential for early diagnosis of thromboembolic diseases.

0865

LOW-MOLECULAR-WEIGHT HEPARIN (LMWH) TREATMENT IN ACUTE LEUKAEMIA (AL) PATIENTS WITH SEVERE THROMBOCYTOPENIA AND CONCOMITANT VENOUS OR ARTERIAL THROMBOSIS

M. Cedrone,[1] A. Chierichini,[1] P. Anticoli Borza,[1] S. Fenu,[1] V. Bongarzoni,[1] B. Anaclerico,[1] B. Ronci,[1] A. Venditti,[2] D. Venditti,[2] L. Maurillo,[3] P. De Fabriitis,[3] L Annino[1]

[1]San Giovanni Hospital, ROME; [2]Dept. of Hematology, Policlinico Tor Vergata,, ROMA; [3]Dept. of Hematology Ospedale S. Eugenio, ROMA, Italy

LMWH has become widely used for initial and long-term treatment of VTE in cancer patients, but there are few data about the safety and efficacy of LMWH in AL patients affected by concomitant severe thrombocytopenia. AL patients, referred between January 2005 and May 2007 at three Haematology Departments in Rome, were retrospectively analyzed in order to identify those with at least one episode of thrombosis treated with LMWH. We identified 22/240 (8,3%) patients (20 AML, 1 APL, 1 ALL) with at least one episode of thrombosis. They were 10 males/12 females, and median age was 64 years (range 25-74). Twenty-one patients developed venous thrombosis: 14(66%) venous catheter-associated thrombosis of the upper extremity, 5(24%) DVT of the leg, 2(10%) inferior cava vein thrombosis. One patient had iliac and femur-popliteal arterial thrombosis. At onset of thrombosis the median platelets number was 53.500/mm^3 (range 10.000-284.000), but in 9/22(40%) patients platelets count was below 30.000/mm^3. All patients were treated with subcutaneous enoxaparin 100 IU/Kg twice a day for the first month, followed by 100-150 IU/kg once a day for 3 months or as long as they received antineoplastic chemotherapy. Patient with arterial thrombosis underwent thrombectomy before LMWH therapy. The LMWH treatment median duration was 3 months (range 1-12). During anti-thrombotic treatment all patients promptly recovered; neither VTE recurrence nor minor or major spontaneous bleedings episodes were observed. Two patients died while on LMWH therapy because of leukaemia progression. The patient with arterial thrombosis discontinued treatment early, after thrombosis resolution, because of traumatic large cutaneous haematoma. On this experience enoxaparin treatment revealed as an effective and safe approach in AL patients with concomitant acute venous or arterial thrombosis and severe thrombocytopenia.

0866

MIGRAINE AND THROMBOPHILIA, ARE THEY RELATED? A CLINICAL STUDY IN CHILDREN

A. Koren, M. Kutai, R. Raviv, C. Levin, Y. Hugeirat, S. Shalev, L. Zalman

Ha'Emek Medical Center, AFULA, Israel

Migraine is a common reason for headaches in children and in recent years a possible connection between migraine and stroke was also described in children after previous reports in adults. Why migraine is associated with an increased risk of ischemic events is not known. An explanation for this association could be a hypercoagulable state. We studied the subject of thrombophilia in 43 migraine children. Factor V Leiden (FVL) and the Prothrombin mutation (G2010A) were found to be significantly more frequent among children of Jewish origin, compared to the control group of the same ethnic origin (p=0.02 and 0.05 respectively). In patients from Arab origin no significant increased frequency of genetic thrombophilic factors was found, probably due to the high frequency of FVL in this population. Increased factor VIII was found in 12 patients and factor IX in four. These factors are considered as acute phase reactants and could be the result rather than the cause of the event. We feel that there is enough evidence to suggest that secondary hemostasis is altered during and between migraine attacks, with a shift towards coagulation.

Table 1. Rate of mutations in migraine patients and controls.

	Jews		Arabs		Total	
	Patients	Controls	Patients	Controls	Patients	Controls
Factor V Leiden (A1691G)	6 / 32¶ *	0 / 28	2 / 10	13 / 47	8/ 42	13 /78
Prothrombin Mutation (G2010A)	5 / 33**	0 / 31	0 / 10	6 /41	4 / 43	6 /78
MTHFR Homozygous (T677C)	4 / 33	4 / 31	2 /10	8 /47	6 / 43	12 / 78
Total	13/33*	4/31	3/10	19/47	16/44	23/78

¶: Six patients FV Leiden and another one low APCR (1.7).
*: p = 0.02 – Jewish patient's vs controls.
**: p = 0.053 – Jewish patient's vs controls.

0867

PORTAL VEIN THROMBOSIS AFTER SPLENECTOMY IN PATIENTS WITH HAEMOGLOBINOPATHIES

M. Hadjigavriel,[1] M. Sitarou,[2] E. Pangalou,[2] E. Savvidou,[2] S. Christou[2]

[1]Thalassemia Center, Limassol Hospital Cyprus, LIMASSOL; [2]Thalassemia Center, LARNACA, Cyprus

Background. Patients with thalassaemia and other haemoglobinopathies undergo splenectomy to reduce their transfusion requirements. Portal vein thrombosis (PVT) is a rare but well - recognized complication of splenectomy. The incidence of this potentially life - threatening complication is estimated around 1%. *Aims.* The aim of this study was to determine the incidence and outcome of symptomatic PVT after splenectomy in patients with haemoglobinopathies. *Methods.* 61 patients with haemoglobinopaties (beta thalassaemia- 55, alpha thalassaemia- 4, sickle/beta thalassaemia- 2), underwent splenectomy between 1994 and 2007 at 3 thalassaemia centers. 5 patients who developed PVT after splenectomy were evaluated for clinical features and outcome. 4 out of 5 patients were transfused regularly before splenectomy. *Results.* During a period of 13 years, there were 59 open splenectomies and one laparoscopic. There was also one conversion from laparoscopic surgery to open surgery because of bleeding. 5 cases of PVT (8%) were identified. Indications for splenectomy in patients with PVT were increased annual blood consumption, with low platelets and/or wbc count (n=3), huge spleen (n=1), idiopathic thrombocytopenic purpura associated with hepatitis C (n=1). Presenting symptoms included abdominal pain (5), fever

(3) and ileus (1). The median interval between splenectomy and diagnosis of PVT was 10 days. Diagnosis was confirmed by abdominal CT scanning (2) and Doppler ultrasonography (3). Splenic mass ranged between 850g to 3000g (mean 1600g). Platelet count at the time of the event was more than 1×10^6/μL in 4 patients, and 9×10^5/μL in one patient. Thrombophilia testing was determined in 4 patients, and all were diagnosed to have one or more markers of inherited thrombophilia. All patients developed PVT despite receiving prophylactic aspirin postoperatively. Anticoagulation was initiated immediately. During a mean follow up period of 50 months (range 6 to120), all patients were found to be free of thrombosis. *Conclusions.* The incidence of portal vein thrombosis after splenectomy in patients with haemoglobinopathies is 8%. Portal vein thrombosis should be suspected in patients with abdominal pain after splenectomy. Thrombocytosis and thrombophilia seem to be possible risk factors. Aspirin may not protect from thrombosis and antithrombotic therapy should be considered after splenectomy.

0868

THE POTENTIAL BIOMARKERS FOR DEEP VENOUS THROMBOSIS DETECTED BY SELDI-TOF-MS

X.P. Zhang, Y. Hu, M. Hong, H.F. Wang, T. Guo, H. Mei, W.J. He

Institute of Hematology of Union Hospital, WUHAN, China

Background. Few studies were concerned about searching for specific biomarkers for venous thromboembolism by the use of Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF-MS). *Aims.* The purpose of our study was to determine whether there were certain biomarkers in plasma useful for accurate diagnosis of deep venous thrombosis (DVT). *Methods.* We screened for potential biomarkers in 160 plasma samples, including samples from 100 patients with lower-limb DVT and 60 healthy controls without a history of thromboembolism. Pretreated plasma samples were analyzed on the Protein Biology System IIc plus SELDI-TOF-MS (Ciphergen Biosystems, Fremont, CA). Proteomic spectra of mass to charge ratio (m/z) were generated by the application of plasma to weak cation exchange (CM10 ProteinChip) arrays. *Results*. A pattern, consisting of three biomarkers (m/z: 28 027, 13 754, and 5 827 Da, respectively) with a total accuracy of 77.5%, was selected based on their collective contribution to the optimal separation between patients with DVT and healthy controls. The differential pattern had a sensitivity of 73.0% and specificity of 85.0%, respectively. *Conclusions*. Plasma proteomic profiling with SELDI-TOF-MS and ProteinChip technologies shows some potential in discriminating patients with lower-limb DVT and healthy subjects. The discovered biomarkers might show great potential for early diagnosis of DVT.

0869

DIAGNOSING HEPARIN INDUCED THROMBOCYTOPENIA: CURRENT PRACTICE AMONGST PARTICIPANTS OF THE UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME FOR BLOOD COAGULATION

S. Walker,[1] S. Kitchen,[1] I. Jennings,[1] K. Horner,[2] D. Kitchen,[1] T.A.L. Woods[1]

[1]UK NEQAS, SHEFFIELD; [2]Royal Hallamshire hospital, SHEFFIELD, UK

We report here the results of a survey of testing for Heparin Induced Thrombocytopenia (HIT), to assess current practice and to inform development of a proficiency testing programme. In May 2007 a questionnaire requesting information on HIT was distributed to 1012 centres who regularly participate in the UK National External Quality Assessment Scheme for Blood Coagulation (600 UK centres and 400 non UK centres, mainly elsewhere in Europe). Responses were received from 213, Around 75% of these were within the UK. The pattern of responses was similar in UK and non UK centres so the following analysis includes both groups. Forty-two % of responding centres were unaware of any involvement in assessing patients with possible HIT (but around 60% of the questionnaires were completed without input from a clinician). Where assessments were made, 20% made a clinical judgement without laboratory testing. The 4Ts system (Thrombocytopenia, Timing, Thrombosis, oTher causes of thrombocytopenia) was used by half, with a further 1/4 of centres unaware whether this system was employed or not. One quarter indicated that the 4Ts were not in use. The most commonly used assay was the Diamed test used by half of centres. Around a quarter used platelet aggregation and a quarter used ELISA. Only 3 centres used washed platelets in platelet activation assays, reported as having higher sensitivity. A UK guideline[1] indicates that only IgG needs to be measured, but only 10% of centres using ELISA methods employed a test specific for IgG antibody, with most using kits which detect IgG, IgM or IgA antibody. Around 60% of these ELISA users did not report the optical density obtained despite guidelines indicating that this is useful. Only 1/3 determined locally the optical densities for normal subjects to define the cut-off between positive and negative. Furthermore, 56% of ELISA users did not include high dose heparin to demonstrate inhibition/correction even though this has been recommended. The survey thus indicates that there are a number of areas where current practice deviates from recommendations. We are commencing distribution of samples to provide proficiency testing in this area.

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0870

ASSOCIATION BETWEEN FACTOR V LEIDEN MUTATION AND CEREBRAL VENOUS THROMBOSIS IN WESTERN IRAN

Z. Rahimi, H. Mozafari, A. Amiri Bigvand, R. Mohammad Doulabi, N. Razazian, D. Afshari, M. Rezaei

Kermanshah University of Medical Sciences, KERMANSHAH, Iran

Background. Cerebral venous thrombosis (CVT) is an infrequent condition with a large variety of causes. Predisposition to CVT also has a genetic basis and factor V Leiden (FVL) is the most common genetic risk factor. *Aims*. The present study was aimed to investigate the association between factor V Leiden mutation and prothrombin G20210A with cerebral venous thrombosis in Western Iran. *Methods*. Nineteen patients with CVT including 4 males and 15 females with the mean age of 36.1±12.7 years (22-70 years) and 100 age matched healthy individuals (50 males and 50 females) from Kermanshah Province of Iran were studied for factor V Leiden mutation and prothrombin G20210A by PCR-RFLP method using Mnl I and Hind III restriction enzymes, respectively. *Results*. Factor V Leiden was found in heterozygous form in 4 of the 19 patients with CVT (21.1%) and in 2 of the 100 control subjects (2%), p=0.006. A significant association was found between factor V Leiden mutation and CVT with odds ratios (OR) of 13.6 (95% confidence intervals [CI] 2.19-77.65, p=0.006). No prothrombin G20210A was found among patients. However, the prevalence of this mutation was 1% among healthy individuals. *Conclusions*. Our findings indicate a significant association between the factor V Leiden and CVT among Iranian patients and suggest that factor V Leiden mutation is a risk factor for cerebral venous thrombosis.

0871

STUDY ON BIOLOGICAL ACTIVITY OF THE FIRST EPIDERMAL GROWTH FACTOR-LIKE DOMAIN OF RAT COAGULATION FACTOR VII

H. Mei, Y Hu, H.F. Wang, Y.H. Zhang, T. Guo, X.P. Zhang, W.N. Wei

Institute of Hematology of Union Hospital, WUHAN, China

Background. The first epidermal growth factor-like (EGF1) domain of coagulation factorVII (FVII) is essential for binding with tissue factor (TF) in man. We hypothesized that the function of rat FVII (rFVII) EGF1 domain might be the same as the human being's. The clone and expression of rFVII EGF1 domain would be helpful in studying the anticoagulant drugs base on FVII/TF interaction. *Aims.* To explore the affinity of rat EGF1 binding to TF by EGFP- EGF1 fusion protein expression. *Methods.* The model of rat TF expression *in vitro* was established by lipopolysaccharide induction. We amplified the EGF1 domain from a rat liver by RT-PCR, a fusion expression vector named pET28a-EGFP-EGF1 was constructed, after expression in Ecoli BL21 cells, the EGF1/EGFP fusion proteins were analyzed for binding affinity to rat TF by flow cytometry and confocal microscope, and biological activity was tested by prothrombin time assay using FVII depleted human plasma. *Results.* The resulting plasmid expressed fusion protein EGFP/EGF1 in the soluble form in E. coli BL21, and the recombinant protein was purified successfully with a major band at 36KD in SDS-PAGE. Binding affinity of the EGF1/EGFP fusion proteins was either depressed or statistically unchanged *vs* mFVII (wide type) (76.2% *vs* 56.3%, p<0.05). However, the proteins lost the activity of coagulation. *Conclusions.* The rat EGF1 region could specifically bind to TF without the ability of coagulation, and it might facilitate the development of molecular target study in antithrombosis treatment.

0872

THE INFLUENCE OF ANTIOXIDANTS ONTO PLATELET FUNCTION

A. Sobotková, J. Štikarová, P. Májek, R. Kotlín, J. Suttnar, J.E. Dyr

Institute of Hematology and Blood Transfusion, PRAGUE 2, Czech Republic

Background. Human blood platelets play a critical role in haemostasis and contribute to essential processes in wound repair. Platelet interaction with the vessel wall serves to numerous physiological and pathophysiological functions. Exposure to the subendothelial matrix induces adhesion, rapid platelet activation, shape changes and aggregation. During activation platelets secrete and generate various species including reactive oxygen species (ROS)- hydrogen peroxide, hydroxyl radical and superoxide.[1] These unstable molecules participate in activation cascade as second messenger and can also modify other molecules (e.g. proteins) in their close vicinity. *Aims.* In this present work we studied influence of antioxidants resveratrol (trans-3,5,4'-trihydroxystilbene) and trolox (vitamin E derivative) on platelet aggregation, static and dynamic adhesion.[2,3] We also monitored changes in platelet proteome and secretome, especially rising of new modification (oxidation, nitration, phosphorylation) in the presence of various concentrations of antioxidants. *Methods.* Platelets were isolated from the blood of healthy volunteers who had not taken any antithrombotic drugs. Washed platelets were activated by three different types of agonists: thrombin, collagen and arachidonic acid. Platelets were incubated for 30 minutes with different concentration of resveratrol or trolox before activation. Platelets aggregation was measured photometrically at 37°C under continuous stirring. Static adhesion was evaluated using acid phosphatase (ACP) activity measurement. Dynamic adhesion was studied by using cone and plate(let) analyzer. For detection of differences in platelet proteome and secretome, we have used two-dimensional gel electrophoresis (2-DE) followed by immunochemical detection. *Results.* We found that antioxidant resveratrol inhibits platelet aggregation dose dependently with increasing concentration. The static adhesion of platelets onto immobilized fibrinogen in the presence of the resveratrol was also significantly lowered. In the presence of trolox we have found significant decrease of aggregation response and of adhesion of platelets on the immobilized fibrinogen. *Conclusions.* These findings suggest that antioxidants have an important role in the regulation of platelet function. These observations indicate that especially resveratrol exerts important effects on platelet functions.

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0873

STUDY OF VENOUS THROMBOEMBOLISM PROPHYLAXIS ACROSS MULTIPLE DISCIPLINES IN A SINGLE INSTITUTION

M. Loingsigh, R. Afghan, D. O'Keeffe

Health Service Executive, LIMERICK, Ireland

Background. Venous thromboembolism (VTE) remains a leading cause of in-hospital mortality partly due to under-recognition of the at-risk population. The risk factors for VTE are well recognised and several risk scores have been published to calculate an individual's risk of VTE. Despite this, many high-risk patients continue to receive inadequate prophylaxis. *Aims.* To assess across multiple disciplines the current practice of VTE prophylaxis in our institution and to compare local practice to current guidelines. *Methods.* 300 patients were assessed, 50 inpatients randomly chosen from each of the following specialities: General Surgery, Orthopaedics (Trauma), Gynaecology, General Medicine, Medicine for the Elderly and Haematology/Oncology. Data was collected over 12 consecutive weeks from October 2007 to January 2008. Verbal consent was obtained from all patients. Patients were stratified using the Caprini risk assessment score into low, moderate, high and very high risk groups. Exclusion criteria were: contraindication to LMWH, therapeutic anticoagulation and inability to communicate. Thromboprophylaxis, if any, was recorded for each patient. *Results.* 134 and 135 patients were categorised as high risk or very high risk in medical and surgical groupings, respectively. 32/134 (24%) of medical patients received LMWH compared to 113/135 (84%) of surgical patients. Compression stockings were fitted on 18/134 (13%) medical patients compared to 122/135 (90%) surgical patients. 45 medical patients had an antiplatelet agent prescribed whilst four of the surgical patients received antiplatelet agents at the time of assessment. *Conclusions.* It is clear that in spite of recent evidence suggesting VTE is a significant cause of mortality and morbidity in medical patients, that prophylaxis remains inadequate. Urgent measures are required to improve VTE prophylaxis in medical patients.

0874

RISK FACTORS FOR AND INCIDENCE OF VENOUS THROMBOEMBOLISM IN PATIENTS WITH AUTOIMMUNE HAEMOLYTIC ANAEMIA: A RETROSPECTIVE ANALYSIS OF 57 PATIENTS

V. Boulton-jones, A. Kerr

Ninewells Hospital Dundee, DUNDEE, UK

Background. There is a known association between autoimmune haemolytic anaemia (AIHA) and thrombosis but it unclear as to whether there is a particular sub-group of patients that are at increased risk. *Aims.* We aimed to establish the incidence of venous thromboembolic (VTE) disease in patients in our centre with a diagnosis of AIHA and establish whether any patient subgroup was at increased risk of VTE. *Subjects and Methods*. We performed a retrospective study of all patients seen by the haematology department with a diagnosis of AIHA between 1994 and December 2007. Evidence of VTE, the time of the thrombotic event and predisposing factors to these thrombotic events were sought. We compared the rate of thrombosis in the following groups -Patient age <60 (n=20) *vs* >60 (n=37), idiopathic AIHA *vs* AIHA associated with a lymphoproliferative disorder, splenectomy or not, clinical course of chronic haemolysis *vs* haemolysis resolving rapidly with first line treatment. Groups were compared using the Fisher Exact Test. During the study period patients with AIHA and no history of VTE only received thromboprophylaxis at times when they were an in-patient.

Table 1. Summary of incidence of thrombosis

group	Incidence of thrombosis- absolute	Incidence of thrombosis - %	P value
Age<60	8/37	21	NS
Age>60	2/20	10	
Coexistent LPD	5/19	26	NS
No coexistent LPD	5/38	13	
1 treatment	1/23	4	P=0.03
>1 treatment	9/34	26	
splenectomy	7/19	37	P=0.01
No splenectomy	3/38	8	
Chronic on going haemolysis	9/30	30	P=0.009
No chronic ongoing haemolysis	1/27	4	

Results. 57 patients with AIHA were identified. The average age was 63. 10(17%) had an episode of thrombosis (4 had deep venous thrombosis and 6 had pulmonary emboli) with 3 deaths as a result of thrombosis. 19 had a lymphoproliferative disorder and 10 had previous or co-existent immune thrombocytopenia. Only 19 patients had an uncomplicated course requiring a single episode of treatment with tapering of oral prednisolone. 19 patients required a splenectomy. Of those 10 patients with VTE, 3 were in-patients at the time of thrombosis and 5 patients had an underlying lymphoproliferative disorder (NS). The mean age was 67 (NS). 7 had had a splenectomy. Four had the splenectomy after the thrombosis and 2 more than 1 year prior. In only1 case was splenectomy temporally associated with the episode of thrombosis. 30/57 had a clinical history of prolonged haemolysis rather than a single treated event or a remitting and relapsing course. The incidence of thrombosis in patients with a chronic course is therefore 9/30 (30%). The p value of the incidence of thrombosis with chronic haemolysis compared to the remainder of the group is p=0.009. Results are summarised in Table 1. *Summary*. Our study confirms the previously reported high risk of thrombosis and associated mortality in

patients with AIHA. We have found that patients with chronic ongoing haemolysis are a particular risk group. A high index of suspicion should be maintained for the investigation of thromboembolic disease and thromboprophylaxis should be considered.

0875

HEPARIN-INDUCED THROMBOCYTOPENIA: A SINGLE CENTRE EXPERIENCE WITH 9 CASES TREATED WITH LEPIDURIN

K. Kwon, A. Rodriguez Huerta, C. Pascual, G. Perez Rus, C. Munoz, J. Sanchez, J.L. Diez Martin

Hospital Gral. Univ. Gregorio Marañon, MADRID, Spain

Background. Heparin-induced thrombocytopenia (HIT) is a clinical-pathological syndrome due to IgG antibodies against heparin-PF4 complexes, which activate platelet aggregation after heparin exposure. HIT frecuency is variable depending on the type of heparin used (0.25-2%), being higher with unfractionated heparin. HIT therapy has been difficult until the introduction of antithrombotic agents like lepidurin. We present our experience with 9 HIT treated with lepidurin. *Methods.* 9 HIT cases diagnosed between 2000 and 2007. The diagnosis was performed following pretest clinical score (4 T´s) and by detection of anti PF4-heparin antibodies (ELISA and/or Diamed®). For all the cases, it was the first HIT episode and all of them received initial therapy with lepidurin (APTT range 1,5-2,5 times control) followed by another antithrombotic therapy for at least 4 weeks. *Results.* From the 9 patients, 5 were male, with a median age of 73 years old (47-82). Four were surgical patients: 2 underwent cardiac surgery, 1 orthopedic surgery and 1 abdominal surgery. At diagnosis, 5 patients showed thrombotic events, 4 of them arterial and 1 venous thrombosis. Most of the cases were exposed to unfractionated heparin (7). The median time of heparin exposure until HIT diagnosis was 7 days (4-26), and the median time to platelet count recovery after heparin withdrawal and lepidurin introduction was 6 days (4-26). Table 1 shows the main characteristics of all the cases. No complications of lepidurin therapy were observed in the 9 cases. *Conclusions.* In our experience, lepidurin therapy is effective and safe for the treatment of HIT. Lower dosis than recommended were required in all cases to achieve targeted APTT. Interestingly, arterial thombotic episodes were more frecuent than venous thrombosis. This observation could be due to underdiagnosed HIT.

Table 1.

	Age	Sex	Risk Group	Heparin used	Exposure days	Thrombotic event	Previous Plt/μL	Plt/μL at diagnossis	Clinical Score	Diamed\ Elisa	Lepidurin mg/kg/h bolus	mt	Days tp Plt recovery
1	70	F	Cardiac Sg	NFH	10	Arterial	200.000	25.000	8	pos	0.09	0.05	4
2	69	M	Cardiac Sg	NFH	4	NO	167.000	27.000	3	pos	0.08	0.05	4
3	73	F	Orthopedic Sg	LMWH	14	Arterial	376.000	15.000	6	pos	NO	0.04	12
4	80	F	Medical	LMWH	26	PE	347.000	70.000	6	pos	NO	0.08	8
5	80	M	Neurological	NFH	5	NO	141.000	80.000	6	neg	NO	0.008	6
6	82	F	Surgical	NFH	7	NO	132.000	52.000	6	neg	NO	0.05	6
7	47	M	Medical	NFH	5	Arterial	159.000	35.000	7	pos	NO	0.05	26
8	78	M	Medical	NFH	7	NO	179.000	61.000	5	pos	NO	0.06	8
9	51	M	Neurological	NFH	10	Arterial	450.000	116.000	7	pos	NO	0.05	6

Sg, Surgical; NFH, non fractioned heparin; LMWH, low molecular weight heparin; PE, pulmonary embolism; Plt, platelets; mt, maintenance

0876

ANTICOAGULANTS AND THEIR INTERACTION: A COHORT STUDY

G. Martini, R. Volpi, R. Del Bono, A. Jager, L. Caimi

Spedali Civili, BRESCIA, Italy

Oral anticoagulants (OA's) are effective in prevention and treatment of deep vein thrombosis and to prevent arterial embolism in patient with atrial fibrillation, prosthetic heart valves, and coronary artery disease. The potential for OA's to interact with other drugs, food, diet and lifestyle resulting in changes in their anticoagulant effect, is widely recognized. The aim of this study is to provide information on the incidence and type of possible OA's interactions. This cohort study sample was drown from 1500 patients attending our Anticoagulation Clinic (AC). To be included, a patient must have been a member of the AC from at least one year and must have had a variation of his/her INR which responded to the following criteria: a) INR outside therapeutic range and b) variation of INR > 1 between two consecutive visits. To be specific to the evaluation of interaction, responses to criteria a) and b) required that intensity of patient's anticoagulation had to be stable, e.g. within the therapeutic range, for more than 65% of time in the last 12 months. We applied the linear interpolation method to measure the time in range for each patient. Whenever a significant INR variation occurred we conducted a structured interview with the patient to elicit the possible causes of interaction and filled up a data collection sheet. The data were aggregated and analyzed with Microsoft Excel and MedCalc for Windows was used for statistical purpose. A total of 407 significant INR variations were recorded between October 21, 2005 and September 20, 2007. As far as more than one significant variation occurred in the same patient during the course of the study we had a total final number of 355 patients involved: 184 the first year and 171 the second year. Apparently, variations occurred independently in both patients taking Warfarin and Acenocoumarol, but when the chi-square test was performed there was a significant difference between patients taking Warfarin and those taking Acenocoumarol, being the last group more susceptible to interactions (Odds ratio: 1,52, CI 95%: 1,19-1,94, p<0,001). Moreover, the difference persisted when we compared Acenocoumarol 1mg toward Warfarin 5mg (Odds ratio: 2,37, CI 95%: 1,07-5,24, p=0,04), while there was no difference between Acenocoumarol 1 mg and Acenocoumarol 4mg (Odds ratio: 1,59, CI 95%: 0,72-3,5, p=0,34). We found INR variations more frequently in: Acenocoumarol users, older patients and in the first two years of treatment. The most common medications which interfered with anticoagulants were: anti-infectives (n=48), analgesics (n=37), antiarrhythmics (n=7). There were two variations due to papaya powder and red ginseng assumption. In spite of the noteworthy number of interactions we didn't have any bleeding complication or thrombosis recurrence. Fifty-one patients had significant variations in which no causes could be identified but we still have to adjust for liver disease and hyperthyroidism, conditions that could explain these events. This study is a *real-life* picture on potential interactions in a population we are still treating: with data derived from this study, we mean to design targeted interventions intended to reduce future possible interactions.

0877

SEROEPIDEMIOLOGICAL STATUS AND INFECTIOUS BURDEN, INFLAMMATORY MARKERS AND THE VENOUS THROMBOEMBOLISM

J. Gonzalez-Ordonez,[1] E. Gonzalez,[2] C. Fernandez-Canal,[2] M. Moran-Alcala,[1] R. Venta-Obaya,[1] J. Medina,[1] D. Macias,[1] M. Peliz[1]

[1]*Hospital San Agustin, AVILES, Spain;* [2]*Hospital de Cabueñes, GIJON, Spain*

Background. The inflammation is a key component for vascular diseases and thrombosis whereas the seroprevalence for certain subclinical or latent infections such as Chlamydia pneumoniae was clearly related with the venous thromboembolism (VTE) (*Lozinguez et al, Thromb Haemost 2000; 83: 887*) although other studies did not confirm such association (*Koster et al, Lancet 2000; 335: 1694*) or find a minor one (*Sant Martin et al, Presse Med 2004; 33: 1493*). However the serological associations neither prove causality nor indicate pathogenic mechanisms. Simultaneous searching for intermediate inflammatory phenotypes could be highly convenient. We aim to know any possible association between the serostatus to the main long-term or lifelong subclinical infections (causing endothelial activation) haemostatic risk factors or adhesion molecules and the VTE. *Population and methods*. We studied 585 individuals, 308 consecutive patients with an objectively diagnosed VTE and 277 healthy controls of similar gender and age [in overall 61.7(14.1) y, 50.1% males]. Among the patients, 113 (36.7%) have suffered a pulmonary embolism (PE) with or without an associated DVT and 68 (22.1%) a recurrent thrombotic episode. We assayed the serological status for Chlamydia pneumoniae (IgG/ IgA antibodies), Mycoplasma pneumoniae (IgG/ IgA), Helicobacter pylori (IgG/ IgA), CMV, Herpes simplex (1 / 2 types), Epstein-Barr virus (IgG/ IgA) and the hepatitis A virus by the enzyme-linked immunosorbent assay (ELISA) specific methods. The soluble fractions of several vascular adhesion molecules apparently related (E-selectin, P-selectin, ICAM-1 and VCAM-1) were also measured by ELISA, the C-reactive protein by a high-sensitive turbidometry (hs-PCR) and the factor VIIIc by the standard coagulative method. *Results*. The soluble fractions of E-selectin, P-selectin and ICAM-1 did not show association with the VTE in opposite to the hs-CRP, FVIIIc and s-VCAM-1 ($p<0.0001$). We obtained a higher seroprevalence for anti-Chlamydia pneumoniae (for IgA antibodies) among the patients than the controls (55.4 *vs* 46.2%) as well as for anti-Mycoplasma pneumoniae (IgG antibodies) (46.8 *vs* 38.3%) ($p<0.05$). In fact, the IgA anti-Chlamydia levels higher than P90 strongly associated with the VTE [OR=2.21 (CI95%: 1.36-3.57), $p=0.001$] or with higher FVIIIc activity ($p<0.05$) and sVCAM-1 concentration ($p<0.01$). Likewise, the IgG anti-Mycoplasma levels higher than the P80 were associated with the VTE [OR=1.51 (CI95%: 1.03-2.26), $p<0.05$]. Unexpectedly, the seroprevalence for anti-CMV (IgG) in patients (80%) was lower than in controls (87%) [OR=0.62(0.39-0.97) ($p<0.05$)]. The serological status (seroprevalence) for antibodies against the Helicobacter pylori, Herpes simplex (1 and 2 types), Epstein-Barr virus and the hepatitis A virus did not associate to the VTE. Therefore, the score for total infectious burden was similar for patients and controls outside the above mentioned associations. *Conclusion*. The serological status for Chlamydia pneumoniae (IgA) and Mycoplasma pneumoniae (IgG) seem to be positively associated to the VTE. Despite the simultaneous relationship of the IgA serostatus for *Chlamydia* with some inflammatory markers as could be the high levels of the sVCAM-1 and the factor VIII we can not establish a pathogenic role even though the association is very suggestive and deserves further specific studies.

Funding. This work was supported by the grants FIS#031553 and #041550

0878

BEST PRACTICES IN QUALITY AND SAFETY OF PATIENT CARE: ROSWELL PARK CANCER INSTITUTE QUALITY INITIATIVE IN VENOUS THROMBOEMBOLISM PROPHYLAXIS (VTE) FOR THE ONCOLOGY POPULATION

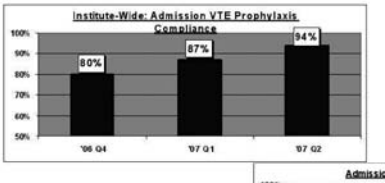
S. Padmanabhan, K. Klein, B. Kuvshinoff, D. Jenkins, J. Smith

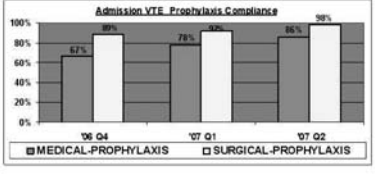
Roswell Park Cancer Institute, NY, USA

Backround. Venous Thromboembolism (VTE) is a serious risk and concern for cancer patients. Roswell Park Cancer Institute (RPCI), a comprehensive cancer center that has over 24,000 patients under active care, began an Institute-Wide Quality Improvement Initiative in 2006 to improve the rates of VTE prophylaxis for all adult inpatient admissions. Initial evaluations based on surveys revealed that a process improvement plan was needed, due to suboptimal prophylaxis on the medical oncology services and while the surgical services were more accustomed to ordering pre-operative pharmacologic and mechanical prophylaxis, there was room for improvement continuing prophylaxis post-operatively. We referred to the National Comprehensive Cancer Network (NCCN) Guidelines to develop the framework for the process improvement plan. *Aims*. The pathophysiologic explanations of VTE in cancer patients are multifactorial, including known hypercoagulability, vessel wall damage related to vascular access devises, or stasis from direct venous compression or invasion. According to the NCCN Guidelines, the actual prevalence of cancer related VTE is likely underestimated, and recommends prophylactic anticoagulation therapy and mechanical prophylaxis for all inpatients with a diagnosis of active cancer, or mechanical prophylaxis alone if contraindication to anticoagulation therapy exists. *Project Goals*. Establish and promote organizational commitment to preferred practices, by reporting quarterly practice performance to the Institute Quality Improvement Committee and dissemination of practice performance reports to the medical/surgical staff. Promotion of VTE awareness and staff education via informational materials, field in-services, seminars and additional resources. Standardize processes and drive continuous quality improvement. Implement NCCN Guideline based Admission DVT Prophylaxis Assessment and Order Form, for selection of pharmacologic VTE prophylaxis or mechanical prophylaxis if the patient status meets the exclusion criteria. Development of a mandatory Computerized Physician Order Entry (CPOE) for VTE Prophylaxis, with expectation that implementation will further standardize and improve practice performance across all services. *Methods*. Audit: preferred Practice Performance - random sample audit of appropriate VTE Prophylaxis, pharmacologic and/or mechanical for adult admissions with a diagnosis of cancer.

Table 1. Audit of VTE admission prophylaxis.

VTE PROPHYLAXIS (INPATIENT ADMISSIONS) PRACTICE PERFORMANCE			
MEDICAL	2006 - Q4	2007 - Q1	2007 - Q2
ASSESSORDER FORM UTILIZATION	66%	78%	78%
APPROPRIATE PROPHYLAXIS	67%	78%	86%
SURGICAL	2006 - Q4	2007 - Q1	2007 - Q2
ASSESSORDER FORM UTILIZATION	63%	62%	56%
APPROPRIATE PROPHYLAXIS	89%	92%	98%





Results. The practice performance for appropriate Admission VTE Prophylaxis is shown in Table 1 and Graphs 1 & 2. Both reveal a consistent improvement trend, with overall Institute-Wide preferred practice increasing from 80% to 94% following implementation of the initiative. The incidence of confirmed Venous Thromboembolism events are shown in Table 1, and shows a stable incidence between 0.39 and 0.37 for the first three Quarters following initiation of the project , with Quarter 2 of 2007 showing a decline to 0.24%. We noted that 79% (116/147) of the VTE events have occurred on the Medical Services, compared

with 21% (31/147) on the Surgical Services. Outpatient VTE events comprised 74% (109/147) compared to 26% (38/147) of events for Inpatients. The three Medical Oncology Services of G.I. Medicine, Hematologic/Lymphoma, and G.Y.N. accounted for 53% of the events. *Conclusions*. Implementation of a VTE Prophylaxis Initiative using NCCN Guidelines resulted in improved practice performance. Medical oncology patients make up the majority of VTE events, but often meet exclusion criteria for pharmacologic prophylaxis and therefore use of mechanical prophylaxis in this high risk population should be considered. We will present the updated summary of our CPOE effort of preventing thrombosis in cancer patients in the upcoming EHA meeting.

0879

PATTERN RECOGNITION OF BLOOD SAMPLES WITH PROLONGED ACTIVATED PARTIAL THROMBOPLASTIN TIME BY AN AUTOMATED COAGULATION ANALYZER

A. Ohsaka,[1] T. Yamamoto,[2] K. Ishii,[2] Y. Kuno,[2] T. Horii[2]

[1]Juntendo University School of Medicine, TOKYO; [2]Juntendo University Hospital, TOKYO, Japan

Background. Assessment of blood samples with a prolonged activated partial thromboplastin time (APTT) requires a mixing experiment with normal plasma, and an additional sample may need to be collected from the patient for further analysis. *Aims*. The aim of this study was to evaluate the performance of an automated coagulation analyzer that was designed to automatically measure blood samples with a prolonged APTT. *Methods.* We used a novel fully-automated multiparameter coagulation analyzer equipped with a photo-optical clot detection unit that was designed to perform coagulation, chromogenic, and immunologic assays. Mixing experiments were performed automatically by using a commercially available APTT reagent and factor-deficient plasma samples (factors VIII, IX, XI, and XII) or patient plasma samples. In these experiments, test plasma was mixed with normal plasma and the assay was repeated, noting the correction (if any). *Results*. With factor-deficient plasma samples, the plot of mixing experiment data obtained by the automated analyzer was similar to that for manual measurement. Twenty-five patient samples with a normal prothrombin time (PT) and a prolonged (≥50 seconds) APTT were analyzed and categorized into the following three types based on their patterns: deficiency type (concave pattern) in 12 cases, inhibitor type (convex pattern) in 4 cases, and suspected inhibitor type (straight pattern) in 9 cases. In 10 out of 12 deficiency type samples, the plasma concentration of factor VIII or factor IX measured by the same automated analyzer was less than 30% of normal. All 4 inhibitor type samples and 7 out of 9 suspected inhibitor type samples were positive for lupus anticoagulant (LA) also measured by the automated analyzer. The estimated time and plasma volume required for a mixing experiment were 25 minutes and 0.175 mL with the automated analyzer *vs* 145 minutes and 1.05 mL for the manual method, respectively. The plasma volume needed for all measurements, including routine PT and APTT, a mixing experiment, LA detection, and assay of coagulation factors, was approximately 1 mL (2 mL of whole blood). *Summary and Conclusions.* This automated coagulation analyzer may be applicable for assessment of blood samples with a prolonged APTT by pattern recognition. The advantages of the automated analyzer are a shorter measuring time, smaller blood volume needed, and less operator-dependent variation compared with a manual method. When a plasma sample shows a prolonged APTT, a mixing experiment and further analyses (i.e., LA detection) can be done by the same automated analyzer without needing an additional patient sample. Measurement of blood samples with a prolonged APTT by this automated coagulation analyzer may rapidly provide data on the probable cause, even at a patient's first visit.

SIMULTANEOUS SESSION II

Chronic myeloid leukemia - Clinical II

0880

DASATINIB EFFICACY IN PATIENTS WITH IMATINIB-RESISTANT/-INTOLERANT CHRONIC MYELOID LEUKEMIA IN BLAST PHASE: 24-MONTH DATA FROM THE START PROGRAM

G. Saglio,[1] H. Dombret,[2] D. Rea,[2] S. Corm,[3] J. Cortes,[4] D.W. Kim,[5] F.T. Garzon,[6] P. Paliwal,[6] M. Baccarani,[7] G. Martinelli,[7] C. Gambacorti-Passerini[8]

[1]University of Torino, ORBASSANO-TORINO, Italy; [2]Hôpital Saint-Louis, PARIS, France; [3]Hôpital Huriez, LILLE, France; [4]MD Anderson Cancer Center, HOUSTON, USA; [5]St Mary's Hospital, The Catholic University of Korea, SEOUL, South-Korea; [6]Bristol-Myers Squibb, WALLINGFORD, USA; [7]S. Orsola-Malpighi Hospital, University of Bologna, BOLOGNA, Italy; [8]University of Milano - Bicocca, MONZA, Italy

Background. The prognosis for patients with myeloid-blast phase (MBP) or lymphoid-blast phase (LBP) chronic myeloid leukemia (CML) is poor, and more than 90% of patients with blast phase CML develop imatinib resistance. Dasatinib is the most potent inhibitor of BCR-ABL (325-fold more potent than imatinib and 16-fold more potent that nilotinib *in vitro*). Dasatinib also inhibits important tyrosine kinases (e.g. Src family kinases) that may play a role in imatinib resistance and CML disease progression. During the START program, dasatinib was demonstrated to be an effective treatment for patients with imatinib-resistant or -intolerant CML in any phase. *Aims.* To investigate response durability with dasatinib 70 mg BID in patients with MBP- or LBP-CML, data from patients treated during START trials are reported with a minimum follow-up of 24 months. *Methods.* Patients with MBP- or LBP-CML and imatinib resistance or intolerance were enrolled between January and June 2005. Dose reductions (to 50 or 40 mg BID) or interruptions were permitted for management of toxicity and dose escalations (to 100 mg BID) were permitted for lack of response. Objectives included the evaluation of rates and duration of hematologic and cytogenetic responses, progression-free and overall survival, and safety of the treatment. *Results*. Of 157 patients with CML-MBP (n=109) or CML-LBP (n=48), 91% and 88% had been recruited following imatinib resistance and approximately half had received imatinib 800 mg/d. During prior imatinib treatment, a major cytogenetic response (MCyR) had been achieved in 44% of each population. Baseline BCR-ABL mutations were documented in 41% and 64% of patients with CML-MBP or -LBP, including T315I in 5% and 9%. Following dasatinib treatment, response rates (MBP and LBP, respectively) were: CHR, 26% and 29%; MCyR, 34% and 52%; and CCyR, 27% and 46%. Rates of MCyR in patients with any/no BCR-ABL mutation at baseline were 29%/38% in patients with MBP, and 48%/63% in patients with LBP. Median duration of MCyR among responding patients was 16.8 months (MBP) and 4.1 months (LBP). In patients with MBP and LBP, respectively, median progression-free survival was 5.6 months and 3.1 months, while 24-month overall survival rates were 38% and 26%. Sixteen patients (10%) were able to undergo SCT following dasatinib treatment. Dasatinib was generally well tolerated, with low rates of discontinuation following toxicity (10%). Grade 3/4 thrombocytopenia and neutropenia occurred in 76% and 83%, respectively. Treatment-related nonhematologic adverse events that occurred at grade 3/4 included pleural effusion (15%), gastrointestinal bleeding (8%), diarrhea (6%), and dyspnea (5%); other nonhematologic side effects were mostly mild to moderate. Dasatinib dose was reduced in 34% and interrupted in 62% of patients, most typically as a result of nonhematologic toxicities. Dose was escalated in 45% of patients. The median treatment duration was 3.4 months (0.03-29.4) for all patients. *Summary and conclusions*. Extended follow-up confirms the efficacy of dasatinib in patients with blast phase CML following imatinib failure, with long-term survivors evident among patients with MBP or LBP. Dasatinib treatment is associated with an acceptable safety profile among patients with a poor prognosis and limited therapeutic options.

0881

EFFICACY OF DASATINIB IN PATIENTS (PTS) WITH PREVIOUSLY UNTREATED CHRONIC MYELOGENOUS LEUKEMIA (CML) IN EARLY CHRONIC PHASE (CML-CP)

J. Cortes,[1] G. Borthakur,[1] D.M. Jones,[1] S. O'Brien,[1] C.A. Koller,[1] C. Nicaise,[2] G. Garcia-Manero,[1] A. Ferrajoli,[1] H.M. Kantarjian[1]

[1]MD Anderson Cancer Center, HOUSTON; [2]Bristol-Myers Squibb, WALLINGFORD, USA

Background. Dasatinib (BMS-354825) is a multi-targeted kinase inhibitor of BCR-ABL and SRC with significant activity in pts with CML-CP resistant or intolerant of imatinib (IM). We initiated a phase II trial of dasatinib in pts with previously untreated CML-CP. *Aims.* To study the efficacy and safety of dasatinib in this setting. *Methods.* The primary objective was to estimate the proportion of pts attaining major molecular response at 12 months (mo). Pts with previously untreated CML-CP were eligible and received dasatinib 100 mg/day, randomized to either 50 mg-twice-daily (BID) or 100 mg-once-daily (QD). *Results.* Forty pts have been enrolled (21 on the QD schedule, 19 BID). Median age was 41 years (yrs) (range 18-76 yrs). The rate of complete cytogenetic response (CCyR) at 3, 6 and 12 mo compares favorably to those observed in historical controls treated with imatinib 400 mg or 800 mg daily (Table 1). At 12 mo, 8/25 (32%) evaluable pts had achieved a major molecular response. There was a trend for improved molecular response with the QD schedule. Grade 3-4 non-hematologic toxicity (regardless of causality) included fatigue (5%), headache (3%), and rash (3%). Pleural effusion occurred in 5 (13%) pts (all grade 1-2). Grade 3-4 hematologic toxicity rates (transient) were thrombocytopenia 10%, neutropenia 5%, and anemia 3%. With median follow-up of 18 mo, 18 (46%) pts required transient treatment interruption and dose reduction. The actual median daily dose for all pts was 100 mg. There is no significant difference in grade 3-4 toxicity by treatment schedule. *Summary/Conclusions.* Rapid CCyR occurs in most patients with previously untreated CML-CP treated with dasatinib frontline therapy with a favorable toxicity profile. Accrual to this trial continues.

Table 1.

	Percent with CCyR (No. evaluable)			
Months on therapy	Dasatinib	Imatinib 400mg	Imatinib 800mg	P value
3mo	72(38)	37 (49)	62 (202)	0.0003
6mo	88 (34)	54 (48)	82 (199)	<0.0001
12mo	100 (26)	65 (48)	86 (197)	0.0001

0882

MARKED TELOMERE EROSION OF PH-NEGATIVE MYELOID CELLS AFTER SUCCESSFUL TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA INDICATES SEVERE PROLIFERATIVE STRESS AND POTENTIAL PERMANENT DAMAGE OF HEMATOPOIESIS

M. Ladetto, C. Lobetti Bodoni, E. Genuardi, M. Genuardi, V. Giai, A. Rocci, L. Monitillo, D. Drandi, A. Risso, B. Mantoan, S. Ferrero, R. Critelli, C. Tarella, M. Boccadoro, D. Ferrero

Università di Torino, TORINO, Italy

Background. Most chronic myelogenous leukemia (CML) patients (pts) experience long-term restoration of non-neoplastic hematopoiesis following successful disease treatment with tyrosine kinase (TK) inhibitors. However little is known on the functional and genetic integrity of Ph-negative cells repopulating the bone marrow after successful disease control. Indeed, they might have suffered considerable stress due to the interaction with the malignant clone and/or the subsequent effort of bone marrow (BM) repopulation. This hypothesis is also suggested by the occurrence of cytogenetic abnormalities (CA) in Ph-negative cells in about 10% of these subjects. *Aims.* Telomere restriction fragment length (TRF-L) analysis is an effective tool to monitor the proliferative stress of hemopoietic cells as shown by several transplantation studies. Aim of this study was to use TRF-L analysis to verify the presence and degree of proliferative stress suffered by the hematopoietic compartment repopulating bone marrow following successful disease control. *Patients and methods.* 53 chronic phase CML pts in complete cytogenetic remission (CR) lasting one year or more were analyzed. 43 pts were treated with

Imatinib and 10 pts with INF associated or not to ara-C. Median age was 61 (23-88), M/F ratio was 1.5, median time from diagnosis and from complete CR were 70 (12-217), and 40 months (6-150), respectively. 24 pts had low Sokal score, 19 intermediate, and 10 high. 7 pts carried an acquired CA. Complete molecular responders were 20 of 43. 86 healty donors with a comparable age and M/F were used as controls. TRF-L analysis was performed by Southern Blotting as previously described (Ladetto M *et al.,* Blood 2004), both on peripheral blood (PB) polymorphonucleates (PMN) (isolated as described in Tarella C *et al.*, Eur J of Cancer 1991) and on monocyte depleted PB mononuclear cells (MD-PBMC) (obtained as described by Ferrero D *et al.,* J Clin Lab Imm 1998). This allowed to assess both the myeloid and lymphoid compartment. *Results.* Figure 1 a shows the comparison between CML pts and healthy subjects. In CML pts, both PMN and MD-PBMC displayed shorter TRF-L, although the finding is more striking in PMN (mean telomeric loss in PMN 1932 pb; in MD-PBMC: 830 pb). Telomeric loss is more severe in young pts compared to older ones, resulting in loss of the association between TRF-L and age, typically seen in healthy subjects (Figure 1b). We found no correlation between TRF-L and previously mentioned clinical and demographic parameters. Telomere shortening was observed in both pts treated with or without TK inhibitors. When a multivariate analysis on pts and healthy controls was performed, the presence of CML resulted a stronger predictor of telomeric damage compared to age. Conclusions Ph-negative hematopoietic cells repopulating the BM after successful treatment of CML display severe telomere erosion compared to healthy subjects. Indeed the previous CML history and treatment induced a proliferative stress comparable to that obtained in 38 years of physiological aging. The functional and genetic consequences of this premature aging are currently under investigation.

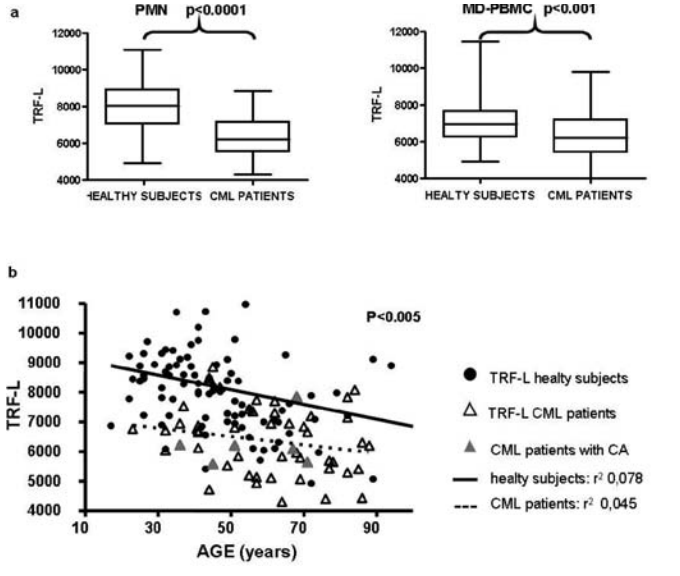


Figure 1.

0883

NILOTINIB IN CHRONIC MYELOGENOUS LEUKAEMIA IN CHRONIC PHASE (CML-CP) PATIENTS WITH IMATINIB-RESISTANCE OR INTOLERANCE: UPDATED PHASE 2 RESULTS

H.M. Kantarjian,[1] F.J. Giles,[2] K.N. Bhalla,[3] R.A. Larson,[4] N. Gatterman,[5] O.G. Ottmann,[6] A. Haque,[7] N. Gallagher,[7] M. Baccarani,[8] P.H. Le Coutre[9]

[1]M.D. Anderson Cancer Center, HOUSTON, USA; [2]CTRC at the UT Health Science Center San Antonio, SAN ANTONIO, USA; [3]Medical College of Georgia Cancer Center, AUGUSTA, USA; [4]The University of Chicago Hospitals, CHICAGO, USA; [5]University of Düsseldorf, DÜSSELDORF, Germany; [6]Medizinische Klinik III, FRANKFURT, Germany; [7]Novartis Pharmaceuticals, EAST HANOVER, USA; [8]University of Bologna Institute of Hematology and Medical Oncology Seragnoli, BOLOGNA, Italy; [9]Campus Virchow, Charité, Universitätsmedizin, BERLIN, Germany

Background. Nilotinib, a potent and highly selective BCR-ABL inhibitor, has been approved in several countries including the US and Europe for the treatment of patients (pts) with Philadelphia chromosome-positive chronic myelogenous leukaemia (Ph^+ CML) in chronic phase (CP) and accelerated phase (AP) who are resistant or intolerant to prior therapy including imatinib. *Methods.* This phase 2, open-label study was designed to evaluate the efficacy and safety of nilotinib in pts with Ph^+ CML-CP who are resistant or intolerant to imatinib. Nilotinib was administered at 400 mg twice daily (BID), with the possibility to dose escalate to 600 mg BID for inadequate responses, in the absence of safety concerns. The primary endpoint was the rate of major cytogenetic response (MCyR). Key secondary endpoints included complete cytogenetic response (CCyR), complete haematological response (CHR), survival and safety. *Results.* Included in this analysis were 321 pts (71% imatinib-resistant; 29% imatinib-intolerant). The majority of study pts (72%) had received prior imatinib doses >600 mg. Median age was 58 years, median dose intensity was 790 mg/day and median nilotinib exposure was 13 months. Of the 206 pts who did not have CHR at baseline, 158 (77%) achieved CHR during nilotinib therapy. Overall, MCyR was observed in 184 pts (57%) and 41% had CCyR. MCyR was observed in 125 (55%) of the 227 imatinib-resistant pts and in 59 (63%) of the 94 imatinib-intolerant pts. The majority (84%) maintained MCyR for at least 18 months. Of 227 pts evaluated, 60 (26%) achieved major molecular response (MMR) at 12 months. MMR occurred in 23% of imatinib-resistant and 34% of imatinib-intolerant pts. At 18 months, the estimated overall survival rate was 91%. Treatment with nilotinib is ongoing in 168 (52%) pts. The most frequent grade 3/4 laboratory abnormalities were thrombocytopenia (28%), neutropenia (30%), anaemia (10%) and asymptomatic serum lipase elevation (15%). Grade 3/4 non-haematological AEs were rare and included rash, headache, and diarrhoea. A low incidence (<1%) of QTcF prolongation >500 msec was observed. Grade 3/4 fluid retention and bleeding events were also rare, occurring in <1% of all pts. The safety profile of nilotinib has not changed with increased follow up. There was minimal cross-intolerance from nilotinib in pts intolerant to prior imatinib treatment. *Conclusions.* Nilotinib induces significant and durable responses in CML-CP pts with imatinib-resistance or -intolerance. Nilotinib is well tolerated, with minimal occurrence of grade 3/4 AEs and is an effective treatment option for this pt population.

0884

DOSE FINDING STUDY OF IMATINIB IN COMBINATION WITH INTRAVENOUS CYTARABINE: FEASIBILITY AND EFFICACY IN NEWLY DIAGNOSED PATIENTS WITH CHRONIC MYELOID LEUKEMIA

W. Deenik,[1] B. van der Holt,[1] G.E.G. Verhoef,[2] W.M. Smit,[3] M.J. Kersten,[4] J.C. Kluin-Nelemans,[5] L.F. Verdonck,[6] A. Ferrant,[7] A.V.M.B. Schattenberg,[8] J.J.W.M. Janssen,[9] P. Sonneveld,[1] M. van Marwijk Kooy,[10] S. Wittebol,[11] R. Willemze,[12] P.W. Wijermans,[13] P.H.M. Westveer,[1] H.B. Beverloo,[1] P. Valk,[1] B. Löwenberg,[1] G.J. Ossenkoppele,[9] J.J. Cornelissen[1]

[1]Erasmus University Medical Center, ROTTERDAM, Netherlands; [2]University Hospital Gasthuisberg, LEUVEN, Belgium; [3]Medical Spectrum Twente, ENSCHEDE, Netherlands; [4]Academic Medical Center, AMSTERDAM, Netherlands; [5]University Medical Center Groningen, GRONINGEN, Netherlands; [6]University Medical Center Utrecht, UTRECHT, Netherlands; [7]University Hospital St-Luc, BRUSSELS, Belgium; [8]Radboud University Nijmegen Medical Center, NIJMEGEN, Netherlands; [9]VU Medical Center, AMSTERDAM, Netherlands; [10]Isala Clinic - Sophia, ZWOLLE, Netherlands; [11]Meander Medical Center, AMERSFOORT, Netherlands; [12]Leiden University Medical Center, LEIDEN, Netherlands; [13]Haga Hospital, THE HAGUE, Netherlands

Background. The introduction of imatinib mesylate has been a major breakthrough in the treatment of patients with chronic myeloid leukemia (CML). Most patients develop a complete cytogenetic response, but complete molecular responses are rare. In view of the synergistic and dose dependent effects of imatinib and cytarabine, the question was raised if combination therapy of both drugs may improve response rates and prevent resistance. *Aims.* The HOVON-51 was set out to investigate the feasibility and efficacy of escalated imatinib and intravenous cytarabine in patients with early chronic phase CML. *Methods.* Patients received two cycles of intravenous cytarabine (200 mg/m^2 or 1000 mg/m^2 days 1-7) in conjunction with imatinib (200 mg, 400 mg, 600 mg or 800 mg), according to one of seven predefined, successive dose levels. Primary endpoints were dose limiting toxicity (DLT) and molecular response as assed by real-time quantitative PCR (RQ-PCR). *Results.* From August 2001 to November 2005 165 patients entered the study after written informed consent was obtained, three patients were subsequently excluded. The median age of the patients was 47 (20-65) years. The Sokal score at diagnosis was low, intermediate or high, in 58 (36%), 51 (31%), 43 (27%) patients, respectively, and unknown in 10 (6%) patients. All dose levels proved feasible. Seven dose limiting toxicities (DLTs) were observed in 302 cycles of chemotherapy. Intermediate-dose cytarabine (1000 mg/m^2) prolonged time to neutrophil recovery and platelet recovery as compared to a standard-dose (200 mg/m^2). High-dose imatinib (600 mg or 800 mg) extended the time to platelet recovery as compared to a standard-dose (400 mg). The actuarial probability of a complete hematological response was 94%, of a complete cytogenetic response 64%, of a major molecular response 46% and of a complete molecular response 13% at 1 year. With longer follow-up the complete molecular response rate increased to 40% at 36 months from diagnosis. With a median follow-up of 44 months 118 patients are still on protocol treatment. Fourteen patients have progressed during follow-up, including 7 patients who developed accelerated phase or blast crisis, 3 patients who had a complete loss of hematologic response and 4 patients who lost their major cytogenetic response. Patients without a major molecular response were prospectively evaluated for ABL-kinase domain mutations and 11 mutations were detected during follow-up. Seven of these 11 patients with a point mutation have subsequently progressed. Sixteen patients have died, including 10 out of 24 patients, who received an allogeneic hematopoietic stem-cell transplantation. The estimated 5-year survival rate was 88%. Updated results will be presented. *Conclusions.* Combination therapy of imatinib and cytarabine is feasible and induces a high rate of molecular response.

Stem cell transplantation

0885

HAPLO-SCT WITH TK+ LYMPHOCYTES PRODUCE LEUKEMIA FREE SURVIVAL COMPARABLE TO UNRELATED BONE MARROW AND CORD BLOOD TRANSPLANTATION IN HIGH RISK ACUTE LEUKEMIA: 'TK007' PHASE II STUDY FINAL ANALYSIS

M.T. Lupo Stanghellini,[1] F. Ciceri,[1] C. Bonini,[1] A. Bondanza,[1] C. Traversari,[2] M. Salomoni,[2] L. Turchetto,[2] S. Colombi,[2] M. Bernardi,[1] J. Peccatori,[1] A. Pescarollo,[1] P. Servida,[1] Z. Magnani,[1] S.K. Perna,[1] V. Valtolina,[1] F. Crippa,[1] L. Callegaro,[1] E. Spoldi,[2] R. Crocchiolo,[1] K. Fleischhauer,[1] M. Ponzoni,[1] L. Vago,[1] A. Santoro,[3] E. Todisco,[3] J. Apperley,[4] S. Slavin,[5] E.M. Weissinger,[6] B. Hertenstein,[6] M. Bregni,[1] C. Gallo Stampino,[2] P. Bruzzi[7]

[1]San Raffaele Scientific Institute, IRCCS, MILAN, Italy; [2]MolMed, S.p.A., MILAN, Italy; [3]I.C. Humanitas, ROZZANO, Italy; [4]Hammersmith H, LONDON, United Kingdom; [5]Hadassah, JERUSALEM, Israel; [6]Hannover Medical Scholl, HANNOVER, Germany; [7]Istituto Nazionale per la Ricerca sul Cancro, GENOVA, Italy

Background. Haploidentical family donors represent the ideal solution to offer to every patient with high risk leukemia the potential cure of marrow stem cell transplantation. Extensive application of haploidentical stem cell transplantation (haplo-SCT) is limited by high rate of late transplant related mortality (TRM) and relapse associated with the delayed immune reconstitution secondary to the procedures for severe graft-vs-host-disease (GvHD) prevention. *Method.* In a haplo-SCT phase I-II multicenter, open, no-randomized, trial sponsored by MolMed SpA, we infused donor lymphocytes genetically engineered to express the suicide gene herpes simplex thymidine kinase (TK-DLI) to induce early immune reconstitution, while selectively controlling GvHD. *Results.* Between September 2002 and September 2007, 51 patients (pts) -median age 48- with high-risk hematologic malignancies were enrolled, 29 out of 51 pts were in complete remission (CR). After myeloablative conditioning regimen, 48 pts received a median 13×10^6/kg CD34$^+$ and 1.0×10^4/kg CD3$^+$ (median time to engraftment: 2 weeks). No immune reconstitution were observed in absence of TK-DLI. Twenty-seven pts received TK-DLI: 22 pts obtained prompt immune reconstitution with CD3$^+$>100/mcl at day+75 (median) from haplo-SCT and day+23 from TK-DLI. Eleven pts developed GvHD (10 acute GvHD grade I-IV and 1 chronic GvHD) that was always abrogated by the suicide gene induction. The 1-year TRM was 26% for pts treated with TK-cells, and 69% for pts who didn't receive TK-cells (p<0.0001). Immune reconstitution obtained with TK-cells infusion correlated with: 1. rapid development of a wide T-cell repertoire; 2. detection of high frequencies of T-cells specific for opportunistic pathogens; 3. abatement of the incidence of infectious adverse events (AE) and serious AE. In the ITT analysis, the 3 years leukemia free survival (LFS) was 5% for pts transplanted in relapse, 43% for pts in CR (p:0.007). The 3 years LFS was 45% for pts who achieved immune reconstitution and 9% for pts who failed immune reconstitution (p:<0.0001). *Conclusions.* This strategy is feasible and effective in providing immune reconstitution in haplo T-cell-depleted setting. In uni- and multi-variate analysis both status at transplant and immune reconstitution are significant risk factor. Infusion of TK-cells could significantly extend the application of haplo-SCT. A randomized phase III study comparing TK-DLI versus any T cell repletion strategy after haplo-SCT in high risk acute leukemia is now starting.

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HLA-DP AS SPECIFIC TARGET FOR GRAFT VERSUS LEUKEMIA REACTIVITY IN HLA-CLASS II EXPRESSING HEMATOLOGICAL MALIGNANCIES

C.E. Rutten, S.A.P. van Luxemburg-Heijs, M. Griffioen, W.A.F. Marijt, I. Jedema, M.H.M. Heemskerk, F.M. Posthuma, R. Willemze, J.H. Falkenburg

Leiden University Medical Center, LEIDEN, Netherlands

HLA-mismatched stem cell transplantation (SCT) can result in a strong alloimmune response since alloreactive T cells recognizing mismatched HLA-alleles are present in high frequencies in peripheral blood. Therefore, patients are preferably transplanted with a donor matched for HLA-A, -B, -C, -DR and -DQ. The role of HLA-DP as transplantation antigen is less clear. HLA-DP has been associated with GVHD following HLA-DP mis-

matched unmanipulated SCT. However, following T cell depleted SCT, HLA-DP has been associated with a decreased risk of disease relapse without GVHD, suggesting a role in GVL reactivity. We hypothesize that HLA-DP reactive T cells administered at the time of SCT may result in GVHD as a consequence of upregulated HLA-DP expression on non-hematopoietic cells caused by an inflammatory environment due to conditioning regimens or infections. In contrast, postponed donor lymphocyte infusion (DLI) after HLA-DP mismatched T cell depleted SCT may selectively induce a GVL response since late after SCT HLA-DP expression is anticipated to be restricted to hematopoietic cells. To analyze whether HLA-DP specific T cells could mediate GVL reactivity without GVHD, we analyzed the immune response in a patient with chronic B lymphocytic leukemia responding to DLI following HLA-DP mismatched T cell depleted SCT. Patient and donor were fully matched but differed for both HLA-DP alleles (donor HLA-DPB1* 0402,0501; patient HLA-DPB1* 0201,0301). Following non-myeloablative conditioning the T cell depleted SCT resulted in mixed chimerism (75% donor) without GVHD. After SCT persistent disease was observed and the number of malignant cells gradually increased to 58% in bone marrow. A single dose of DLI was administered 7 months after SCT, causing a profound anti-leukemic effect resulting in complete remission and conversion to full donor chimerism in the absence of GVHD. During the clinical response, the emergence of leukemia reactive $CD4^+$ T cells was demonstrated using IFN-γ ELISPOT analysis. The leukemia reactive $CD4^+$ T cells were clonally isolated from peripheral blood and bone marrow. All $CD4^+$ T cell clones (n=21) produced INF-γ in response to patient leukemic cells but not to donor cells. A CFSE based cytotoxicity assay showed that eleven $CD4^+$ T cell clones specifically lysed the leukemic cells. Using blocking studies, panel studies and retroviral transduction experiments of both mismatched HLA-DPB1 alleles, we identified HLA-DPB1*0201 and HLA-DPB1*0301 as the targets of this immune response. Next, recognition of skin-fibroblasts as targets for GVHD was determined. The T cell clones did not recognize unmanipulated fibroblasts. However, incubation with IFN-γ for three days as a model to mimic an inflammatory environment induced upregulated HLA-DP expression and thereby recognition by the T cell clones. This suggests that HLA-DP reactive T cells may cause GVHD in an inflammatory environment as a consequence of upregulated HLA-DP expression. However, we showed that the administration of HLA-DP reactive T cells at a later time point may selectively induce GVL reactivity without GVHD when HLA-DP expression may be restricted to hematopoietic cells. Therefore, HLA-DP may be used as a specific target for immunotherapy following T cell depleted SCT in patients with HLA-class II expressing hematological malignancies.

0887

G TO C TRANSITION AT POSITION -173 OF MIF GENE OF THE RECIPIENT IS ASSOCIATED WITH IMPROVED OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

G.C. Hildebrandt,[1] Y.Y. Chang,[1] H.T. Greinix,[2] A.M. Dickinson,[3] D. Wolff,[4] G.H. Jackson,[3] R. Andreesen,[1] E. Holler[1]

[1]University of Regensburg Medical Center, REGENSBURG, Germany; [2]Department of Internal Medicine, Vienna Mdical University, VIENNA, Austria; [3]School of Clinical and Laboratory Science, University of Newcastle, NEWCASTLE, UK; [4]University of Rostock Medical School, ROSTOCK, Germany

Background. Allogeneic stem cell transplantation (allo-SCT) is an important therapeutic option for a number of malignant and nonmalignant diseases. However, its use is limited by several complications, most importantly, the development of acute and chronic graft-versus-host disease (aGVHD and cGVHD). Macrophage migration inhibitory factor (MIF) was reported to be associated with several inflammatory diseases and based on its pro-inflammatory and dendritic cell activating capacities, we hypothesized that MIF is a potential mediator of GVHD. Specially, a G/C single nucleotide polymorphism(SNP) at position -173 of the MIF coding region has been described, and MIF-173*C was shown to influence MIF promoter activity in T lymphoblast cell lines and was linked to the development or severity of various immunological diseases. Aim and Method: To investigate if this specific genetic difference in humans alters the complex immunological events after allo-SCT, we tested, whether MIF -173 G/C gene polymorphism contributes to changes in inflammation and alloreactivity after allo-SCT by analyzing 454 donor-recipient pairs for presence -173*C allele and transplantation outcome. *Results.* Donor -173 G/C polymorphism did not change overall survival (OS), disease-free survival (DFS), relapse and GVHD, whereas patient -173*C allele led to improved OS (5 years: 60.8% versus 46.3%, p=0.042) and DFS (5 years: 55.4% versus 39.5%; p=0.014). Interestingly, this beneficial effect of SCT outcome was due to a reduction not in GVHD development but in relapse (20.5% versus 32.9%; p=0.01). Multivariate analysis showed that MIF-173*C is an independent risk factor for relapse (p=0.015) after including cofounders such as patient age, severe aGVHD, cGVHD, stem cell source, conditioning regimen intensity, T cell depletion, donor type, stage of disease. In addition, the beneficial effect on survival rate (5-year OS: 83.6% versus 46.4%; p=0.004) and relapse (16.7% versus 41.6%; p=0.03) was most prominent in patients with lymphoma and multiple myeloma, but not in patients with acute leukemia or myeloproliferative diseases. Again, patient MIF -173*C allele proved as an independent factor for reducing relapse in this disease group. Further, CC/GC genotype resulted in higher MIF serum levels at time of transplantation. When dividing patients into two groups according to their levels of pre-SCT serum MIF, a clear trend towards a reduction in relapse rate could be recognized in association with increased pre-SCT serum MIF levels (23.1% versus 44.4%; p=0.085). *Conclusions.* Our data suggest a strong association between MIF and the graft versus leukemia effect, that only applies to certain disease entities, and, therefore, patient MIF -173*C allele may be used as a prognostic marker to improve patient-tailored counseling and therapy in allo-SCT.

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BONE MARROW TRANSPLANTATION IN PATIENTS WITH HOMOZYGOUS THALASSEMIA

S. Santarone,[1] E. Di Bartolomeo,[1] P. Bavaro,[1] P. Olioso,[1] G. Papalinetti,[1] P. Di Carlo,[1] A. Angelone,[2] M. Di Nicola,[3] P. Di Bartolomeo[1]

[1]Bone Marrow Transplant Center, PESCARA; [2]Anatomia Patologica, Ospedale Civile, PESCARA; [3]Dipartimento Scienze Biomediche, Laboratorio Biostatistica, Università degli Stu, CHIETI, Italy

In this study we report the long-term results of bone marrow transplantation (BMT) in 120 patients (M 61, F 59) with homozygous thalassemia who were given 128 transplants between May 1983 and October 2007. The median age was 10.02 years (0.11-28.11). The median number of transfusions given before BMT was 127 (2-900). Donors were HLA-genotipically identical siblings in 114 cases, HLA-phenotipically identical relatives in 4, and matched unrelated donors in 2. One patient received HLA-identical sibling cord blood cells and 119 were given marrow cells. Liver biopsies were performed on 84 patients before BMT. Liver fibrosis was absent in 4 patients, mild in 41, moderate in 26 and severe in 13. All patients received the same preparative therapy consisting of Busulphan (BU) (13-14 mg/Kg) and Cyclophosphamide (200 mg/Kg), preceded by an hypertransfusion regimen for 2-3 weeks. For graft-versus-host disease (GvHD) prophylaxis, 39 patients were given Cyclosporine (CSA) alone and 81 received CSA and short course Methotrexate (MTX). The median number of transplanted nucleated cells was 5.0×10^8/Kg (2.3-10.1). Sustained engraftment was documented in 115 patients (95.8%). The median time to achieve 0.5×10^9/L neutrophils and 50×10^9/L platelets was day 19 (range, 11 to 37) and day 24 (range, 10 to 55) respectively. The cumulative incidence of graft rejection was 8.3% (3 primary graft failures, 2 delayed graft failures and 5 autologous reconstitutions). In univariate analysis the only factor associated with a higher probability of graft rejection was the GvHD prophylaxis with CSA as compared to the regimen CSA-MTX (13% versus 3%, p=0.04). The Kaplan-Meier estimate of probability of developing grade I to IV acute GvHD was 37%. The patients who received the CSA-MTX prophylaxis had a significantly lower probability of developing acute GvHD compared with those who were given CSA alone (27% versus 54%, respectively, p=0.001). The Kaplan-Meier estimate of probability of developing chronic GvHD was 17% (7% limited, 10% extensive). Transplant-related mortality at 1 year was 8.3%. Ten patients died for BMT-related causes: pneumonia in 4, heart failure in 3, encephalopathy in 2, aGvHD in 1. Two late deaths occurred, one for septic shock 54 months post-BMT and one for parotitis carcinoma 138 months after BMT. As of February 2008, 108 patients are alive. The 20-year Kaplan-Meier estimates of overall survival and disease-free survival were 90,1% (95% CI 82.2-96.2) and 86% (95% CI 77.6-93.8) respectively after a median follow-up of 15 years (4 months to 24 years). In 103 cases BMT was successful with definitive transfusion independence. Five patients are now living and receive regular transfusion treatment following recurrence of thalassemia. In multivariate analysis, no adverse risk factor affecting survival was identified among recipient-donor age and sex, number of pre-BMT transfusions, level of ferritin, severity of chronic hepatitis, grade of liver fibrosis, serum ALT level, HBV and HCV serology, dose of BU, type of GvHD prophylaxis, marrow cell dose. In conclusion, this study demonstrates that BMT can cure the majority of patients with homozygous thalassemia with an acceptable margin of risk.

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MONITORING OF VIRUS-SPECIFIC IMMUNE RECONSTITUTION IN PATIENTS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

S. Borchers, S. Luther, B. Grabow, A. Ganser, E.M. Weissinger

Hannover Medical School, HANNOVER, Germany

Background. The reactivation of latent viruses like Cytomegalovirus (CMV) contributes significantly to morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Especially CMV-seropositive recipients transplanted with cells from a CMV-seronegative donor have a high risk for recurrent reactivation. Risk factors include the conditioning regimen, prophylaxis as well as therapy of acute and chronic graft versus host disease (GvHD). *Aims and Methods.* Virus specific T-cells play a major role in controlling reactivation, thus we monitored the immune reconstitution with CMV-reactive cytotoxic T-cells using MHC-I-peptide-tetramers for the major HLA class I groups. All transplanted patients matching at least one commercially available CMV-tetramer were included. In parallel, the functionality of the tetramer positive T-cells was evaluated using intracellular staining for IFN-γ. Samples were taken prior to HSCT, on days +50, +80, +100, + 180 and +365 or weekly in case of CMV reactivation or increased immunosuppressive therapy. *Results.* To date we have included 97 patients (median age 49 y, range 18-70 y). The majority had acute myeloid leukemia (AML= 40%, 17% secondary AML). The CMV serostatus for recipients (R) and donors (D) was: R^+/D^+ 49%, R^+/D^- 10%, R^-/D^+ 9% and R^-/D^- 31%. The majority of patients (72%) did not reactivate CMV, 28% reactivated CMV at least once. Mean day of the first CMV reactivation was day +43 (range +11 to +136). For 58% of the patients at least 2 matching tetramers (46% =2, 10%=3, 2%=4) were available, while 41% could be screened with 1 tetramer. The mean follow-up data of 71 living patients range from (range: +4 to +930 days after HSCT). In all patients of the R^+/D^+ setting, CMV-reactive T-cells were detected; cut off for protection against CMV was around 10 cells /µL. In case of reactivation in 11 of 14 patients of R^+/D^+ group an increase of CMV-tetramer-positive T-cells was detected. In the R^+/D^- group 67% reactivated CMV, 33% more than once. 2 of the 4 haploidentical transplanted patients reactivated CMV, 1 three time though he had a positive donor. In patients not reactivating CMV or controlling CMV reactivation we could show that tetramer-positive T-cells were also secreting IFN-γ upon stimulation with recombinant pp65 protein. T-cells from patients reactivating CMV despite the presence CMV-tetramer binding cells did not secrete IFN-γ. On the other hand, patients with no matching tetramers could be screened with intracellular staining. *Conclusions.* These results indicate that screening patients for virus-specific immune reconstitution after HSCT will help to identify patients at risk of recurrent reactivations early on. In addition, patients with suitable numbers of IFN-γ secreting, CMV-specific T-cells may be able to control the reactivation without virus suppressing medication. For patients with no matching commercially available tetramer (27%), intracellular staining for IFN-γ can yield comparable results with small numbers of cells. Thus we conclude that screening for CMV-specific T-cells after HSCT may help to improve the survival after HSCT, by selecting antiviral therapy according to the presence of CMV-specific T-cells.

Acute myeloid leukemia - Clinical

0890

AMONAFIDE: A TOPOISOMERASE II INHIBITOR WITH NOVEL PHARMACOLOGICAL PROPERTIES AND UNIQUE ACTIVITY FOR THE TREATMENT OF SECONDARY AML

R.L. Capizzi,[1] A.S.L. Lundberg,[1] M.C. Chau,[1] D.J.F. Fernandes,[2] A.M.A. Ajami[1]

[1]Xanthus Pharmaceuticals, Inc., CAMBRIDGE; [2]Medical University of South Carolina, CHARLESTON, USA

Background. Secondary AML (sAML) portends a poor prognosis due to disease and patient-related factors. Newer therapies are needed. *Aims.* A. Comparison of amonafide to classical TOPO II inhibitors (anthracyclines, mitoxantrone, etoposide): 1. Cytotoxicity in leukemia cells that display the MDR phenotype due to P-glycoprotein (Pgp) over-expression/function; 2. Efflux from patient-derived sAML blasts; 3. Inhibitory effect on TOPO II, DNA damage and leukemia cell apoptosis. B. Clinical trial: combination of amonafide and standard dose ara-C (SDaC) for treatment of sAML. *Methods.* Standard assessments: Pgp expression/function; cell viability; DNA fragmentation; apoptosis. Cell lines: human leukemia K562 and K562/DOX; murine P388 and P388/ADR. The /DOX and /ADR cell lines over-express P-gp and display the MDR phenotype. Primary leukemic blasts: pre-treatment bone marrow samples from patients participating in the phase 2 trial of amonafide and SDaC noted below. *Results.* The IC_{50} (µM) for the classical TOPO II inhibitors in the Pgp over-expressed cell lines, K562/DOX and P388/ADR, increased 1-2 logs compared to the wild-type cells. Amonafide had equal cytotoxicity in both the wild type and MDR cells. Additional studies showed that amonafide was neither a substrate nor an inhibitor of Pgp. Cyclosporin A, an inhibitor of Pgp, effectively reversed resistance to the classical TOPO II inhibitors in the MDR cells and had no effect on amonafide cytotoxicity. Leukemic blasts from 15 sAML patients demonstrated significantly less efflux of amonafide than of daunorubicin: overall 5.2%±3.2 *vs* 16%±2.1; p=0.0083; unfavorable cytogenetics, 0.13%±3.7 *vs* 16%±2.1; p=0.0015; CR patients 4.0%±6.7 *vs* 21%±2.9; p=0.035, respectively. Amonafide did not affect the TOPO II/DNA cleavable complex, a shared key effect related to DNA fragmentation for all classical TOPO II inhibitors. Also, amonafide binds to TOPO II prior to the cleavable complex locus, is ATP-independent, and induces significantly less DNA fragmentation. Amonafide resulted in DNA disorganization by causing the release of chromatin loops from the nuclear matrix leading to apoptosis equivalent to the classical TOPO II inhibitors. *Clinical Trial.* 88 sAML patients (antecedent MDS or tAML) were treated with amonafide, 600 mg/m²/day for 5 days and ara-C, 200 mg/m²/day, 7 day continuous infusion. Median age 63 yrs (range 23-87); prior MDS, 45.5%; tAML 54.5%; unfavorable cytogenetics, 47%. CR: overall 42%; CR in subgroups: age <60 yrs, 39.4%; >60yrs, 43.6%. MDS ' AML without and with prior therapy for MDS (mostly azacytidine), 43.5% & 36%; tAML, 40%. Median duration of CR is >10 months with patients continuing on study. Kaplan-Meier estimate of continuous CR at 12 months is 44%. Safety profile: death within 28 days, 20.5%; marrow recovery to CR averaged 1 month; non-hematologic toxicity 6% moderate diarrhea and skin rash. *Conclusions.* Amonafide has distinctly different pharmacologic properties compared to the classical TOPO II inhibitors; it is not subject to MDR and hence not cross-resistant with classical TOPO II inhibitors. Cytotoxicity relates to intercalation in DNA leading to chromatin disorganization rather than DNA fragmentation. Significant and durable CRs were achieved across poor-risk subsets of sAML. A Phase 3 trial of amonafide and ara-C in sAML is underway.

0891

PATIENT AND DONOR CHARACTERISTICS DETERMINE OUTCOME AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA

C. Craddock,[1] S. Tauro,[2] L. Buckley,[3] P. Kottaridis,[4] E. Thelouli,[5] K. Thomson,[6] D. Milligan,[7] E. Liakopoulou,[8] J. Snowden,[9] J. Marsh,[10] A. Hunter,[11] J. Yin,[5] A. Peniket,[12] M. Cook,[1] S. Mackinnon,[4] N. Russell[13]

[1]Centre for Clinical Haematology, BIRMINGHAM; [2]Division of Pathology & Neuroscience, University of Dundee, DUNDEE; [3]Division of Cancer Studies, University of Birmingham, BIRMINGHAM; [4]Department of Haematology, Royal Free Hospital, LONDON; [5]Department of Haematology, Manchester Royal Infirmary, MANCHESTER; [6]Department of Haematology, University College Hospital, LONDON; [7]Department of Haematology, ; Heartlands Hospital, BIRMINGHAM; [8]Department of Haematology, Christie Hospital, MANCHESTER; [9]Department of Haematology, Royal Hallamshire Hospital, SHEFFIELD; [10]Department of Haematology, St Georges Hospital, LONDON; [11]Department of Haematology, Leicester Royal Infirmary, LEICESTER; [12]Department of Haematology, John Radcliffe Hospital, OXFORD; [13]Department of Haematology, Nottingham City Hospital, NOTTINGHAM, UK

Background. Allogeneic stem cell transplantation performed using a reduced intensity conditioning (RIC) regimen represents an important advance in the treatment of older patients with high risk acute myeloid leukaemia (AML) whose outlook with conventional chemotherapy would be poor. However the factors which influence the survival of patients with AML transplanted using a RIC regimen have not yet been rigorously defined. *Aims.* We wished to characterise patient and donor characteristics determining survival in patients with AML transplanted using a uniform reduced intensity regimen over a ten year period. *Methods.* Long term follow-up data (maximum 114 months, median 30 months) was collected from 178 patients with AML transplanted using a conditioning regimen consisting of fludarabine (30 mg/m^2×5 days), melphalan (140 mg/m^2 × 1 day) and alemtuzemab (10 mg×5 days). The median age was 52 years (range 18-71). 89 patients were in CR1 at the time of transplant, 69 were in CR2/3 and 20 had relapsed or refractory disease. Cytogenetic information was available on 176 patients and of these 133 had intermediate and 43 adverse risk cytogenetics using MRC criteria. 85 transplants were performed using an HLA identical sibling donor and 93 using a volunteer unrelated donor. *Results.* The 3 year overall survival (OS) for the whole group was 45% and 3 year disease free survival (DFS) 42%. 20 patients remain in remission more than five years post-transplant. The 100 day transplant related mortality was 11%. 29% of patients developed Grade II-IV acute GVHD and 22% chronic GVHD. Survival was significantly influenced by status at transplant (p=0.01) and presentation cytogenetics (p=0.005) as determined by multivariate Cox proportional hazards regression. The 3 yr OS for patients transplanted in CR1 or CR2/3 was 49% and 48% respectively compared to 16% for patients with relapsed/refractory disease. The 3 year OS for patients with intermediate risk cytogenetics was 51% compared to 31% for patients with adverse risk cytogenetics. Neither patient age, stem cell source (sibling v unrelated donor) or the presence of acute or chronic GVHD influenced outcome. However the survival of patients transplanted using an unrelated donor was influenced by patient:donor HLA disparity and was decreased in the presence of a single antigen molecular mismatch at HLA A, B, C or DRβ1. The 3 yr OS for patients transplanted using an unrelated donor with no detectable HLA disparity was 56% compared with 16% in patients with a single antigen mismatch (p=0.0009). *Conclusions*. This study confirms the ability of RIC allografts to deliver encouraging long term survival rates in high risk AML. Pre-transplant patient and donor characteristics can be used to predict outcome after a RIC allograft. Patients with adverse risk cytogenetics, active disease at the time of transplant and those lacking a molecularly matched unrelated donor require novel strategies in order to improve long term survival.

0892

PHASE II STUDY OF SINGLE AGENT CLOFARABINE IN PREVIOUSLY UNTREATED OLDER ADULT PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA (AML) UNLIKELY TO BENEFIT FROM STANDARD INDUCTION CHEMOTHERAPY

H. Erba,[1] H. Kantarjian,[2] D. Claxton,[3] M. Arellano,[4] R. Lyons,[5] T. Kovacsovics,[6] J. Gabrilove,[7] S. Eckert,[8] R. Abichandani,[8] S. Faderl[2]

[1]University of Michigan Comprehensive Cancer Center, ANN ARBOR; [2]University of Texas MD Anderson Cancer Center, HOUSTON; [3]Penn State Hershey Medical Center, HERSHEY; [4]Emory University School of Medicine, ATLANTA; [5]Cancer Care Centers of Texas, SAN ANTONIO; [6]Oregon Health and Science University Center, PORTLAND; [7]Mount Sinai School of Medicine, NEW YORK; [8]Genzyme, CAMBRIDGE, USA

Background. Many studies have identified a population of AML patients unlikely to benefit from combination induction therapy ("7+3"anthracycline-based therapy). based on adverse prognostic factors such as advanced age, poor performance status (PS), the presence of an antecedent hematologic disorder (AHD), or an intermediate/unfavorable risk karyotype. *Aims.* This study assessed the efficacy and safety of single-agent clofarabine (CLO) in this population. *Methods.* This was a single-arm, Phase II, open-label, 2 stage study with a planned total enrollment of 109 patients. Eligible patients included adults with untreated AML who were ≥60 years with at least one adverse prognostic factor: ≥70 years, AHD, PS 2, and/or intermediate/unfavorable risk karyotype. CLO was given on days 1-5 as a 1-hour intravenous infusion at dosages of 30 mg/m^2 during induction and 20 mg/m^2 during re-induction/consolidation. Patients could receive a maximum of 6 cycles. The primary endpoint was the overall remission rate (ORR = CR + CRp). Results: A total of 116 patients were enrolled. As of August 2007, safety data were available for 54 patients; efficacy data were available for 40 patients. All patients signed IRB-approved consent forms. Median age was 71 years and 48% were classified as M1 or M2 by FAB classification. Baseline prognostic factors included: 65% with ≥70 years, 37% with AHD (13% yet unreported), 70% with intermediate/unfavorable risk karyotype (30% yet unreported), and 19% with PS 2. All 54 patients initiated at least one cycle; 44% initiated a second cycle as re-induction or consolidation therapy. Thirty-day all-cause mortality was 13%. Drug-related adverse events occurring in >20% of patients were: nausea (41%), vomiting (28%), diarrhea (26%), febrile neutropenia (24%), and rash (20%). Most treatment-related events were Grades 1-2. Treatment-emergent Grade 3 febrile neutropenia occurred in 30% of patients. Treatment-emergent Grade 4 neutropenia was reported in 33% of patients with baseline absolute neutrophil count values available. The ORR was 43% (95% CI: 27.0%, 59.1%): 40% CR (95% CI: 24.9%, 56.7%) plus 3% CRp (95% CI: 0.1%, 13.2%). The ORR by prognostic factor was: 58% for unfavorable risk karyotype, 44% for intermediate risk karyotype; 44% for AHD; 32% for age ≥70, 67% for age <70; and 14% for PS 2. Summary/conclusions: These interim data indicate that single-agent CLO is active and well tolerated in treatment-naïve, older AML patients with ≥1 adverse prognostic factor, especially unfavorable risk karyotype and/or AHD. Safety data from this study are consistent with previously reported studies of CLO in older patients. Safety and efficacy results for all patients will be presented.

0893

PATIENT-REPORTED OUTCOMES IN LEUKEMIA RANDOMIZED CONTROLLED TRIALS. A SYSTEMATIC REVIEW TO EVALUATE THE ADDED VALUE IN SUPPORTING CLINICAL DECISION-MAKING

F. Efficace,[1] G. Kemmler,[2] M. Vignetti,[1] F. Mandelli,[1] S. Molica,[3] B. Holzner[2]

[1]GIMEMA, ROME, Italy; [2]Innsbruck University Hospital, INNSBRUCK, Austria; [3]Azienda Ospedaliera Pugliese-Ciaccio, CATANZARO, Italy

Background. The term Patient-Reported Outcomes (PROs) describes a broader set of parameters that have in common their focus on assessing health outcomes from the patient's perspective. It includes a wide range of measures, ranging from single item instruments assessing a specific symptom (e.g., pain or fatigue) to multidimensional health-related quality of life (HRQOL) measures. *Aims.* While PROs are now considered as key outcomes in cancer research, also from Regulatory Agencies, and their use in clinical trials of patients with solid tumors is quite common, no evidence exist on PRO trial-based studies conducted in patients leukemia. On this ground, we performed a systematic review to investigate traditional clinical and PROs in randomized controlled trials

(RCTs) of leukemia patients. *Methods.* A systematic search of the literature from 1980 to 2007 was undertaken in MedLine, Cancerlit and the Cochrane Controlled Trials Register to retrieve all RCTs having PROs as an endpoint of the study (either primary or secondary). No restriction in the publication language, number of patients enrolled or type of leukemia was performed. Three reviewers independently assessed all studies to consistently evaluate their methodological quality according to a previously developed protocol reviewer. This included a number of methodological and statistical quality criteria such as PRO missing data documentation, timing of assessment, discussion of outcomes in terms of clinical significance and questionnaire used. Both PROs and traditional clinical outcomes were also systematically analyzed to evaluate their consistency and their relevance for supporting clinical decision-making. *Results.* Nine RCTs were identified, involving overall 3010 patients. Only 6 RCTs evaluated HRQOL in a prospective fashion while in 3 studies this was evaluated in a cross sectional fashion. There were 4 RCTs involving acute myeloid leukemia patients (AML), 3 with chronic myeloid leukemia (CML) and 2 with chronic lymphocityc leukemia (CLL). Six studies were published after 2000 and provided fairly robust methodological quality. Imatinib greatly improved HRQOL compared to interferon based treatments in CML patients and fludarabine plus cyclophosphamide seem not to have a deleterious impact on patient's HRQOL compared to standards fludarabine alone or chlorambucil in CLL patients. In addition, fatigue is the major baseline symptom affected by disease burden in CLL when compared to healthy matched controls. Toxicity data in these trials did not translate into predictable HRQOL and symptom domains from the patient's perspective. Three studies provided excellent examples on how PROs can be implemented in a RCT setting yielding additional valuable outcomes to fully evaluate overall treatment effectiveness. *Conclusions.* This study revealed some methodological drawbacks as well as the paucity of PRO research in leukemia patients. Nonetheless, PRO assessment is feasible in RCTs and has the great potential of providing valuable outcomes to further support clinical decision-making. This study also emphasizes the unique information provided by the patient's perspective on the burden of the disease and treatment related effects.

0894

A CLINICAL AND IMMUNOLOGICAL PHASE II TRIAL OF WILMS TUMOR GENE PRODUCT 1 (WT1) PEPTIDE VACCINATION IN PATIENTS WITH AML AND MDS

A.L. Letsch,[1] U. Keilholz,[2] A.M. Asemissen,[2] A. Busse,[2] S. Bauer,[3] I.W. Blau,[2] W.K. Hofmann,[2] L. Uharek,[2] E. Thiel,[2] C. Scheibenbogen[3]

[1]Hematology and Oncology, BERLIN; [2]Hematology and Oncology, Charité CBF, BERLIN; [3]Med. Immunology, Charité CBF, BERLIN, Germany

Background and Aims. To assess the clinical and immunological efficacy and toxicity of vaccination with an HLA-A201-restricted WT1 peptide. *Methods.* Patients with WT1-expressing AML and MDS without curative treatment option were eligible. Vaccination consisted of 62.5 mg granulocyte macrophage colony stimulating factor (GM-CSF) days 1-4, and a mixture of 0.2 mg WT1.126-134 peptide and 1 mg keyhole limpet hemocyanin (KLH) on day 3. Vaccination was repeated biweekly x 4 followed by 4-weekly in the initial 13 patients (cohort 1) and continuously biweekly in subsequent 13 patients (cohort 2). Early disease progression until vaccine # 6 was allowed. T cell responses were measured by tetramer and cytokine flow cytometry. WT1 levels were assessed by qRT-PCR. Clinical response assessment followed IWG-MDS criteria. *Results.* A median of 11 vaccinations was administered to 26 patients, including 24 patients with AML and 2 with RAEB. Treatment was well tolerated. One Complete Remission (CR) (514 days) and 14 Stable Diseases (SD) (101 to 571+ days) were observed, including 4 SD with >50% blast reduction and 3 with hematologic improvement. The CR and 4 SD occurred after initial Progressive Disease (PD). WT1 mRNA-levels decreased at least 3-fold from baseline in 52% of patients In 25 patients evaluable for immune response, the frequency of patients with WT1 tetramer responses increased from 28% prior to vaccination to 76% at week 10 and with peptide specific cytokine responses from 24% to 52%, respectively. *Conclusions.* This study proves immunological, molecular and clinical efficacy of WT1 peptide vaccination in patients with AML warranting further investigations.

Non-Hodgkin's lymphoma - Biology

0895

RBL2/P130 NETWORK ABNORMALITIES IN ENDEMIC BURKITT'S LYMPHOMA EXPLORED BY GENE EXPRESSION ANALYSIS

P.P. Piccaluga,[1] G. De Falco,[2] E. Leucci,[2] W. Mwanda,[3] L. Leoncini,[2] S.A. Pileri[1]

[1]Section of Hematopathology, BOLOGNA, Italy; [2]Department of Human Pathology and Oncology, University of Siena, SIENA, Italy; [3]Department of Pathology, Kenyatta National Hospital, University of Nairobi, NAIROBI, Kenya

Background.. Burkitt's lymphoma (BL), the most common non Hodgkin lymphoma (NHL) type in Africa, is a B-cell tumor typically characterized by translocation t(8;14)(q24;q32), leading to MYC ectopic expression. However, several additional alterations have also been described in BL, including RBL2 gene mutations. It has been previously demonstrated that RBL2 is commonly mutated in endemic BL and less frequently in sporadic cases, with consequent lack of translation or functional inactivation due to cytoplasmic delocalization of the encoded protein, pRb2/p130. On the other hand, HIV-related BL cases usually show high expression of wild type (WT) RBL2. This raises the possibility that RBL2 pathway alteration might play a role in BL pathogenesis by offering, intriguingly, different contribution to the development of the three BL subtypes. *Aim.* We performed a gene expression profile (GEP) analysis of BL cell lines, BL primary cases (including endemic, sporadic and HIV-related forms), B-NHLs, and normal B-cell subpopulations in order to 1) identify possible RBL2 target genes deregulated as a consequence of inactivating mutations, 2) explore the RBL2 network in primary cases and normal B-cells, and 3) assess whether RBL2 network abnormalities are limited to BL or common to other B-NHL. *Methods.* Raji and Daudi BL cell lines were transfected with either WT-RBL2 or empty vector; cell cycle, proliferation and apoptosis were studied by conventional methods (flow citometry, cell counting and Annexin V assay, respectively). Gene expression analysis was carried on Affymetrix microarrays, by studying BL cell lines (eventually transfected with WT-RBL2), BL primary cases (16 sporadic, 10 endemic, and 6 HIV-related), other NHL cases (including 37 diffuse large B-cell lymphoma, DLBCL; 37 follicular lymphoma, FL; and 10 B-chronic lymphocytic leukemia, B-CLL) and normal B-cells (20 samples, including germinal center, naïve and memory subtypes). Validation was performed by quantitative RT-PCR and/or immunohistochemistry. *Results.* First, we found that upon RBL2 transfection, p130 was again properly located in the nuclei, while cell cycle was deeply affected with proliferation decline and apoptosis promotion. Second, we identified several genes whose expression was regulated upon transfection (*RBL2 signature*), allowing a clear discrimination of the samples according to the RBL2 mutational status. Further, by applying a gene set enrichment analysis (GSEA), we found that genes differentially expressed in BL primary cases of different subtypes were significantly enriched in genes included in the *RBL2 signature*. At present, it was not possible to establish whether RBL2 played a different role in the three BL subtypes, as they showed, in general, pretty similar profiles. However, interestingly, the *RBL2 signature* appeared to be particularly relevant in endemic cases (GSEA, $p=4\times10^{-19}$). Notably, in HIV-related cases, carrying WT-RBL2, we provided evidence of p130 functional inactivation. Finally, we found that RBL2 network alterations were not exclusive of BL but could be detected also in GCB-DLBCLs, while FL and B-CLL showed a molecular pattern closer to that of normal B-cell, for what the *RBL2 signature* was concerned. *Conclusions.* Taken together, our results strongly suggest a role of RBL2 deregulation in BL molecular pathogenesis and possible involvement also in DLBCL.

0896

GENOME-WIDE ARRAY-BASED COMPARATIVE GENOMIC HYBRIDIZATION OF HIV-RELATED NON-HODGKIN LYMPHOMA: IDENTIFICATION OF RECURRENT GENETIC LESIONS SPECIFICALLY ASSOCIATED WITH THE DISEASE

D. Capello

University of Eastern Piedmont, NOVARA, Italy

Background. Non-Hodgkin's lymphomas (NHL) represent a frequent complication of HIV infection and a major source of morbidity and mortality among patients affected by acquired immunodeficiency syndrome. Data on the underlying genetics of HIV-NHL are still scarce and mainly based on screening of genes known to be involved in the pathogenesis of lymphoma in immunocompetent (IC) hosts. *Aims.* To improve our

understanding of HIV-NHL pathogenesis using a powerful analysis technique, such as genome-wide DNA profiling. *Methods.* A genome-wide DNA profiling, based on SNP-microarray comparative genomic hybridization, was performed in 70 HIV-NHL, including 59 systemic cases (29 HIV-diffuse large B-cell lymphomas, HIV-DLBCL; 22 HIV-Burkitt lymphomas, HIV-BL; 8 HIV-Burkitt-like lymphomas, HIV-BLL), as well as 4 HIV-primary central nervous system lymphoma (HIV-PCNSL) and 7 HIV-primary effusion lymphomas (HIV-PEL). Twenty-two IC-DLBCL were analysed as a control group. DNA samples, extracted from frozen biopsies, were analyzed using the Affymetrix Human Mapping 250K Nsp arrays. Methylation analysis of selected genes was performed by methylation specific PCR. *Results*. Aberrations occurring in 20% or more of the cases were defined as recurrent. HIV-DLBCL cases had recurrent gains at 2p15, 3q29, 4q13.2, 8p23.1, 10p12.31, 11q22.1-q24.1, 14q11.2, 14q32.33, 12p13.31-pter, 15q11.2, and recurrent losses at 1p36.32-p36.33, 2p11.2, 5p12, 3p14.2, 7q22.1, 8p23.1, 9p23, 9p24.1 12p11.1, 14q11.2, 15q11.2, 17p13.1-p13.3 and 22q11.1. In comparison to IC-DLBCL, significant differences were the lack of 18q gains, and a lower frequency of 6q losses. HIV-BL had recurrent gains in 1q25.2, 3q29, 8p23.1, 15q11.2, 12p12.1-pter, 20q13.12 and recurrent losses at 15q11.2 and 17p11.2-p13.2. Frequent alterations of specific genes were also observed. Deletion of the fragile histidine triad gene (FHIT), in the recurrently lost 3p14 region, was detected in 14/70 (20%) HIV-NHL (6 HIV-DLBCL, 4 HIV-PEL, 2 HIV-BL and 2 HIV-BLL). Deletion of the WW domain-containing oxidoreductase gene (WWOX), at 16q23.1, was observed in 9/70 (13%) cases (4 HIV-DLBCL, 4 HIV-PEL and 1 HIV-BL). In all but one cases, WWOX deletion was associated with FHIT deletion. No IC-DLBCL had interstitial deletions affecting either FHIT or WWOX. Methylation of the FHIT gene was observed in 14/70 (20%) HIV-NHL (6 HIV-DLBCL, 6 HIV-BL, 1 HIV-BLL and 1 HIV-PCNSL), whereas WWOX methylation was observed in 31/70 (44%) cases (15 HIV-DLBCL, 7 HIV-BL, 3 HIV-BLL and 2 HIV-PCNSL). *Conclusions.* Our data show evidence of specific genomic alterations associated to HIV-NHL. FHIT and WWOX are selectively inactivated by DNA loss and/or promoter methylation in about 50% of HIV-NHL. HIV-BL shows less copy number aberrations than HIV-DLBCL. The pattern of gains and losses in HIV-DLBCL is more similar to the genetic profile observed in IC DLBCL derived from germinal center B-cells (GCB) than that of DLBCL derived from activated B-cells (ABC).

0897

TP53 MUTATION IDENTIFIES A HIGH RISK GROUP OF PATIENTS AT DIAGNOSIS OF FOLLICULAR LYMPHOMA AND IS ASSOCIATED WITH SHORTER TIME TO DISEASE PROGRESSION AND OVERALL SURVIVAL

D. O'Shea,[1] C. O'Riain,[1] C. Taylor,[2] R. Waters,[3] E. Carlotti,[1] F. MacDougall,[1] J. Gribben,[1] A. Rosenwald,[4] L. Rimsza,[4] E.B. Smeland,[4] N. Johnson,[4] E. Campo,[4] W.C. Chan,[4] R.D. Gascoyne,[4] L.M. Staudt,[4] T.A. Lister,[1] J. Fitzgibbon[1]

[1]Centre for Medical Oncology, LONDON, UK; [2]Mutation Detection Facility, St James's University Hospital, LEEDS, UK; [3]Centre for Medical Statistics, OXFORD, UK; [4]Lymphoma Leuaemia Molecular Profiling Project, Germany

Background. TP53 mutations are associated with poor prognosis in several types of malignancy and previous reports have suggested a link with Follicular Lymphoma (FL) and transformation to Diffuse large B cell lymphoma. *Aims.* To evaluate the presence of TP53 mutation and its association with clinical characteristics, response to treatment, progression free survival (PFS) and overall survival (OS) in previously untreated patients with FL. *Methods.* DNA from 185 patients with FL was obtained through the LLMPP; these cases were previously characterised by gene expression profiling and single nucleotide polymorphism array genotyping. Mutations of TP53 gene were investigated by PCR, fluorescent single-strand conformation polymorphism (FSSCP) analysis of exons 5-8, and confirmed by direct sequencing. The relationship between TP53 mutation and clinical characteristics, response to treatment, PFS and OS was investigated for 172 cases where complete clinical data was available. *Results.* A single TP53 mutation was detected in 12/185 (6%) cases. All mutations were heterozygous and were reported previously in the IARC database. Eleven mutations were missense [exon 5 (1), exon 7 (5) and exon 8 (5)], and a single mutation arose in the splice site region of intron 7. Comparison of mutated and wildtype groups showed that age >60 years (p=0.02) and high International Prognostic Index (IPI) status (p=0.04; IPI 3-5 *vs* 0-2) were associated with the presence of mutation. Patients were treated according to presentation features with mutation status having no association with initial treatment modality, treatment response or risk of transformation. By univariate analysis, TP53 mutation correlated with a significantly shorter PFS (p<0.0001, Hazard ratio 3.6, 95% CI 1.9 to 6.7) and OS (log rank p=0.001, Hazard ratio 3.2, 95% CI 1.6-6.1). On a multivariate analysis, adjusting for age and stage, TP53 mutation was significantly associated with a shortened PFS (p= 0.0004, Hazard ratio 3.4, 95% 1.7 to 6.7) and OS (p=0.009, Hazard ratio 2.7, 95% CI 1.3 to 5.6). *Conclusions.* Detection of a TP53 mutation, although uncommon, is associated with older age and higher IPI at diagnosis without increasing the risk of transformation. Both PFS and OS are shorter in the mutated group, with TP53 mutation remaining a highly significant variable for both PFS and OS after multivariate analysis. In patients with a TP53 mutation at diagnosis, the disease progresses more rapidly after a good initial response to treatment and so more aggressive treatment approaches should be considered.

0898

BCL-2/IGH PCR VALUES AT THE END OF INDUCTION TREATMENT ARE NOT PREDICTIVE FOR PROGRESSION FREE SURVIVAL IN RELAPSED/RESISTANT FOLLICULAR LYMPHOMA: RESULTS OF A PROSPECTIVE RANDOMIZED PHASE III INTERGROUP TRIAL

M.H.J. van Oers,[1] B.A. van der Reijden,[2] E. Tönnissen,[2] M. van Glabbeke,[3] L. Giurgia,[3] R. Klasa,[4] R.E. Marcus,[5] M. Wolf,[6] E. Kimby,[7] M. van t Veer,[8] A. Vranovsky,[2] H. Holte,[7] A. Hagenbeek[2]

[1]Academic Medical Center, AMSTERDAM, Netherlands; [2]EORTC Lymphoma Group, NIJMEGEN, Netherlands; [3]EORTC Data Center, BRUSSELS, Belgium; [4]NCIC CTG Hematology Group (Canada), VANCOUVER, Canada; [5]NCRI, LONDON, UK; [6]Australasian Leukaemia and Lymphoma Group, MELBOURNE, Australia; [7]Nordic Lymphoma Group, STOCKHOLM, Sweden; [8]HOVON, ROTTERDAM, Netherlands

Background. The predictive value of assessment of minimal residual disease after induction treatment in follicular lymphoma (FL) is still controversial. Last year we published the results of a prospective randomized phase III intergroup trial evaluating the role of rituximab (R) both in remission induction and maintenance treatment of 465 relapsed /resistant FL patients. Major conclusions were that addition of R to CHOP induction yielded an increased ORR and CR rate, and that R maintenance strongly improved median progression free survival (PFS; both after induction with CHOP and R-CHOP) and overall survival when compared to observation (van Oers *et al.* Blood 2006;108:3295). We now report on the Bcl-2/IgH PCR analysis in this study. Study design. Peripheral blood (PB) and bone marrow (BM) samples where obtained before the start of the induction therapy, at the end of the induction therapy and at the end of the 2 years maintenance/ observation period. The percentage of Bcl-2/IgH MBR$^+$ cells was quantified by genomic qPCR with a sensitivity of at least one Bcl-2/IgH MBR$^+$ cell among 10,000 normal cells (primer and probe sequences are available on request. The main question of our analysis was whether the primary endpoints of the study (response rate /quality for the induction phase and PFS for the maintenance phase) were correlated with results of the Bcl-2/IgH PCR in PB or BM. *Results.* Molecular biology data were available from 250 patients, evenly distributed amongst the therapeutic arms, both for induction and maintenance. Before treatment 48.5% and 42.0% of assessable patients had a positive Bcl-2/IgH PCR in BM and PB respectively. At the end of induction this had decreased to 28.6% and 17.3% respectively, and at the end of maintenance/observation to 10.5% and 10.6%. Conversion of positive to negative values were more frequent with R-CHOP induction (BM: p=0.026 and PB: p=0.003), and with R maintenance (BM: p=0.005). Percentages and levels of Bcl-2/IgH positivity in PB and BM correlated well at the three sampling time points. Bcl-2/IgH PCR results at diagnosis did not predict for overall response or complete remission rates after induction treatment, but BM results predicted PFS (p=0.04). Rather surprisingly, Bcl-2/IgH PCR results of BM and PB at the end of induction treatment were not predictive for PFS: 3 years PFS was 46% and 38% in the BM$^-$ and BM$^+$ group respectively (p=0.4), and 51% and 42% in the PB$^-$ and PB$^+$ group respectively (p=0.4).The highly significant improvement of PFS by R maintenance versus observation was observed in both Bcl-2/IgH PCR PB/BM positive and negative groups. Finally, patients who still had a positive Bcl-2/IgH PCR in PB or BM at the end of the 2 years of R maintenance/observation had a significantly shorter PFS (from end of maintenance/observation) than those who were Bcl-2/IgH PCR negative. Conclusion: Bcl-2/IgH PCR results in BM or PB at the end of CHOP or R-CHOP induction treatment are not useful for decisions on subsequent therapy in patients with relapsed/resistant follicular lymphoma.

0899

REAL-TIME QUANTITATIVE PCR ANALYSIS FOR BCL2/IGH IN THE PHASE III FIRST-LINE INDOLENT TRIAL OF (90Y)-IBRITUMOMAB TIUXETAN AS CONSOLIDATION OF FIRST REMISSION IN ADVANCED-STAGE FOLLICULAR LYMPHOMA

L. Goff,[1] K. Summers,[1] S. Iqbal,[1] J. Kuhlman,[2] M. Kunz,[2] T. Louton,[3] A. Hagenbeek,[4] T. Lister,[1] A. Rohatiner[1]

[1]CR-UK Medical Oncology Unit, LONDON, UK; [2]Bayer Schering Pharma AG, BERLIN, Germany; [3]Dr Notghi Contract Research, BERLIN, Germany; [4]UMC Utrecht/HOVON, UTRECHT, Netherlands

Background. A randomised phase III *First-line Indolent Trial* (FIT) was conducted in newly-diagnosed patients (pts) with stage III or IV follicular lymphoma (FL) to evaluate the use of (90Y)-ibritumomab tiuxetan (Zevalin®; Zev) as consolidation of complete (CR/CRu) or partial remission (PR) following first-line induction chemotherapy. *Aims.* Bcl-2 status was investigated using *real-time* quantitative PCR (RQ-PCR) for the major breakpoint region (MBR) Bcl2-IgH rearrangement, at the time of randomisation and at follow-up (after treatment with Zev or observation). *Methods.* Peripheral blood samples were available for RQ-PCR analysis for 414 pts randomised to receive either Zev (n=208) or no further treatment (control group, n=206). Zev treatment comprised: rituximab 250 mg/m^2 on day -7 and day 0, followed on day 0 by Zevalin 0.4 mCi/kg (maximum dose: 32 mCi). Pts were assigned to categories based on the level of Bcl2-IgH+ cells as follows: *high*'(>1 Bcl2-IgH$^+$ cell in 100 normal cells), *intermediate* (1 in 102-103), *low* (1 in 103-105) and negative (<1 in 105). Since not all pts with FL have an MBR rearrangement, the analysis was limited to those showing RQ-PCR positivity at some point. *Results*. Overall, 186 pts fulfilled the above criteria; 127/186 (68%) were RQ-PCR positive at the time of randomisation, 68/90 (76%) and 59/96 (61%) in the Zev and control arms, respectively. Only 2% of pts in both groups had *high* levels; 7% and 10% had *intermediate* levels and 67% and 49% had *low* levels in the Zev and control groups, respectively. At 3 months, the number of Bcl2-IgH$^+$ cells was reduced by at least 1 log in 52/68 (76%) of Zev-treated pts, 51/68 (75%) becoming PCR-ve. At 6 months, 6 additional Zev-treated pts (9%) became PCR-ve, a total of 57 pts (84%) thus converting to PCR negativity. In the control arm, 15/59 (25%) and 3/59 (5%) of pts had a 1-log reduction in Bcl2-IgH$^+$ cells at 3 and 6 months, respectively. At the final evaluation, at a median of 2.4 years for Zev-treated pts, 43/68 (63%) who were PCR+ve at baseline maintained at least a 1-log reduction, 42/68 (62%) remaining PCR-ve. In the control arm, at the final evaluation at a median of 1 year, 13/59 pts (22%) maintained a 1-log reduction, 12/59 (20%) remaining PCR-ve. Clinical evidence of recurrence was observed in 24/61 (39%) and 16/21 (76%) of pts who converted to PCR-ve status in the Zev and control arms, respectively. Conversion to PCR negativity with Zev consolidation was associated with prolongation of PFS: the 61 Zev-treated pts who converted to PCR negativity had a median PFS of 3.4 years compared with 2 years for the 21 pts who converted in the control arm (p<0.005). *Conclusions.* Conversion to RQ-PCR negativity was observed in both arms but was more frequent in the Zev arm and had occurred by month 3 in most pts. Treatment with Zev consolidation resulted in 90% of pts converting from RQ-PCR+ve to -ve status and this was associated with significant prolongation of PFS compared with the control arm.

Hemoglobinopathies and paroxysmal nocturnal hemoglobinuria

0900

SERUM DICKKOPF-1 IS INCREASED AND CORRELATES WITH BONE MINERAL DENSITY IN PATIENTS WITH THALASSEMIA-INDUCED OSTEOPOROSIS. REDUCTION POST ZOLEDRONIC ACID ADMINISTRATION

E. Voskaridou,[1] D. Christoulas,[2] C. Xirakia,[1] K. Varvagiannis,[1] G. Boutsikas,[2] E. Terpos[2]

[1]Thalassemia Center, Laikon General Hospital, ATHENS, Greece; [2]Department of Medical Research, [25]1 General Air Force Hospital, ATHENS, Greece

Background-Aims. Osteoporosis represents an important cause of morbidity in adult patients with thalassemia. Its pathogenesis is multifactorial, and includes mainly bone marrow expansion, endocrine dysfunction and iron overload. Although osteoclast function is elevated in thalassemia patients with osteoporosis there is limited data for osteoblast function in this setting. Dicckopf-1 (Dkk-1) protein is an inhibitor of Wnt signaling, which is crucial for osteoblast differentiation. The aim of this study was to evaluate serum levels of Dkk-1 in patients with thalassemia-induced osteoporosis who receive therapy with zoledronic acid (ZOL) and evaluate possible correlations with clinical data. *Patients and Methods.* Sixty-six patients with thalassemia-induced osteoporosis (21M/45F; median age 35.5 years) were studied. Patients were blindly randomized to receive ZOL at a dose of 4 mg, iv, in 15 min infusion, every 6 months (group A, n=23) or every 3 months (group B, n=21), or to receive placebo every 3 months (group C, n=22) for a period of one year. All patients were under oral calcium (500 mg) administration during the treatment period. Dkk-1 was measured at baseline and after 12 months of therapy using ELISA methodology (Biomedica Medizinprodukte, No. BI-20412, Gesellschaft GmbH & Co KG, Wien, Austria) along with a series of serum bone remodeling indices: i) bone resorption markers [C-telopeptide of type-I collagen (CTX), tartrate-resistant acid phosphatase isoform-5b (TRACP-5b)], ii) bone formation markers [bone-alkaline phosphatase (bALP), osteocalcin, and C-terminal propeptide of collagen type-I (CICP)], and iii) osteoclast regulators [receptor activator of nuclear factor-kappaB ligand (RANKL), osteoprotegerin (OPG), and osteopontin]. BMD of the lumbar spine (L1-L4), femoral neck (FN) and wrist (W) was determined using DEXA, before and 12 months after treatment. The above bone markers were also evaluated in 30, age- and gender-matched, healthy controls. *Results.* At baseline, all patients had increased serum levels of Dkk-1 (mean±SD: 39±17.1 pmol/L) compared with healthy controls (27.4±9.7 pmol/L; p<0.0001). Furthermore, thalassemia patients had increased values of CTX (p<0.0001), bALP (p<0.0001), CICP (p=0.003), sRANKL (p=0.02), and OPG (p=0.001) compared with controls. Dkk-1 serum levels correlated with L1-L4 BMD (r=-0.290, p=0.022) and W-BMD (r=-0.415. p=0.001), but also with TRACP-5b (r=0.310, p=0.011) and bALP levels (r=-0.289, p=0.018). As reported previously, patients of group B experienced an increase of L1-L4 BMD, while no other alterations in BMD were observed in the 3 studied groups after 12 months of therapy. Interestingly, patients of groups A+B showed a strong reduction of Dkk-1 after 12 months of ZOL (from 39.6±16.6 to 28.9±16.3 pmol/L; p=0.004); indeed they almost normalized Dkk-1 levels (no difference from control values). In contrast, patients of group C showed a borderline increase of Dkk-1 (from 33.1±16.8 to 40.1±23.2 pmol/L, p=0.08). *Summary and Conclusions.* These results show for the first time in the literature that Dkk-1 is increased in the serum of patients with thalassemia and osteoporosis, correlates with their BMD and is reduced post-ZOL therapy. These increased Dkk-1 levels may be at least partially responsible for osteoblast dysfunction in thalassemia and reveal a novel possible target for the development of new agents for the management of bone loss in these patients.

0901

COMPARISON OF DEFERASIROX, DEFERIPRONE, AND DESFERRIOXAMINE EFFECTIVENESS ON MYOCARDIAL IRON CONCENTRATIONS AND BIVENTRICULAR FUNCTION BY QUANTITATIVE MR IN BETA-THALASSEMIA MAJOR

A. Pepe,[1] A. Ramazzotti,[1] P. Cianciulli,[2] A. Spasiano,[3] M. Capra,[4] C. Borgna-Pignatti,[5] M.C. Putti,[6] A. Lippi,[7] M.A. Romeo,[8] M.G. Bisconte,[9] A. Filosa,[10] V. Caruso,[11] A. Quarta,[12] L. Pitrolo,[13] V. De Sanctis,[14] C. Gerardi,[15] A. Maggio,[16] A. Pietrangelo,[17] G. Rossi,[1] M. Lombardi[1]

[1]Institute of Clinical Physiology, CNR, PISA; [2]Centro Talassemie, Sant'Eugenio Hospital, ROMA; [3]Centro per la Cura delle Microcitemie, Cardarelli Hospital, NAPOLI; [4]Pediatria per le Emopatie Ereditarie, G. Di Cristina Hospital ARNAS, PALERMO; [5]Department of Pediatrics, University of Ferrara, FERRARA; [6]Department of Pediatrics, University of Padova, PADOVA; [7]Centro Talassemie ed Emoglobinopatie, Meyer Hospital, FIRENZE; [8]Department of Pediatrics, University of Catania, CATANIA; [9]Centro di Microcitemia. U. O. Ematologia, Presidio Osp. Annunziata, COSENZA; [10]UOC pediatria, DH thalassemia, Cardarelli Hospital, NAPOLI; [11]Centro Microcitemia Garibaldi Hospital, CATANIA; [12]Ematologia, A. Perrino Hospital, BRINDISI; [13]Pediatria II per le Emopatie Ereditarie, Villa Sofia-CTO Hospital, PALERMO; [14]Department Reproduction and Growth, Pediatric Adolescent Unit, St Anna Hospital, FERRARA; [15]Centro Talassemia Ospedali Civili Riuniti, SCIACCA (AGRIGENTO); [16]Ematologia II con Talassemia, V. Cervello Hospital, PALERMO; [17]Center for Hemochromatosis, University Hospital of Modena and Reggio Emilia, MODENA AND REGGIO EMILIA, Italy

Background. Despite dramatic gains in life expectancy in the desferrioxamine era for thalassemia major patients, the leading cause of death for this young adults population remains iron-induced heart failure. For this reason, strategies to reduce heart disease by improving chelation regimens has of the highest priority in this phase. These strategies include development of novel oral iron chelators to improve compliance. Oral deferipron was proved more effective than subcutaneous desferrioxamine in removing cardiac iron. The novel oral one-daily chelator deferasirox has been recently commercially available but its long-term efficacy on myocardial iron concentrations and cardiac function is unknown. *Aims.* To compare in thassemia major patients the effectiveness of deferasirox, deferipron and desferrioxamine on myocardial and liver iron concentrations and bi-ventricular function by quantitative magnetic-resonance imaging (MRI). *Methods*. Among the 550 thalassemic subjects enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network between September 2006 and September 2007, we selected patients receiving one chelator alone for longer than 1 year. MIOT is an Italian network of 6 MR sites where the cardiac and liver iron status is assessed by validated and homogeneous standard procedures.

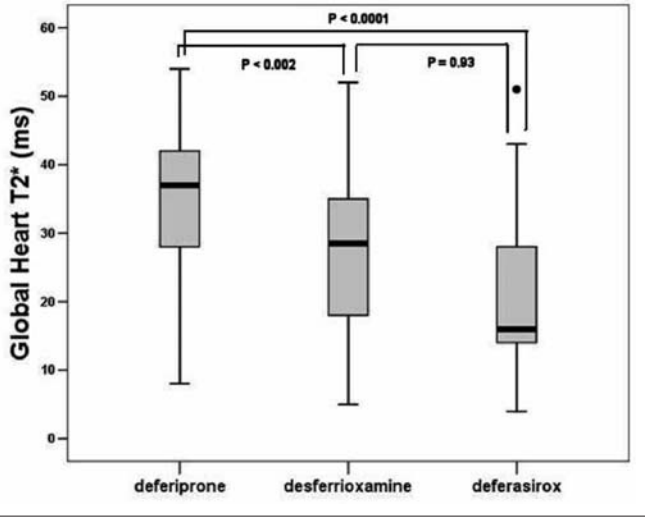

Figure 1.

We identified 3 groups of patients: 24 treated with deferasirox, 42 treated with deferipron and 89 treated with desferrioxamine. The 3 groups were matched for gender, Hb pre-transfusion levels, age of starting chelation, and good compliance to the treatment. The deferasirox group was significantly younger (26±7 years) than the deferipron- (32±9 years) and desferioxamine group (33±8 years) (p=0.0001) and showed significantly higher mean serum ferritin levels (2516±2106 ng/mL) than the deferipron- (1493±1651 ng/mL) and the desferrioxamine group (987± 915 ng/mL) (p=0.0001). Myocardial iron concentrations and distribution were measured by MRI T2* multislice multiecho technique. Biventricular function parameters were quantitatively evaluated by cine-dynamic MRI images. Liver iron concentrations were measured by MR T2* multiecho technique. Written informed consent was obtained from all subjects. *Results*. The global heart T2* value was significantly higher in the deferiprone group (34±11 ms) versus the deferasirox (21±12 ms) and the desferrioxamine group (27±11 ms) (p=0.0001). The T2* in the mid ventricular septum was significantly higher in the deferiprone (36±12 ms) versus the deferasirox (20±12 ms) and the desferrioxamine group (28±13 ms) (p=0.0001).The number of segments with normal T2* value was significantly higher in the deferiprone and the desferrioxamine group versus the deferasirox group (14±2 versus 11±6 versus 7±7 segments; p=0.0001). Among the biventricular function parameters, we found higher left ventricular ejection fractions in the deferiprone and the desferrioxamine group versus the deferasirox group (64±7 versus 62±6 versus 58±7%; p=0.005). Liver T2* values were significantly higher in the desferrioxamine group versus the deferiprone - and the deferasirox group (10±9 versus 6±6 versus 5±5 segments; p=0.002). *Summary and conclusions.* Oral deferiprone seems to be more effective than oral deferasirox and subcutaneous desferrioxamine in removal of myocardial iron with concordant positive effect on left global systolic function.

0902

A HOMOZYGOUS DELETION OF THE MAJOR ALPHA GLOBIN REGULATORY ELEMENT (HS-40) RESPONSIBLE FOR A SEVERE CASE OF HEMOGLOBIN H DISEASE

M.C. Sollaino, M.E. Paglietti, D. Loi, R. Congiu, R. Podda, R. Galanello

Ospedale Regionale Microcitemie, CAGLIARI, Italy

Background. Alpha thalassemia is an inherited blood disorder caused by a decrease in the synthesis of alpha-globin chains due to deletions or point mutations in one or both alpha globin genes, located on human chromosome 16 p13.3. Rarely, alpha thalassemia is due to deletions within the telomeric region flanking the alpha globin locus, which contains positive cis-acting elements required to regulate alpha globin expression. *Aims.* A 11 year old italian boy was admitted to the Hospital for severe hemolytic anemia (Hb 5 g/dL) requiring blood transfusion. He had microcytosis (MCV 64.8 fl) and hypocromia (MCH 17.8 pg), reduced HbA2 (1%), moderate reticulocytosis (4.4%) with RBC HbH inclusion bodies. Hemoglobin electrophoresis revealed the presence of small amount of hemoglobin H (0.8%). Globin chain synthesis ratio was significantly reduced (α/β=0.61). Conventional molecular techniques (GAP-PCR) and DNA sequencing of the alpha globin genes did not detect any deletion or non deletion defect in the proband and his parents. Aim of the study was to identify the molecular defect responsible for this unusual case of HbH disease. *Methods.* Multiplex ligation-dependent probe amplification (MLPA) analysis of the alpha-globin gene cluster was used to identify specific deletions of the telomeric region of chromosome 16 where the alpha cluster is contained. Sequences of polymorphisms along the telomeric region were subsequently studied to define the extension of the deletion. *Results.* MLPA analysis of the alpha-globin cluster suggested the homozygous state for HS-40 region deletion in the proband. Family studies of SNPs along the telomeric region of chromosome 16 confirmed the loss of this region and showed in the paternal chromosome a telomeric deletion extending for about 70 Kb and in the maternal chromosome a deletion of about 7 Kb. *Summary and conclusions.* This is to our knowledge the first case of homozygous HS-40 deletion resulting in a phenotype of HbH disease. It has been previously reported that in cell lines with HS-40 deletion, the basal levels of alpha 1 and alpha 2 globin m-RNA were less than 3% of the control (Bernet *et al.* 1995).The hematological and clinical features of this unusual case of HbH disease confirm that the complete loss of HS-40 region severely downregulates the expression of the alpha-globin genes, but is not associated with a complete absence of alpha m-RNA and alpha chain production.

0903

IMPROVEMENT IN FATIGUE WITH ECULIZUMAB TREATMENT OF PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) OCCURS INDEPENDENT OF CHANGES IN ANEMIA

A. Hill,[1] P. Muus,[2] U. Dührsen,[3] G. Socié,[4] A. Risitano,[5] R. De Paz,[6] E. Van den Neste,[7] A. Zanella,[8] J.S. Lai,[9] P. Hillmen,[10] R. Rother,[11] D. Cella[9]

[1]Teaching Hospitals NHS Foundation Trust, BRADFORD, UK; [2]Radboud University, NIJMEGEN, Netherlands; [3]University Essen, ESSEN, Germany; [4]Hospital Saint Louis and INSERM, PARIS, France; [5]Midiche Federico II University of Naple, NAPLES, Italy; [6]Hospital De La Paz, MADRID, Spain; [7]Ucl St. Luc, BRUSSELS, Belgium; [8]Ospedale Maggiore di Milano, MILAN, Italy; [9]Northwestern University and Evanston Northwestern Healthcare, EVANSTON, IL, USA; [10]Leeds Teaching Hospitals NHS Trust, LEEDS, UK; [11]Alexion Pharmaceuticals, Inc., CHESHIRE, CT, USA

Background. In patients with paroxysmal nocturnal hemoglobinuria (PNH), RBCs undergo chronic complement-mediated hemolysis resulting in serious sequelae including anemia, thrombosis, pain, dyspnea, poor quality of life and disabling fatigue. Fatigue levels in patients with PNH are similar to that experienced by anemic cancer patients, and treatment measures to improve anemia can improve fatigue in both disease settings. In PNH, however, fatigue is also related directly to chronic hemolysis. *Aims.* To elucidate the contribution of hemolysis versus anemia to the improvement in fatigue in eculizumab-treated PNH patients. *Methods.* Patient-reported fatigue was determined utilizing the Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue scale and anemia and hemolysis were objectively recorded in the double-blind placebo controlled phase 3 TRIUMPH and the open label phase 3 SHEPHERD PNH studies. The effect of eculizumab treatment on fatigue in PNH patients was compared to changes in fatigue in anemic cancer patients who received EPO using the same fatigue questionnaire; patients were analyzed as 3 strata: >1 g/dL increase; no change; or a >1 g/dL decrease in hemoglobin during treatment. Effect sizes (ES) indicating large, moderate or small clinical improvements were assessed. *Results.* Treatment independent univariate analysis of PNH patients showed that intravascular hemolysis reduction (decreased lactate dehydrogenase levels) and anemia improvement (increased hemoglobin) were both significantly associated with fatigue improvement (odds ratio 1.11, $p<0.001$ and 1.29, $p=0.005$, respectively).

Table 1. Relationship between hemoglobin levels and FACIT-Fatigue scores.

	Change in Hemoglobin During Treatment			
	Increased by ≥1 g/dL	**No Change**	**Decreased by ≥1 g/dL**	**Overall**
Anemic Cancer patients*				
Number of patients	n=1011	n=303	n=64	n=1378
Change in Fatigue, mean(SD)	6.6±13.7	1.7±11.2	-4.3±12.7	5.02‡
Effect Size (ES)	0.48	0.15	-0.33	
PNH patients†				
Number of Patients	n=59	n=68	n=37	n=164
Change in Fatigue, mean(SD)	11.0±10.9	7.5±10.4	4.7±7.7	8.12
Effect Size (ES)	1.02	0.72	0.61	

*mean time to EPO completion was 89 days (SD±37); Data for anemic cancer patients was obtained from a published report.[1]

†84 days of eculizumab treatment. PNH patients were treated in the TRIUMPH[2] and SHEPHERD[3] studies.

‡Calculated by weighted average of the sub-groups.

An increase in FACIT-Fatigue score denotes an improvement in fatigue while a decrease in score indicates a worsening in fatigue. An increase of 3 or more points is considered a clinically meaningful improvement.

ES is characterized as follows: <0.2 = trivial to non-existent effect; 0.2 to 0.5 = small effect; >0.5 to 0.8 = moderate effect; >0.8 = large effect.[4]

[1]Cella et al. *J Pain Symptom Manage.* 2002;24:547. [2]Hillmen et al. *N Engl J Med.* 2006;355:1233. [3]Brodsky et al. *Blood.* 2008;111:1840. [4]Cella et al. *Ann Oncol.* 2004;15:979-986.

Further, multivariate analysis indicated that hemolysis reduction was predictive of an improvement in fatigue independent of an improvement in anemia (1.07, $p=0.028$). Eculizumab treatment was associated with a significant improvement in fatigue by one week and the improvement has been sustained for over two years. The improvement in fatigue was larger ($p=0.002$) in eculizumab-treated anemic PNH patients compared to EPO-treated anemic cancer patients. Patients treated with eculizumab experienced a large improvement (ES:1.0) when the hemoglobin level increased, and a moderate fatigue improvement when hemoglobin showed no change (ES:0.72) or even decreased (ES:0.61). By contrast, patients treated with EPO experienced a small improvement in FACIT-Fatigue score only when hemoglobin level increased (ES:0.48); fatigue scores in EPO treated patients did not change meaningfully when hemoglobin levels did not change (ES:0.15) and actually showed worsening when hemoglobin levels decreased (ES:-0.33) during treatment (Table 1). *Summary and conclusions.* Taken together, these findings suggest that eculizumab-treated PNH patients experience a larger improvement in fatigue than EPO-treated cancer patients due to the improvement in hemolysis with eculizumab. Also, there is improvement in fatigue with eculizumab that is independent of any improvement in anemia. In contrast to the observations with EPO in which patients did not experience meaningful improvement in fatigue unless they also showed an improvement in anemia, meaningful improvements in fatigue were experienced with eculizumab by both those patients who had an improvement in anemia as well as by patients that had no improvement in anemia. Since improvement in fatigue with eculizumab is associated with relatively rapid reduction in hemolysis and occurs independent of change in anemia, eculizumab can benefit a broader PNH patient population than those who realize improvement in anemia.

0904

MODULATION IN HEMODYNAMIC AND ADHERENCE OF SICKLED RED BLOOD CELLS ON ENDOTHELIAL CELLS TREATED BY HYDROXYUREA

E. Verger,[1] S. Laurance,[2] A. Bruel,[3] D. Schoëvaërt,[3] M.H. Odièvre,[4] O. Fenneteau,[5] C. Claudine,[2] J. Elion[2]

[1]INSERM, PARIS; [2]Inserm, UMR763, Hôpital Robert Debré, PARIS 19; [3]Inserm U553, Plateforme de vidéomicroscopie, Hôpital St Louis, PARIS; [4]Service de pédiatrie générale, Hôpital Louis Mourier, COLOMBES; [5]Service d'Hématologie, Hôpital Robert Debré, PARIS, France

Background. Sickle Cell Disease (SCD) is a severe disorder characterised by a complex pathophysiology and limited therapeutic options. It is characterised by the occurrence of acute episodes and chronic organ damage probably via at least one common mechanism: vascular occlusion. Hydroxyurea (HU) is the only drug to have shown effectiveness in SCD treatment, notably on the occurrence of vaso-occlusive crisis resulting from an increased adhesion of red blood cells (SS-RBCs) on activated endothelial cells (ECs), in a pro-inflammatory context, and to the subsequent RBC sickling. Effects of HU have been largely studied on RBCs, but due to the crucial role of ECs in SCD, our laboratory is investigating their potential responsiveness to HU. We have shown that ECs are indeed a target of HU which modulates endothelial expression of genes implicated in adhesion, inflammation and vascular tone. *Aims*. To explore the functional effects of HU on ECs in a system close to the physiological conditions of the microcirculation, we analysed the hemodynamics of RBCs in a flow chamber lined with EC subjected to various conditions. *Methods*. Human ECs from the micro-(TrHBMEC and HPMEC) and macro-(HUVEC) circulation, treated 24 h by HU, in basal and inflammatory (+cytokines) conditions, constitute the basis of the flow chamber. AA-RBCs from 5 controls and SS-RBCs from 5 homozygous SCD children labelled with the PKH26 fluorophore, were perfused at 1dyne/cm^2 (i.e. the shear stress in postcapillary venules). The individual RBC displacement was followed every 20 msec, and 400 trajectories were constructed for each experimental condition. The number of adherent RBCs at the end of experiment and the adhesion force (resistance to detachment with increasing washing intensity) were also measured. *Results*. Mean velocities of RBCs rolling on HU-treated ECs were always more elevated than on untreated ECs, whether in basal or inflammatory conditions, and whatever the type of EC. This increase was more marked for SS-RBCs on HPMEC (pulmonary microcirculation): +32,4% and +40,6% (HU and cytokines+HU, respectively). RBCs are notoriously heterogeneous and various populations of distinct velocities were observed. EC treatment by HU enriched the highest speed populations and increased their acceleration and deceleration rate by the same factor, in basal and inflammatory state. The adherent SS-RBCs number after perfusion diminished by 63% on HU-treated HPMEC, restoring the adhesion level to that of AA-RBCs. This reduction was observed for all washing forces and with a highest threshold of significance for inflammatory conditions. This decreased adhesion by HU was observed to lesser extent for HUVEC, however these cells exhibited a low level of adhesion even before treatment. *Conclusions*. This study is the first demonstration of the HU effect on ECs in a system close to physiological conditions. These data confronted with our results on HU action on SS-RBC *in vitro* and *in vivo*, suggest that the modulation of EC / SS-RBCs interaction and adhesion is a critical phenomenon in the mode of action of HU in SCD. This modelling tool of blood microcirculation, will permit to elucidate the physiological process induced by HU on its targets, with the ultimate aim of developing safer and more fitted therapies.

Myelodysplastic syndromes

0905

GENOME-WIDE MOLECULAR ALLELOKARYOTYPING USING SNP ARRAY DISCLOSED ASSOCIATION BETWEEN A UNIPARENTAL DISOMY AND HOMOZYGOUS MUTATION IN MYELODYSPLASTIC SYNDROMES

M. Sanada,[1] S. Lee Yung,[2] T. Suzuki,[3] G. Yamamoto,[1] Y. Nannya,[1] M. Sakata,[1] M. Kato,[1] K. Kumano,[1] N. Kawamata,[4] H. Mori,[5] M. Kurokawa,[1] S. Chiba,[1] M. Omine,[5] H.P. Koeffler,[4] S. Ogawa[1]

[1]University of Tokyo, TOKYO, Japan; [2]Chungun Memorial Hospital, TAOYUAN, Taiwan; [3]Jichi Medical School, TOCHIGI, Japan; [4]Cedars-Sinai Medical Center, LOS ANGELES, USA; [5]Showa University Fujigaoka Hospital, YOKOHAMA, Japan

Background. Myelodysplastic syndromes (MDS) are heterogeneous groups of clonal hematopoietic disorders characterized by impaired blood cell production due to ineffective hematopoiesis and high propensity to acute myeloid leukemias. One of the prominent features of MDS is the high frequency of unbalanced chromosomal abnormalities that result in genetic imbalances and copy number (CN) alterations. Loss of heterozygosity (LOH) in tumor genomes has been also implicated in the development of MDS. *Aim*. In this study, we performed genome-wide profiling of CN abnormalities and allelic imbalances in MDS genomes in order to clarify the genetic changes that characterize MDS and to reveal molecular basis of MDS pathogenesis. *Method.* We analyzed a total of 171MDS and MDS/MPD specimens, including 7 RA/RARS, 23 RCMD/RCMD-RS, 6 5q-syndrome, 30 RAEB-1, 40 RAEB-2, 4 therapy related-MDS/AML, 5 MDSu, 17 CMML-1, 16 CMML-2, 24 overt AML, using high-density SNP arrays. The data were analyzed by CNAG/AsCNAR software we specifically developed for allele-specific CN analysis and sensitive LOH detection. *Results*. MDS showed characteristic CN profiles in SNP array analysis. Of particular interest is the finding of high frequency of CN-neutral LOH (Uniparental disomy,UPD), which were observed in 51 of 171 (30%) MDS cases. They preferentially involved 1p, 1q, 4q, 7q, 11q, 17p and other chromosomal segments, which were associated with homozygous mutations of both loss-of-function mutations and gain-of function mutations of tumor suppressor genes and cellular oncogenes, including TP53 (17p UPD), AML1/RUNX1 (in 21q UPD), Nras and cMPL (1p UPD), JAK-2 (9p UPD), and FLT3 (13q UPD). In addition, we identified a new gene target in MDS, which are tightly associated with 11qUPD. This gene, or mds11, harbored homozygous mutations in 8 of 9 MDS cases with 11q UPD (CMML=5, RAEB=3, overt leukemia=1), but very rare in cases without11qUPD (1/162). The mds11 mutants can transform NIH3T3 in a dominant manner, and when introduced into c-kit$^+$Sca1$^+$Lin$^-$ murine bone marrow cells, it prolonged replating capacity of these hematopoietic progenitors. This is another example showing that duplication of a dominant mutation (11qUPD) with exclusion of the wild-type allele is an important mechanism of tumorigenesis in hematopoietic cancers. *Conclusions.* In conclusion, UPD is an important mechanism of development of MDS, in which both gain-of-function and loss-of-function mutations are duplicated with concomitant exclusion of wild-type allele. Analysis of 11q UPD disclosed novel gain-of-function mutations. Identification of the targets of UPDs in 1q, 4q and 7q should also be important to gain a novel insight into the pathogenesis of MDS.

0906

PROGNOSTIC IMPACT OF ADDITIONAL CHROMOSOMAL ABERRATIONS TO 5Q- IN PATIENTS WITH PRIMARY MYELODYSPLASTIC SYNDROMES

M. Mallo,[1] J. Cervera,[2] J. Schanz,[3] B. Espinet,[1] E. Such,[2] E. Luño,[4] C. Steidl,[5] M.L. Martín,[5] U. Germing,[6] I. Granada,[7] M. Pfeilstöcker,[8] J.M. Hernández,[9] T. Noesslinger,[8] M.J. Calasanz,[10] P. Valent,[11] R. Collado,[12] C. Fonatsch,[11] E. Bureo,[13] M. Lübbert,[14] R. Ríos,[15] R. Stauder,[16] E. Arranz,[17] B. Hildebrandt,[6] J.C. Cigudosa,[18] C. Pedro,[1] M. Salido,[1] L. Arenillas,[1] G.F. Sanz,[2] MA. Sanz,[2] A. Valencia,[2] L. Florensa[1]

[1]Hospital del Mar, BARCELONA, Spain; [2]Hospital Universitario La Fe, VALENCIA, Spain; [3]University of Göttingen, GÖTTINGEN, Germany; [4]Hospital Universitario Central de Asturias, OVIEDO, Spain; [5]Hospital Universitario 12 de Octubre, MADRID, Spain; [6]University of Düsseldorf, DÜSSELDORF, Germany; [7]Hospital Universitari Germans Trias i Pujol, BADALONA, Spain; [8]Hanusch Hospital, VIENNA, Austria; [9]Centro de Investigación del Cáncer, Universidad de Salamanca, SALAMANCA, Spain; [10]Universidad de Navarra, PAMPLONA, Spain; [11]Medical University of Vienna, VIENNA, Austria; [12]Consorcio Hospital General Universitario, VALENCIA, Spain; [13]Hospital Universitario Marqués de Valdecilla, SANTANDER, Spain; [14]University of Freiburg Medical Center, FREIBURG, Germany; [15]Hospital de los Pedroches, POZOBLANCO, Spain; [16]Innsbruck Medical University, INNSBRUCK, Austria; [17]Hospital Universitario La Princesa, MADRID, Spain; [18]Centro Nacional de Investigaciones Oncológicas (CNIO), MADRID, Spain

Background. Deletion of the long arm of chromosome 5 is the most frequent chromosomal abnormality in MDS (10-15% of MDS cases). Patients with del(5q), particularly those with the '5q- syndrome' have a much better prognosis than other MDS subtypes. Presence of abnormalities additional to del(5q) has been suggested to negatively influence this favourable outcome. *Aim.* To analyse the prognostic value of cytogenetics aberrations additional to del(5q) in a large series of MDS treated with supportive care. *Patients and methods.* Three-hundred and seven MDS patients with del(5q) were selected from a 3128 cases database that included 1004 patients from the Spanish Haematological Cytogenetics Working Group (GCECGH) (Solé *et al.*, 2005) and 2124 patients from the German-Austrian MDS Study Group (Haase *et al.*, 2007). Patients were separated into two groups: group A (n=204), all del(5q) cases as a single abnormality and group B (n=101), those with additional cytogenetic anomalies. Group B was sorted according to the number of additional anomalies (1 to ≥5 anomalies) and the type of additional cytogenetic aberrations: chromosomes 1 and 3, monosomy 7, 7q-, trisomy 8, trisomy 11, trisomy 13, 12p-, involvement of chromosome 17, -18/18q-, 20q-, trisomy 21, loss of X/Y chromosome, and unrelated clones. *Results.* The series includes 90 males (29%) and 217 females (71%) with a median age of 66 years (range: 3-92 yr). Using FAB criteria (n=294): 52% had RA, 9% RARS, 30% RAEB, 8% RAEB-t and 1% CMML. WHO classification was available for 217 patients: 52% had '5q- syndrome', 1% RA, 0% RARS, 2% RCMD, 2% RSCMD, 13% RAEB-1, 20% RAEB-2, 1% CMML, 8% AML and 1% were unclassifiable. Overall, 204 (67%) of the patients presented del(5q) isolated, 52 (17%) del(5q) with one additional abnormality, 10 (3%), 6 (2%), 7 (2%) and 26 (9%) with 2, 3, 4 and 5 or more additional abnormalities, respectively. Fourteen patients showed additional clones unrelated to del(5q). Follow-up data were available for 273 patients (89%). Median survival was 48 months for all. Median survival for patients with isolated del(5q), with one additional abnormality and with two or more additional abnormalities (complex karyotypes) was 69, 55 and 8 months, respectively (p<0.0001). However, no statistical differences were found between patients with isolated del(5q) and patients with one additional abnormality (p=0.35). Complex karyotypes showed a very adverse outcome. None of the single additional anomalies analysed showed a particular better or worse prognosis. The presence of unrelated clones to del(5q) was not associated with a worse survival. The same results were obtained when the analysis was restricted to patients with the '5q- syndrome'. *Conclusions.* 1- Patients with del(5q) associated with two or more additional chromosomal abnormalities have a significantly worse overall survival than patients with del(5q) as a single anomaly. 2- Patients with del(5q) as a sole anomaly and those with one additional abnormality show a similar survival. 3- Neither any of the single additional abnormalities analysed nor the presence of unrelated clones to del(5q) conferred a particular poor prognosis. 4- Our results do not support the exclusion

of patients with one single additional chromosomal abnormality and typical bone marrow features from the '5q- syndrome' WHO category. This is work is presented on behalf of the Grupo Cooperativo Español de Citogenética Hematológica (GCECGH), German-Austrian MDS Study Group (GASMSG), International Working Group on MDS Cytogenetics of the MDS Foundation.

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0907

CHARACTERISTICS OF DISEASE PROGRESSION IN 3144 PATIENTS WITH MYELODYSPLASTIC SYNDROME

F. Zohren,[1] C. Strupp,[2] A. Kündgen,[2] A. Giagounidis,[3] B. Hildebrandt,[4] U. Suter,[2] R. Haas,[2] U. Germing[2]

[1]Heinrich-Heine Universität Duesseldorf, DUESSELDORF; [2]Department of Hematology, Oncology, Heinrich-Heine University, DUESSELDORF; [3]Department of Hematology, Oncology, St. Johannes Hospital Duisburg, DUISBURG; [4]Institute of Human Genetics; Heinrich-Heine University, DUESSELDORF, Germany

Myelodysplastic Syndromes (MDS) are associated with increased risk of developing Akute Myeloid Leukemia (AML). Some patients gradually progress to more advanced MDS subtypes while others have an apparently immediate AML onset without transition period. In order to get a better understanding of these different types of MDS progression, we retrospectively analyzed the data of 3144 patients included into the MDS registry Düsseldorf. As assessed by bone marrow examination a disease progression to AML or to advanced MDS subtype was observed in 25% of the patients. The progression rate was lowest in the unilineage dysplasia group (RA/RARS: 10%) as compared to 5q- (26%), multilineage dysplasia (17%), RAEB I (27%) and RAEB II (36%). The progression rate in CMML I was 17%, in CMML II 28%, in RARS-T 9% and in the former RAEB-T group 58%. We then evaluated the survival-time of the progressive patients. In the entire group, patients who progressed had a median survival of 18 months compared to 30 months in those with stable disease (p=0.0005). In the group of patients with less than 5% of medullary blasts at time of diagnosis, those who progressed had a median survival of 30 months compared to 48 months in those who did not progress (p=0.00005). In the group of patients who had >5% of medullary blasts at diagnosis, the progression did not influence survival substantially (14 vs.16 months, p=0.04). The cumulative risk of AML evolution at 2 and 5 years after initial diagnosis was lowest in unilineage dysplasia (4% and 8%), multilineage dysplasia (11% and 19%), 5q- (10% and 18%), RAEB I (26% and 44%), RAEB II (50% and 74%), CMML I (14% and 24%), CMML II (33% and 74%), RARS-T (5% and 5%) and RAEB -T (70% and 77%). In the following we investigated the course of MDS progression in those patients who did not develop AML. Fifty-three% of the patients in the unilineage group (RA/RARS) progressed to RAEB I or RAEB II, 10% to RCMD and 3% developed a 5q- syndrom. In the multilineage group 41% of the patients transformed into RAEB I or II, while 40% of the patients with 5q- progressed to RAEB I or RAEB II. In the RAEB I group, 28% of the patients developed RAEB II and 18% of the CMML I patients ended up as CMML II. Finally, we analyzed the effect of progression within the IPSS groups with regard to survival. Patients within the low risk group who progressed (22%) had a median survival of only 48 months, compared to 88 months among those who did not progress (p=0.002). This correlation was also significant among patients within the intermediate I group (29% progressive patients, 27 vs. 36 months, p=0.001). In both, the intermediate II and the high risk group progression was not associated with a shorter survival. Conclusions. 1. About 25% of the patients progress to a more advanced MDS type or to AML. 2. A substantial part of the patients with 5q- Syndrome as well as patients with multilineage dysplasia and RAEB types show disease progression. 3. Progression is only associated with a shorter survival among patients within the IPSS low and Intermediate I risk group.

0908

AUTOCRINE GDF15 IS NECESSARY FOR NORMAL ERYTHROID DIFFERENTIATION AND ITS OVEREXPRESSION IN RARS PATIENTS CONSTITUTES A POSSIBLE COMPENSATORY MECHANISM FOR INEFFECTIVE ERYTHROPOIESIS

J.M. Ramirez,[1] O. Shaad,[2] S. Durual,[3] D. Cossali,[3] M. Docquier,[2] P. Beris,[3] P. Descombes,[2] T. Matthes[3]

[1]Hôpital Universitaire de Genève, GENÈVE; [2]University Medical Center, GENEVA; [3]University Hospital Geneva, GENEVA, Switzerland

The myelodysplastic syndromes (MDSs) are a heterogeneous group of preleukemic hematological diseases, characterized by a disturbed hematopoiesis. The classification of MDSs is largely based on morphologic criteria (FAB classification, 1982; WHO classification, 1997) and distinguishes two risk groups, depending on survival rates and evolution into acute leukemia. Refractory anemia with ring-sideroblasts (RARS) is part of the low risk MDSs. In addition to abnormal proliferation/apoptosis of hematopoietic precursors, patients with RARS present the particularity of a disturbed iron metabolism in erythroid precursors, leading to the characteristic appearance of *ring-sideroblasts* in the bone marrow. To gain insight into these pathophysiologic mechanisms we compared the gene expression profile (GEP) of erythroid precursors from 11 patients with RARS to the GEP of normal erythroid precursors. 364 probe sets were found unregulated (e.g.: TRIB3, TP53, GDF15..) and 253 probe sets were found down-regulated (e.g.: ACSL6, MBNL3, CTSE, ..) in RARS cells. Most interestingly, Growth Differentiation factor 15 (GDF15), a cytokine from the TGFß family with a pro-apoptotic function in solid tumors, was dramatically upregulated in all RARS patients. Measurement of GDF15 protein in the sera from twenty RARS patients as well as from patients with other forms of ineffective erythropoiesis showed significantly increased GDF15 levels (7.2-fold). To test the erythroid specific expression of GDF15 mRNA, we performed *in vitro* experiments in which we differentiated $CD34^+$ cord blood cells into erythroid or myeloid lineage cells. Whereas GDF15 mRNA and secreted protein became detectable in erythroid cultures at day five and increased with ongoing erythroid differentiation, myeloid cultures did not show any detectable GDF15 mRNA or protein production. Subsequently, we studied various cytokines stimulating GDF15 production in short-term cultures of erythroid progenitors: only erythropoietin was found to lead to any significant increase in GDF15 mRNA and protein (9-fold). In parallel experiments we found that apoptotic stimuli (e.g.: arsenic) increased GDF15 production in a similar way (3-fold). Finally, we could show in $CD34^+$ cultures that inhibition of endogenous GDF15 production by specific siRNAs results in inhibition of erythroid differentiation (2-fold). In summary, our findings indicate that high GDF15 serum levels in RARS patients originate from erythroid precursors and may be the result of a combination of high erythropoietin levels and apoptotic stimuli. We show moreover that GDF15 is necessary for normal erythroid differentiation. Therefore, we propose that high GDF15 levels in RARS patients probably constitute a compensatory mechanism to counter regulate ineffective erythropoiesis.

0909

IMPACT OF CYTOGENETIC ABNORMALITIES ON OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR THERAPY-RELATED MYELODYSPLASTIC SYNDROME (T-MDS) AND ACUTE MYELOID LEUKEMIA. A REPORT FROM THE MDS SUBCOMMITTEE OF THE CHRONIC LEUKEMIA WORKING PARTY OF THE EUROPEAN GROUP FOR BLOOD AND MARROW TRANSPLANTATI

N. Kröger,[1] R. Brand,[2] A. Van Biezen,[2] A. Zander,[3] J. Dierlamm,[3] D. Niederwieser,[3] A. Devergie,[3] R. Trapani,[3] J. Cornish,[3] P. Van der Borne,[4] P. Ljungman,[3] A. Gratwohl,[3] C. Cordonnier,[5] D. Beelen,[3] E. Deconinck,[6] A. Symoneidis,[3] T De Witte[3]

[1]University Hospital Hamburg, HAMBURG, Germany; [2]University, LEIDEN, Netherlands; [3]University Hospital, HAMBURG, Germany; [4]University Hosital, LEIDEN, Netherlands; [5]Hopital Henri Mondor, CRETEIL, France; [6]Hopital Jean Minjoz, BESANCON, France

Allogeneic stem cell transplantation (SCT) can cure patients with therapy related MDS or AML. To predict outcome we analysed 461 patients (pts) with t-MDS or t-AML underwent allogeneic SCT and were reported to the EBMT registry. The median age of the pts was 40 years (r., 3-69) and primary disease were solid tumor (n=173), malignant lymphoma(n=135), or other haematological diseases (n= 45). The

median time from primary diagnosis to t-MDS/t-AML was 54 months (r., 1-416). Diagnosis were: RA/RARS (n=28), RAEB (n=49), RAEB-T (n=44) and t-AML (n=308). In a multivariate analysis, being in "non CR" was a significant risk factor for relapse (HR:2.20; 95% CI: 1.44-3.36, $p<0.001$), event-free (EFS) (HR: 1.78; 95% CI 1.34-2.36; $p<0.001$) and overall survival (OS): (HR: 1.57; 95% CI 1.18-2.09; $p<0.001$). A marked reduction in non-relapse mortality (NMR) was seen per calendar year (HR: 0.93; 95% CI: 0.90-0.96, $p<0.001$), which also results in improved EFS (HR: 0.96; 95% CI: 0.94-0.98, $p=0.004$) and OS (HR: 0.95; 95% CI: 0.93-0.98, $p=0.001$). Age influenced NRM (HR: 2.67; 95% CI: 1.68-4.25, $p<0.001$), EFS (HR: 1.95; 95% CI: 1.36-2.78, $p<0.001$) and OS (HR: 2.13; 95% CI: 1.47-3.08, $p<0.001$). Intermediate and high risk cytogenetic negatively influenced relapse (HR: 1.78; 95% CI: 1.03-3.07; $p=0.04$) and EFS (HR: 1.41; 95% CI: 1.01- 1.99; $p=0.05$) in comparison to low risk cytogeneic (normal karyotyp and t(8;21); inv 16 and t (15;17)). Based on these three variables we create a simple risk factor: age > 40y =+2, abnomal cytogenetic=+1 and non CR=+2, which allows to distinguish four risk categories: low (0-+1), moderate(+2), strong (+3 to+4) and high (+5). This risk model separates overall survival from 60% (low risk) 45% (moderate) to 31% (strong) and 21% (high risk) ad non-relapse mortality from 21% (low risk) to 25% (moderate) to 42% (strong) and 44% (high).

Thrombosis

0910

ENOXAPARIN VERSUS ASPIRIN VERSUS LOW-FIXED-DOSE OF WARFARIN IN NEWLY DIAGNOSED MYELOMA PATIENTS TREATED WITH THALIDOMIDE-CONTAINING REGIMENS: A RANDOMIZED, CONTROLLED TRIAL

A. Palumbo,[1] M. Cavo,[2] S. Bringhen,[1] A. Zaccaria,[2] A. Spadano,[2] S. Palmieri,[2] N. Pescosta,[2] T. Caravita,[2] M. Spriano,[2] C. Cangialosi,[2] L. Castagna,[2] F. Morabito,[2] R. Ria,[2] E. Zamagni,[3] P. Tacchetti,[3] G. Benevolo,[2] M. Aragno,[2] V. De Stefano,[2] F. Elice,[2] M. Boccadoro[1]

[1]A.O.U. San Giovanni Battista, TORINO; [2]Italian Multiple Myeloma Network, GIMEMA; [3]Istituto di Ematologia e Oncologia Medica Seràgnoli, Università di Bologna, BOLOGNA, Italy

Background. The risk of venous thromboembolism (VTE) is high in newly diagnosed myeloma (MM) patients who receive thalidomide-containing regimens. Anticoagulant prophylaxis is recommended but it's not clear which is more appropriate. *Aims.*In this prospective, multicenter phase III trial we evaluated the safety and the efficacy of low-molecular weight heparin (LMWH) or low-dose aspirin (ASA) or low-fixed dose warfarin (WAR) as anticoagulant prophylaxis. *Methods.* In a GIMEMA study, newly diagnosed MM patients were randomized to VTD (Velcade 1.3 mg/m^2 d 1,4,8,11; Thalidomide 200 mg/d; Dexamethasone 320 mg/21 d) or TD (Thalidomide 200 mg/d; Dexamethasone 320 mg/21 d) or VMPT (Velcade 1.3 mg/m^2 d 1,8,15,22; Melphalan 9 mg/m^2 d 1-4; Prednisone 60 mg/m^2 d 1-4; Talidomide 50 mg/d) or VMP (Velcade 1.3 mg/m^2 d 1,8,15,22; Melphalan 9 mg/m^2 d 1-4; Prednisone 60 mg/m^2 d 1-4). In a sub-study, patients treated with VTD or TD or VMPT were randomly assigned to receive LMWH (Enoxaparin 40 mg/d) or ASA (Aspirin 100 mg/d) or WAR (Warfarin 1.25 mg/d) for the duration of the induction therapy. Patients treated with VMP did not receive any prophylaxis and were used as controls. End-points were incidence of VTE, acute cardiovascular events, sudden death, bleeding and any other serious adverse events. A total of 950 patients will be included in this study. *Results.* 435 patients were randomly assigned to VTD (148 patients) or to TD (150 patients) or to VMPT (66 patients) or to VMP (71 patients). 17 patients were excluded from sub-study because of indication for anticoagulant/antiplatelet therapy or high-risk of bleeding. A total of 347 patients (median age 59 years) were analyzed: 115 patients were randomized to LMWH, 112 to ASA and 120 to WAR. Patient characteristics were similar in all groups. All patients completed at least 3 cycles of therapy. The incidence of VTE was 2/115 (1.7%) in the LMWH group, 6/112 (5.4%) in the ASA group and 4/120 (3.3%) in the WAR group (p not significant). VTEs were 2/71 (2.8%) in the VMP group. The cumulative incidence of VTE was 5/197 (2.5%) in patients treated with Velcade plus Thalidomide, and 7/150 (4.7%) in those treated with TD ($p=0.28$). One grade 3-4 acute cardiovascular event (0.9%) was observed in the ASA group. No sudden deaths were reported. The incidence of all grades bleeding was 1/115 (0.9%) in the LMWH group, 3/112 (2.7%) in the ASA group and 2/120 (1.7%) in the WAR group. The distribution of the major risk factors was comparable in the 3 arms, 42% of patients had at least 2 risk factors. Of note, in the LMWH and WAR groups all patients who developed VTE had at least 1 risk factor while in the ASA group VTE was also observed in patients with no risk factors. *Conclusions.* The overall incidence of VTE was less than 10% in all groups. ASA patients had higher frequency of VTE; LMWH patients had lower risk of bleeding; patients who received Velcade had lower frequency of VTE. An update of these data will be presented at the meeting.

0911

SYNERGISTIC EFFECTS OF HYPOFIBRINOLYSIS AND GENETIC AND ACQUIRED RISK FACTORS IN VENOUS THROMBOSIS

M.E. Meltzer,[1] T. Lisman,[2] C.J.M. Doggen,[3] P. De Groot,[4] F. Rosendaal[3]

[1]University Medical Center Utrecht/Leiden University Medical Center, UTRECHT/LEIDEN; [2]University Medical Center Utrecht/University Medical Center Groningen, UTRECHT/GRONINGEN; [3]Leiden University Medical Center, LEIDEN; [4]University Medical Center Utrecht, UTRECHT, Netherlands

Background. Several genetic and acquired risk factors are known to increase the risk of venous thrombosis. The occurrence of multiple risk factors in a single patient may be associated with a thrombotic risk

which exceeds the sum of the individual risks, as is for example seen with oral contraceptive use and factor V Leiden. Previously we have shown that reduced fibrinolytic potential as measured by a plasma-based assay increases the risk of venous thrombosis. *Aims.* To investigate the thrombotic risk in individuals with hypofibrinolysis overall and in combination with established genetic and acquired risk factors. *Methods.* We included 2090 Patients with a first deep vein thrombosis of the leg or a pulmonary embolism and 2564 controls of the Multiple Environmental and Genetic Assessment (MEGA) of risk factors for venous thrombosis study, a large population-based case-control study. Participants filled in a questionnaire and provided a blood sample. Lysis of a tissue factor-induced clot by exogenous tissue-type plasminogen activator was studied by monitoring changes in turbidity during clot formation and subsequent lysis. *Results.* Overall, using quartiles of clot lysis time (CLT) based on the values found in the control subjects, we found an increase in risk of venous thrombosis with each increasing quartile of CLT. The odds ratio (OR) (95% confidence interval (95% CI)) for individuals with hypofibrinolysis (i.e., CLT in the fourth quartile) was 2.4-fold higher (95% CI 2.0-2.8) than in those in the first quartile (adjusted for age and sex). While hypofibrinolysis in those without factor V Leiden increased the thrombotic risk 2.4-fold (95% CI 2.0-2.9), and factor V Leiden in those without hypofibrinolysis increased the risk 3.5-fold (95% CI 2.3-5.5), the joint presence of factor V Leiden and hypofibrinolysis led to a 8.1-fold (95% CI 5.3-12.3) increased risk compared to those with neither risk factor. Similar analyses for immobilization (surgery, bed rest, plaster cast) gave ORs of 2.4 (95% CI 1.9-2.9) for hypofibrinolysis only, 4.3 (95% CI 3.2-5.8) for immobilization only, and 10.3 (95% CI 7.7-13.8) for the combination. The largest joint effect was seen for the combination of hypofibrinolysis and oral contraceptive use in women below 50 years. Hypofibrinolysis in women not using oral contraceptives increased the risk of venous thrombosis 1.9-fold (95%CI 1.1-3.3). Oral contraceptive use in those without hypofibrinolysis increased the risk 2.6-fold (95%CI 1.6-4.0). In women using oral contraceptives with hypofibrinolysis an OR of 21.8 (95%CI 10.2-46.7) was found. The joint presence of hypofibrinolysis and the FII 20210A mutation did not substantially increase the risk of venous thrombosis. *Conclusions.* The combination of hypofibrinolysis and factor V Leiden, immobilization, or oral contraceptive use results in synergistic effects on the risk of venous thrombosis.

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0912

ARSENIC TRIOXIDE (AS2O3) MODULATES THE PROCOAGULANT ACTIVITIES OF FRESHLY ISOLATED ACUTE PROMYELOCYTIC LEUKEMIA (APL) BLASTS: A COMPARISON WITH ALL-TRANS RETINOIC ACID (ATRA)

D. Balducci, M. Marchetti, L. Russo, A. Vignoli, I. Previtali, T. Barbui, A Rambaldi, A Falanga

Ospedali Riuniti, BERGAMO, Italy

Background. Complete remission of human APL obtained by differentiating therapy with ATRA is associated with resolution of the life-threatening coagulopathy of this disease. One mechanism resides in the capacity of ATRA to modulate APL cell procoagulant activities [i.e. tissue factor (TF) and cancer procoagulant (CP)]. As_2O_3 also induces complete remission of both relapsed and newly diagnosed APL with minimal toxicity. It produces an improvement of the coagulopathy as well. Little information is available on TF and CP sensitivity to As_2O_3, particularly in freshly isolated blasts. *Aims.* We wanted to: 1. Compare the effects of As_2O_3 and ATRA on modulating the procoagulant activities of freshly isolated APL blasts from bone marrow of 10 consecutive patients and on the APL cell line NB4; and 2. Evaluate whether the modulation of the two procoagulants was associated with differentiation and/or apoptosis in both APL cell types. *Methods.* APL cells were incubated for 24h with 0.1 µM As_2O_3 or 1µM ATRA or a combination of the two drugs, or vehicle (control cells). Cells were tested for TF expression (by chromogenic and immunological assays, and mRNA), CP activity (by chromogenic assay), the differentiation of blasts into mature neutrophils (by cytofluorometric analysis of the CD11b surface-antigen expression), and apoptosis (by Annexin V staining). *Results.* As_2O_3 significantly reduced TF activity in both APL cells (versus control: blasts: 21% reduction, NB4: 35% reduction; $p<0.05$). ATRA was more effective than As_2O_3 in reducing TF (versus control: blasts: 52%, $p=0.001$, NB4: 68%, $p<0.05$). These results were confirmed by antigenic and molecular assays of TF in both APL cell types. As_2O_3 reduced CP expression of blasts (34% reduction, $p<0.05$) and NB4 cells (26% reduction, p=n.s.) to a lesser extent than ATRA (% reduction: blasts = 47%, NB4 = 62%; $p<0.05$). The As_2O_3/ATRA combination was as effective as ATRA alone on both procoagulants. Modulation of the expression of the two procoagulants induced by ATRA alone or in combination with As_2O_3 was associated to a significant cellular differentiation in both APL blasts and NB4 cells, as measured by a 10 to 15 times increment of the percentage of CD11b positive cells. Differently, As_2O_3 alone did not induce significant cellular differentiation. Finally, nor ATRA nor As_2O_3 had a measurable pro-apoptotic effects in these cells at the doses utilised in this study. *Conclusions.* These data indicate that, similarly to ATRA, As_2O_3 can modulate both TF and CP activities in freshly isolated human APL blasts as well as in NB4 cells. The modulation of procoagulants by ATRA, but not by As_2O_3, parallels the occurrence of cellular differentiation. As the molecular basis for activation of clotting in malignancies becomes better elucidated, these data suggest a potential bifunctional role for As_2O_3 as well as for ATRA, in targeting both the malignant process and the resultant coagulopathy in APL.

0913

THROMBOPHILIC ALTERATIONS AND RISK OF VENOUS THROMBOEMBOLISM IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE AND HIGH-DOSE DEXAMETHASONE

E. Zamagni,[1] L. Valdrè,[2] M. Cini,[2] C. Legnani,[2] P. Tosi,[1] P. Tacchetti,[1] M. Ceccolini,[1] F. Patriarca,[3] L. Catalano,[3] A.F. Casulli,[3] S. Volpe,[3] G. Perrone,[1] L. Masini,[3] A. Ledda,[3] S. Falcioni,[3] A. Brioli,[1] A. Gozzetti,[3] C. Califano,[3] M.C. Pallotti,[1] C. Cellini,[3] L. Pantani,[1] A. Petrucci,[1] A. Carubelli,[3] M. Baccarani,[1] G. Palareti,[2] M. Cavo[1]

[1]Istituto di Ematologia Seragnoli, BOLOGNA; [2]Dipartimento di Angiologia, Ospedale S. Orsola-Malpighi, BOLOGNA; [3]Bologna 2002 Study Italian Myeloma Network, BOLOGNA, Italy

Venous thromboembolism (VTE) is a major adverse event of primary induction therapy with thalidomide (thal) and dexamethasone (dex) for newly diagnosed multiple myeloma (MM). Aim of the present study was to investigate the relationship between thrombophilic alterations and the risk of VTE in 266 patients who received four months of therapy with thal (200 mg/d) and pulsed high-dose dex in preparation for double autologous transplantation. The rate of VTE in the whole group of patients was 11.6%. The risk of VTE was 26.3% (86.2% patient-years) among the first 19 patients who entered the study and did not received any prophylaxis against thrombosis. The corresponding value among the remaining 247 patients who received thromboprophylaxis with fixed low-dose (1.25 mg/d) warfarin during the four months of thal-dex therapy was 10.6% (35.5% patient-years) (p=0.04). Episodes of VTE occurred at a median of 53 days from the start of thal therapy and, with the exception of 3 patients, were observed after at least a partial response to thal-dex was documented. No VTE events were recorded during the first two months after the end of the induction phase. After VTE occurrence, the majority of patients went on with thal treatment plus full anticoagulation, without evidence of progression of thrombosis. One hundred and ninety patients were evaluated for the presence of thrombophilic alterations at baseline and at the end of thal-dex therapy. The prevalence of factor V Leiden (3.2%) or g20210A prothrombin (2.1%) polymorphism in patients with MM was similar to that observed in 183 healthy controls (3.3%, p=0.81; 3.8%, p=0.50, respectively). The relative risk of VTE for patients carrying one of these thrombophilic alterations was 20% compared with 9.4% for patients who lacked both of them (p=0.58). Reduced protein C and S activities or acquired activated protein C resistance (aAPCR) were recorded at baseline in 11% and 7.4% of MM patients, respectively, and increased levels of factor VIII was found in 33.1% of patients as compared to 5.5% of healthy controls ($p<$ 0.0001). Abnormal values at baseline normalized almost completely at the end of treatment with the exception of factor VIII which showed a significantly decrease after the induction therapy but remained elevated in 1/3 of patients. Carriers of aAPCR and/or of reduced levels of natural anticoagulants and/or of elevated levels of factor VIII at baseline did not have a significantly higher risk of VTE compared with normal patients (15.2% *vs* 9.3%; p=0.49). In conclusion, no significant relationship was found between baseline thrombophilic alterations and the risk of thal-related VTE. Prophylaxis with fixed low-dose warfarin was associated with an apparent decrease in the rate of VTE in comparison with a subgroup of patients who did not receive any thromboprophylaxis. Supporting our data, an interim analysis of the italian prospective phase III study comparing low molecular weight heparin with fixed low-dose warfarin with aspirin as prophylaxis against the risk of thal-related VTE for patients with newly diagnosed MM did not show significant differences between the three arms.

0914

ABSENCE OF RESIDUAL VEIN THROMBOSIS AFTER AN EPISODE OF IDIOPATHIC DEEP VEIN THROMBOSIS: SHORT-TERM ANTICOAGULATION IS SAFE. THE 'EXTENDED DACUS STUDY'

S. Siragusa

University Hospital of Palermo, PALERMO, Italy

Background. The optimal duration of Oral Anticoagulant Therapy (OAT) for Deep Vein Thrombosis (DVT) can be tailored by Residual Vein Thrombosis (RVT) (Siragusa S *et al.* Blood 2003;102(11):OC183), a marker able to assess the individual risk for recurrent thrombosis. However, in patients with idiopathic DVT the safety of early interruption of OAT, because of absence of RVT, is still debated. Objective of the study. In the present study, we evaluated the safety of withholding OAT, in patients with idiopathic DVT and without RVT, three months after the index thrombotic episode. Study design. Prospective controlled study with two groups: patients without RVT stopped OAT after 3 months while those with RVT continued for additional 3 months. Materials and *Methods.* Consecutive patients with a first episode of idiopathic DVT of the lower limbs. Patients with cancer or known thrombophilia were excluded. At the third months of OAT, RVT was assessed as previously described; briefly, RVT was considered absent when a clot occupying less than 40% of the vein lumen was detected by compression ultrasonography. Events, classified as recurrent DVT and/or Pulmonary Embolism and/or major and minor bleeding were evaluated; all patients were followed-up for at least 12 months after OAT discontinuation. *Results.* During the period 1999-2006, 518 patients were included in the study. In 206 (39.7%) RVT was considered absent (RVT negative group) and they stopped OAT; the remaining 312 patients continued anticoagulants for additional 3 months (RVT positive group). Total duration of follow-up (FU) was 184.7 years for RVT negative group (with a mean FU of 3.0+0.83 years) and 191.3 years for RVT positive group (with a mean FU of 3.1+0.89 years). The recurrent events [n/100 person-year (%)] between patients with and without RVT were 63/191.3 (32.9%) and 2/184.7 (1.08%), respectively. This difference was statistically significant (p<0.0005). Major bleeding occurred in 3/312 (0.9%) of patients who continued OAT for 1 year; no events were recorded in those who stopped anticoagulation after 3 months. In Figure 1 is reported the Kaplan-Mayer curve of recurrent events between RVT positive and negative group. *Conclusions.* This investigation shows that in patients without RVT, three months of OAT are safe even after an episode of idiopathic DVT. This hold for at least 30% of the entire DVT population and has an important clinical impact; in fact, it is possible to select a group of patients with a very low risk for recurrency over a period of 3 years. This approach carries also a negligible risk for bleeding.

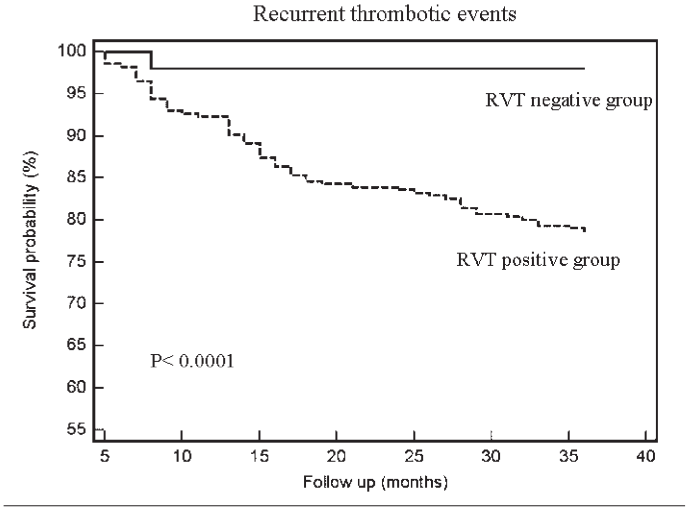


Figure 1.

Myeloma and other monoclonal gammopathies - Clinical

0915

MELPHALAN 200 MG/M2 (MEL200) VERSUS MELPHALAN 100 MG/M² (MEL100) IN NEWLY DIAGNOSED MYELOMA PATIENTS: A PROSPECTIVE, RANDOMIZED PHASE III STUDY

M. Boccadoro,[1] S. Bringhen,[1] M.T. Petrucci,[2] A. Falcone,[2] A.M. Liberati,[2] S. Morandi,[2] A. Gabbas,[2] A. Capaldi,[2] V. Callea,[2] V. De Stefano,[2] V. Bongarzoni,[2] M. Rizzo,[2] E. Calabrese,[2] F. Cavallo,[1] F. Gay,[1] P. Omedè,[1] P. Musto,[2] R. Foà,[2] A. Palumbo[1]

[1]A.O.U. San Giovanni Battista, TORINO; [2]Italian Multiple Myeloma Network, GIMEMA, Italy

Background. Several trials have shown the superiority of high-dose melphalan (usually 200 mg/m², MEL200) versus standard therapy in myeloma patients. Intermediate-dose melphalan (100 mg/m², MEL100) was also superior to the standard dose, but MEL100 has not been clinically compared with MEL200 in a randomized study. In this prospective, randomized, phase III trial, we compared the efficacy and toxicity of MEL200 and MEL100. *Aims.* The primary end points were complete remission (CR) rate, event-free survival (EFS) and incidence of gastrointestinal toxicity, infections and treatment-related mortality (TRM). *Methods.* Inclusion criteria were previously untreated myeloma, aged ≤65 and Durie and Salmon stage II or III. Exclusion criteria were abnormal pulmonar, cardiac, liver and renal function, HBV, HCV, or HIV positivity, concomitant cancer or psychiatric disease. The institutional review board approved the protocol and written informed consent was obtained from all patients. All patients received 2 cycles of 28-day-dexamethasone-doxorubicin-vincristine (doxorubicin 50 mg/m² day 1, vincristine 1 mg day 1, dexamethasone 40 mg days 1-4) and 2 cycles of cyclophosphamide (4 g/m², day 1) followed by stem cell harvest. MEL200 patients was conditioned with 2 cycles of melphalan 200 mg/m² and MEL100 patients with 2 courses of melphalan 100 mg/m2. All MEL courses were followed by stem cell reinfusion. *Results.* Two-hundred and ninety-eight patients (median age 57) were randomly assigned either to MEL200 (149 patients) or to MEL100 (149 patients). All patients were evaluated for response, EFS and OS in intention-to-treat analysis. Patient characteristics were similar in both groups with the exception of chromosomal 13 deletion, present in 69% of MEL200 and 45% of MEL100 patients (p=0.02). Ninety-six patients completed tandem MEL200; 103 tandem MEL100. The very good partial response rate was higher in MEL200 group (37% *vs* 21%, p=0.003), but CR was 15% in the MEL200 group and 8% in the MEL100 group (p=0.07). The median follow-up for censored patients was 30.5 months. The 3-years EFS was 46% in the MEL200 and 26% in the MEL100 group (HR=0.7, 95% CI 0.51-0.97, p=0.03). The 3-years overall survival (OS) was 81% in the MEL200 and 73% in the MEL100 group (HR=0.69, 95% CI 0.42-1.13, p=0.14). Duration of grade 4 neutropenia and thrombocytopenia was comparable, but a higher proportion of MEL200 patients required platelet transfusions (p=0.002). Grade 3-4 non-hematologic adverse events were reported in 38% of MEL200 patients and in 19% of MEL100 patients (p<0.0001). The incidence of grade 3-4 mucositis was 16% after MEL200 and 3% after MEL100 (p<0.0001). The incidence of severe gastrointestinal toxicity was 19% after MEL200 and 2% after MEL100 (p<0.0001). The incidence of grade 3-4 infections and of TRM was similar in both groups. *Conclusions.* In conclusion, MEL200 resulted in a significantly higher very good partial response rate. This translated in a superior EFS, but not OS. Mel200 was associated with less gastrointestinal toxicity, including mucositis.

0916

ORAL MELPHALAN, PREDNISONE AND THALIDOMIDE IN ELDERLY MULTIPLE MYELOMA PATIENTS: UPDATED RESULTS OF A RANDOMIZED CONTROLLED TRIAL

A. Palumbo,[1] S. Bringhen,[1] A.M. Liberati,[2] T. Caravita,[2] A. Falcone,[2] V. Callea,[2] C. Cangialosi,[2] M. Grasso,[2] M. Galli,[2] F. Rossini,[2] L. Catalano,[2] V. De Stefano,[2] S. Morandi,[2] M.T. Petrucci,[2] P. Falco,[1] A. Larocca,[1] V. Magarotto,[1] G. Ciccone,[3] M. Cavo,[2] M. Boccadoro[1]

[1]A.O.U. San Giovanni Battista, TORINO; [2]Italian Multiple Myeloma Network, GIMEMA; [3]Epidemiologia dei Tumori, A.O.U. San Giovanni Battista and CPO Piemonte, TORINO, Italy

Background. The initial analysis of the oral combination melphalan,

prednisone and thalidomide (MPT) in newly diagnosed myeloma patients showed significantly higher response rate and longer progression-free survival (PFS) in comparison with the standard melphalan and prednisone (MP) and suggested an overall survival (OS) advantage. *Aims.* The primary end points were response rates and PFS. Secondary end points included OS, prognostic factors and incidence of any grade 3 or higher adverse events. In this updated analysis, efficacy and safety endpoints were revised. *Methods*. Inclusion criteria were previously untreated myeloma patients, age >65 years of age or younger but excluded from transplant procedure, Durie and Salmon stage II or III myeloma and measurable disease. The institutional review board approved the protocol and written informed consent was obtained from all patients. Experimental therapy (MPT) consisted of 6 4-week cycles of oral melphalan at 4 mg/m^2 on days 1 to 7, oral prednisone at a dose of 40 mg/m^2 on days 1 to 7 and oral thalidomide (Pharmion LTD, Windsor, UK) at 100 mg/day continuously during the 6 MPT cycles and then at 100 mg/day, as maintenance therapy, until confirmed evidence of relapse or refractory disease. The dose of thalidomide was reduced 50% on the occurrence of any non-haematological grade 2 toxicity and it was discontinued for any non-haematological grade 3 toxicity. After the randomization of 65 MPT patients, enoxaparin at 40 mg/day was delivered subcutaneously during the first 4 cycles of therapy, as anticoagulation prophylaxis. Standard therapy (MP) consisted of 6 4-week cycles of oral melphalan at 4 mg/m^2 on days 1 to 7 and oral prednisone at a dose of 40 mg/m^2 on days 1 to 7. *Results.* Three-hundred and thirty-one patients with newly diagnosed multiple myeloma were randomly assigned to receive oral MPT or MP alone. After a median follow-up of 38.1 months, 3-year PFS rates were 30.7% for MPT and 17.9% for MP (HR 0.63, 95% CI 0.48 to 0.81, p=0.0004). 4-year OS rates were 45.4% for MPT and 49.3% for MP (HR 1.04, 95% CI 0.76 to 1.44, p=0.79). In different patient subgroups, MPT improved PFS regardless of age, serum levels of β2-microglobulin or high ISS disease stage. The median survival from progression/relapse was shorter (11.5 months) in the MPT group and longer (24.3 months) in the MP group (HR 1.56, 95% CI 1.09 to 2.24, p=0.01). The administration of thalidomide or bortezomib as salvage regimens in relapsing patients significantly improved survival after progression in the MP group (HR 0.25, 95% CI 0.12 to 0.51, p=0.0002) and less in the MPT group (HR 0.75, 95% CI 0.42 to 1.35, p=0.34). The MPT safety profile was not significantly modified from the initial report. *Conclusions*. These data confirm activity of MPT on PFS but failed to show any survival advantage. The introduction of new agents in the management of the relapsed disease may explain it.

0917

A NOVEL STAGING SYSTEM FOR LIGHT CHAIN AMYLOIDOSIS INCORPORATING FREE LIGHT CHAIN LEVELS

K. Kumar, A. Dispenzieri, M.Q. Lacy, S.R. Hayman, F.K. Buadi, S.R. Zeldenrust, N.L. Leung, M.R. Ramirez-Alvarado, R.A. Kyle, S.V. Rajkumar, M.A. Gertz

Mayo Clinic, ROCHESTER, USA

Background. Primary systemic amyloidosis (AL) is a monoclonal gammopathy characterized by multiple organ dysfunction secondary to deposition of light chain derived amyloid fibrils. The degree of cardiac dysfunction has been the primary determinant of outcome in these patients and hence existing staging systems have been developed based on cardiac biomarkers. However, significant heterogeneity in outcome exists with in these groups, which could be potentially explained by the plasma cell characteristics rather than the degree of the end organ damage. *Aims.* To develop an staging system that allows better prognostic stratification. *Methods.* We examined the baseline clinical and laboratory data from 2119 patients with AL who were seen at our institution with in 90 days of their diagnosis. The data were collected from a prospectively maintained data base, and additional testing was done for free light chain levels (FLC) on stored sera from patients seen before the routine introduction of FLC testing. Cox proportional hazards analysis was performed to estimate the prognostic values of different variables. *Results*. Variables related to the plasma cell characteristics (Difference between involved and uninvolved FLC, bone marrow plasma cell%, plasma cell labeling index, beta 2 microglobulin (B2M), circulating plasma cells) and organ involvement (troponin-T, BNP, NT-Pro-BNP, ejection fraction, septal thickness, 24 hour urine protein, creatinine, alkaline phosphatase) were all found to be prognostic for overall survival using normal value cutoffs prognostic value. In a multivariate analysis incorporating all variables that were significant on univariate testing, using a step wise selection, we arrived at four variables that had maximum impact on the outcome (FLC difference, troponin-T, brain natiuretic peptide (BNP), and B2M). We then identified cutoffs that grouped a third to half of the patients into a higher risk category, at the same time conforming to the existing prognostic cutoffs and hence easy to incorporate into a new staging system. Patients were assigned a score of 1 for presence of each characteristic (FLC difference >35 mg/dL, troponin-T >0.035 ng/mL, BNP >350 pg/mL, and B2M >3.5) or 0 if the value was at or below the cutoff. The scores were added to obtain a composite prognostic score that grouped the 268 patients (who had all the variables available for analysis) into 5 groups with very different outcomes (Figure 1). *Conclusions.* Incorporation of plasma cell related measurements into the existing staging system using cardiac biomarkers allows better risk stratification of patients with AL amyloidosis. The system has the advantage of easily available laboratory values and can be widely adopted. Incorporation of this system into clinical trials will allow prospective validation and potentially better choice of therapies.

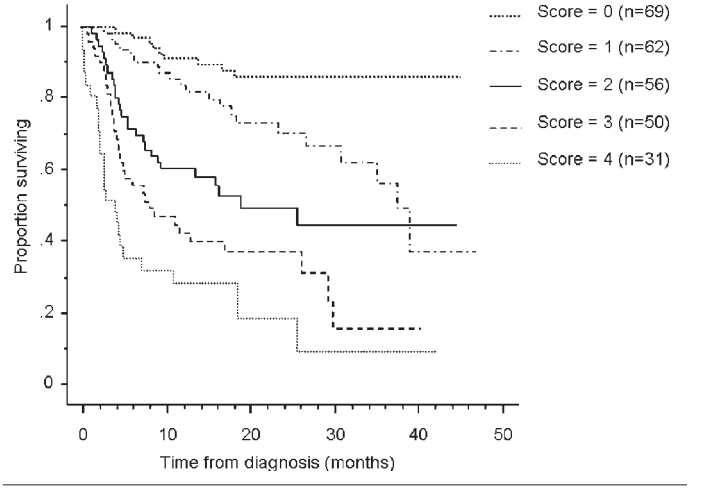


Figure 1. Overall survival based on number of risk factors.

0918

BORTEZOMIB, LOW DOSE INTRAVENOUS MELPHALAN AND DEXAMETHASONE FOR PATIENTS WITH RELAPSED MULTIPLE MYELOMA

R. Popat,[1] H. Oakervee,[2] C. Williams,[3] M. Cook,[4] C. Craddock,[4] S. Basu,[5] C. Singer,[6] L. Odeh,[1] N. Foot,[1] S. Joel,[2] S. Hallam,[1] S. Harding,[7] G. Mead,[7] J. Cavenagh[1]

[1]St. Bartholomew's Hospital, LONDON; [2]Centre for Experimental Cancer Medicine, Barts and the London, LONDON; [3]Nottingham University Hospitals, NOTTINGHAM; [4]University Hospitals Birmingham, BIRMINGHAM; [5]Royal Wolverhampton Hospitals, WOLVERHAMPTON; [6]Royal United Hospital, BATH; [7]The Binding Site, BIRMINGHAM, UK

Background. The outcome of patients with relapsed multiple myeloma (MM) is generally poor however the advent of novel agents such as bortezomib and lenalidomide has challenged this. Combining bortezomib with chemotherapy is attractive due to the observed in-vitro synergistic cytotoxicity and ability to overcome drug resistance. *Aims.* This multicenter phase I/II clinical trial sought to determine the maximum tolerated dose (MTD) of intravenous (IV) melphalan with bortezomib and to assess response, progression-free survival (PFS), overall survival (OS), and safety. IV melphalan was chosen to overcome the known variability in oral absorption. *Methods.* Patients with relapsed MM received bortezomib 1.3 mg/m^2 on days 1,4,8, 11; i.v. melphalan 7.5 mg/m^2 on day 2 and dexamethasone 20 mg on the day of and day after each bortezomib dose (for stable or progressive disease) in a 28 day cycle. The melphalan dose was based on the MTD from the phase I dose escalation component (2.5 to 10 mg/m^2). Serum free light chain (SFLC) analysis was performed on Day 1 of each cycle. The study was approved by local ethics committees and was in accordance with the declaration of Helsinki. *Results*. 53 patients were enrolled with a median of 3 prior lines of therapy including thalidomide (64%), bortezomib (9%) auotologous (85%) and allogeneic (4%) stem cell transplant. Dexamethasone was added for 27 patients as per protocol. Responses were rapid (median 1 cycle), and independent of cytogenetic abnormality. The overall response rate (≥PR) by modified EBMT criteria across all melphalan levels was 69% (29% ≥VGPR) and 78% at the MTD of 32 patients (40% ≥VGPR). The medi-

an PFS at the MTD was 12 months (vs 8.5 months at other melphalan levels ($p<0.05$)), but 14 months for those in nCR or CR *vs* 10 months for those not ($p<0.05$). The median OS was 28 months for all patients but not reached for those responding to therapy or treated at the MTD. 66% of the evaluable SFLC samples were abnormal at baseline by International Response Criteria. Those that normalised their SFLC ratio had a median of 6 months longer duration of response (DOR) and a median of 3 months longer PFS than those that failed to do so. In addition, patients with a >80% reduction in SFLC levels after the first cycle also had a superior DOR and PFS. Toxicities were acceptable and predominantly haematological - grade 3/4 thrombocytopenia (62%) and neutropenia (57%) with GCSF administered in 36% of cases for grade 4 toxicity. Grade 3/4 neuropathy was observed in 15% of patients with a median cycle of onset of 3. *Conclusions.* This regimen was safe, highly effective and resulted in durable responses for patients with relapsed MM. Patients treated at the MTD and those achieving CR had significantly longer PFS and a trend towards better overall survival than others. The MTD for melphalan was lower than expected presumably due to the sensitising effects of bortezomib, and the higher responses obtained compared to the oral melphalan studies may be attributable to the better bioavailability of the intravenous route.

0919

A COMPARISON OF REDUCED-INTENSITY CONDITIONING FOR ALLOGRAFTING (RICT) FOLLOWING AUTOGRAFTING (ASCT) *VS* DOUBLE AUTOGRAFTING FOR NEWLY MULTIPLE MYELOMA (MM) PATIENTS

A.M. Carella, M. Spriano, G. Catania

Hematology Division, GENOA, Italy

Background. Allografting is a possible curative approach for patients with MM. Unfortunately this procedure is only an option for younger patients with HLA-identical siblings. Recently RICT demonstrate stable engraftment of allogenic cells. High-dose therapy/ASCT followed shortly thereafter by RICT might improve outcomes in MM as compared to ASCT or conventional allografting used alone. *Aims.* we compared hematopoietic stem cell ASCT followed by RICT with a protocol of double ASCT. The sole criterion for the assignement of treatment in patients with newly MM was the presence of absence of an HLA-identical sibling. *Methods.* We enrolled 132 consecutive patients 65 years of age or younger with stage II or III myeloma. One hundred seven patients had siblings and 93 patients and their siblings underwent HLA typing. Thirty-four of them had an HLA-identical sibling. All patients were initially treated with induction chemotherapy consisting of 3-4 courses of VAD regimen or modifications of VAD regimen. Soon after, peripheral blood stem cells (PBSC) were collected after 3-4 gr of cyclophosphamide per square meter of body-surface area. G-CSF was given 4-5 days after chemotherapy. Daily aphereses was continued until at least $>3\times10^6$ CD34 cells/kg were collected. After the first ASCT, patients who had an available HLA-identical sibling donor were offered RICT. Patients without an HLA-identical sibling donor underwent a second ASCT. The conditioning regimen of ASCT-1 and ASCT-2 consisted of melphalan 200 mg/m^2 infused over 30 minutes. The RICT consisted of fludarabine 30 mg/m^2 daily for 3 days and TBI (2Gy or melphalan 70 mg/m^2). Graft-versus-host prophylaxis consisted of cyclosporin A and short-course methotrexate. *Results.* The rate of CR was significantly higher in RICT arm (RICT: 54,1%; ASCT: 28,5%). Nine (37,5%) of 24 patients who received RICT and 4 (11,1%) of 35 patients who received double ASCT are in continuous remission after a median of 57 months (range, 14-88 months) and 56 months (range, 28-70 months), respectively. Thirteen (54,1%) patients in the ASCT/RICT group and 20 (56,8%) in the double ASCT are alive at a median of 57 months (range, 14-88 months) and 49 months (range, 13-147 months), respectively. The cumulative incidence rate of acute and chronic GVHD after RICT was 41% and 54%. Four patients (19%) died of extensive chronic GVHD in RICT arm; no patient died in ASCT arm. *Conclusions.* In our experience the tandem ASCT-RICT protocol has demonstrated rapid and sustained engraftment with durable complete donor chimerism and tolerable toxicity. The high CR rate achieved with ASCT/RICT is encouraging but this did not determine an increased DFS and OS with respect to double ASCT. At the light of our and other results, it is not really clear whether the use of ASCT/RICT strategy could improve the outcome of newly diagnosed MM patients.

Acute lymphoblastic leukemia - Clinical

0920

TREATMENT OF ADULTS WITH NEWLY DIAGNOSED ACUTE LYMPHOBLASTIC LEUKEMIA WITH MULTIPLE DOSES OF INTRAVENOUS PEGYLATED ASPARAGINASE IN AN INTENSIFIED PEDIATRIC REGIMEN

P. Srivastava,[1] K. Watkins,[1] L. Mark,[1] A. Mohrbacher,[1] A.S. Yang,[1] V. Avramis,[2] D. Douer[1]

[1]USC/Keck School of Medicine, LOS ANGELES; [2]Children's Hospital Los Angeles, LOS ANGELES, USA

Introduction. Pediatric ALL regimens are more intense and usually contain higher doses of asparaginase (ASP) than adult regimens. In large randomized pediatric ALL trials multiple doses of E.Coli ASP given throughout the post remission phase are associated with improved outcome. It has been suggested that in adults, pediatric protocols with more ASP may improve outcome. PEG-asparaginase (PEG-ASP) is a modified E.coli ASP, with fewer hypersensitivity reactions and longer half life. In adult ALL a single IV dose of PEG-ASP during induction produces a long duration of asparagine depletion with similar toxicity to equivalent multiple doses of E.coli ASP (Douer *et al.* Blood 19:2744,2007). We now report the feasibility of using an intensive pediatric regimen containing multiple doses of PEG-ASP in adults with newly-diagnosed previously-untreated ALL. *Methods.* The backbone of our protocol is an augmented BFM pediatric ALL regimen consisting of 8 cycles of multi-agent chemotherapy, followed by maintenance. PEG-ASP (2000 U/m^2/dose) is given IV once on day 15 of the following cycles (total 6 doses): induction phase I (cycle 1), induction phase II (cycle 2), two cycles of consolidation (cycles 3 and 6), and two cycles of delayed re-induction (cycles 5 and 8). *Results.* 37 patients, aged 19-57 (median 33) years, (precursor B cell - 32, T cell-5, Ph$^+$ 7) were studied. Median WBC at diagnosis - 21,000/cumm (range 1,900-512,000). CR rate: 36 (97%) pts., after induction phase I. Thirteen patients discontinued the protocol for: allogeneic stem-cell transplantation - 7, refusal -1, pancreatitis - 4, grade 3 DVT-1. Two additional patients died in CR from neutropenic sepsis. Patients with elevated liver enzymes, high bilirubin, or hyperglycemia continued on the study. To date 8 patients received all 6 doses of PEG-ASP having completed all consolidation cycles. The other patients are still being treated. So far the number of PEG-ASP doses given is: 6-8pts, 5-2 pts., 4-2 pts., 3- 6 pts., 2-7 pts., 1-12 pts. Total number of doses was 110 and none had an allergic reaction. The number of pts. with grade 3/4 toxicities during PEG-ASP cycles were: elevated liver enzymes - 20 (54%), hyperbilrubinema - 7 (19%), hyperglycemia -11 (30%), pancreatitis -4 (11%), fatigue -3 (8%), hypertriglyceridemia-2 (6%), catheter thrombosis- 2 (6%), neuropathy-1. All toxicities were reversible. With a median follow up of 15 months EFS at 3 yrs is 65% (Ph$^-$ patients 68%) without difference between patients above or below age 35 years. *Conclusions.* Administration of multiple doses of PEG-ASP IV to adults (ages 19-57 years) in an intensified BFM-based pediatric-like strategy is feasible and provides long term asparagine depletion. Although the follow up is short the EFS appears to be relatively high. Such approach may benefit adults with ALL

0921

SHORT-COURSE IMATINIB (IM) ADDED TO CHEMOTHERAPY IMPROVES EARLY BUT NOT LONG-TERM OUTCOME IN ADULT PH/BCR-ABL$^+$ ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): 5-YEAR FOLLOW-UP RESULTS OF STUDY NILG-09

R. Bassan,[1] T. Intermesoli,[1] E. Oldani,[1] G. Rossi,[2] E. Borlenghi,[2] E.M. Pogliani,[3] E. Terruzzi,[3] P. Fabris,[4] E. Di Bona,[4] G. Gianfaldoni,[5] C. Romani,[6] A. Cortelezzi,[7] D. Mattei,[8] O. Spinelli,[1] A. Rambaldi[1]

[1]Ospedali Riuniti, BERGAMO; [2]Spedali Civili, BRESCIA; [3]Ospedale S.Gerardo, MONZA; [4]Ospedale Civile, BOLZANO; [5]Ospedale Univ. Careggi, FIRENZE; [6]Ospedale Oncol. Businco, CAGLIARI; [7]Ospedale Maggiore, MILANO; [8]Ospedale S.Croce e Carlo, CUNEO, Italy

Background. Northern Italy Leukemia Group program 09 for Ph/BCR-ABL$^+$ ALL was modified in 2003 adding to chemotherapy short intermittent IM pulses (IM$^+$), with the aim to minimize the risk of resistance induction and to sensitize Ph$^+$ cells to other drugs. *Aims.* The reduction of early failures, leading to higher stem cell transplantation (SCT) rates and improved survival in comparison with the cohort treated on the same schedule without IM, was the study objective. *Methods.* IM 600 mg/d po. was added for 7 days to each chemotherapy cycle (C), starting

from day 15 on C1, and from day -3 on C2 to C8 (C1,8: IDR/VCR/PDN+ASP; C2,3,4,6: IDR/VCR/CY/DEX; C4,7: HD-MTX/Ara-C). All patients were eligible to allogeneic SCT a.s.a.p., or alternatively to HD cycles with autologous support and long-term maintenance (again with intermittent IM). *Results.* Between 2000-6, 68 of 280 study patients had Ph/BCR-ABL$^+$ ALL (24%). M/F ratio was 1.19 and median age 48 years, with range 19-66. 35 and 33 patients belonged to successive IM- and IM$^+$ cohorts, respectively. Outcome to induction therapy was improved in IM$^+$ group because of lower NR rate (borderline statistical significance): CR 30/33 (91%) vs. 28/35 (80%), NR 1 (3%) *vs* 6 (17%) (p=0.05), ED 2 (6%) vs. 1 (3%). The ability to perform a SCT in CR1 was also increased, from 43% in IM- group(n=15: 11 allogeneic, 4 HD plus autologous; 43% overall) to 67% (n=22: 18 allogeneic, 4 HD plus autologous) in IM$^+$ group (p=0.049). This result was put in relation to the absence of early relapses i.e. during the first 6 months of therapy (p=0.039 in favor of IM$^+$ group for cumulative incidence of relapse), so that more patients completed the donor search and reached planned allogeneic/autologous SCT/HD phases. In terms of molecular response, the positive clinical effect from IM was not associated with greater reduction of BCR-ABL transcripts. RQ-PCR in marrow samples taken at weeks 10, 16 and 22 in 13 IM- and 12 IM$^+$ cases revealed a major molecular response (absent/<10^{-4} PCR signals) in 14/28 (50%) determinations in IM- group vs. 8/25 (32%) in IM$^+$ group, with median log reductions to 0,0009-0,0000 *vs* 0,0011-0,0001 (all P=NS between 0.28-0.84). With regard to post-SCT course and long-term outcome, there were 7 SCT-related deaths (6 in IM$^+$ group) and a cumulative posttrasplantation relapse rate of 50%. Consequently, despite the improved early outcome in IM$^+$ cases, 5-year DFS/overall survival results were similar to IM- group (median 17/15 months and probability 0.31/0.27, P=NS). *Conclusions.* TK inhibitors such as IM play a major role in improving the early outcome of patients with Ph/BCR-ABL$^+$ ALL, who may thus have larger access to curative SCT treatments. In this regard, the present, short IM schedule conferred a therapeutic benefit, despite the unimproved molecular response from the retrospective comparative analysis. However, the problems of SCT-related toxicity and postgrafting relapse need to be addressed further in order to obtain better long-term survival and cure rates.

0922

DASATINIB MONOTHERAPY AS 1ST LINE TREATMENT OF PH$^+$ ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS: UPDATE OF THE GIMEMA LAL1205 STUDY

R. Foà,[1] P. Fazi,[1] A. Vitale,[1] A. Guarini,[1] M.S. De Propris,[1] L. Elia,[1] G. Cimino,[1] M. Luppi,[2] C. Castagnola,[3] S. Sica,[4] R. Nieddu,[5] M.-N. Piersantelli,[6] F. Ferrara,[7] F. Nobile,[1] R. Fanin,[8] G. Martinelli,[9] F. Mandelli,[1] G. Meloni,[1] M. Baccarani[9]

[1]Division of Hematology, ROME; [2]Department of Oncology and Hematology, University of Modena, MODENA; [3]Department of Hematology, Fondazione Policlinico S. Matteo, University of Pavia, PAVIA; [4]Department of Hematology, Catholic University, ROME; [5]Institute of Hematology, University of Sassari, SASSARI; [6]Department of Hematology, University of Ancona, ANCONA; [7]Department of Hematology, NAPLES; [8]Division of Hematology, University of Udine, UDINE; [9]Department of Hematology/Oncology L. and A. Seràgnoli, BOLOGNA, Italy

Background. Dasatinib is a potent, oral inhibitor of the BCR-ABL, c-KIT and SRC kinase family, which has proven to be a more active inhibitor of BCR-ABL and c-KIT than imatinib mesylate in preclinical studies. Clinically, it has been shown to be effective in chronic myeloid leukemia and in Ph$^+$ ALL patients resistant or intolerant to imatinib. *Aims.* Assess the activity, safety and tolerability of dasatinib monotherapy as 1st line treatment for adult Ph$^+$ ALL patients. *Methods.* The GIMEMA LAL 1205 protocol was designed for patients >18 years (no age uplimit) who receive dasatinib po, 70 mg BID. A steroid pre-phase is started 7 days prior to dasatinib administration and continued up to day 31, and then tapered. The pre-phase allows to identify the presence of the BCR/ABL transcript. Dasatinib is given for 12 weeks. Two intrathecal methotrexates are administered at days +22 and +43. All cases are analyzed through a central handling of samples at presentation for morphology, immunophenotype, cytogenetics and molecular biology. Minimal residual disease (MRD) is also centrally investigated by flow-cytometry and Q-RT-PCR at days +22, +43, +57 and +84. *Results.* Thirty-six BCR/ABL$^+$ ALL patients have been enrolled: median age was 56 years (24-74), 15 were females and 21 males. To date, 28 patients are evaluable for response, 7 are too early and 1 withdrew from the study due to medical decision at day +14. All patients (100%) have witnessed a complete hematological response (CHR): 22 (78.6%) at the 1st determination, 4 (14.3%) at the 2nd and 2 (7.1%) at the 3rd. Median time to CHR has been 24 days (range 19-67). No fatalities have been observed; in 10 patients, at least 1 severe adverse event (SAE) has been recorded, for a total of 23 SAEs (1 life threatening, 9 severe, 10 moderate, 3 mild). Overall, the compliance has been good; only 1 patient stopped treatment at day 67 due to toxicity while in CHR. The median follow-up is 8.6 months (range 0.1-14.0). So far, 7 patients have relapsed, at a median of 72 days from the end of induction; 4 patients have died, 2 upon relapse and 2 due to transplant-related complications. Monitoring of MRD has shown a very marked clearance of leukemic cells by day +22, strengthened at the subsequent timepoints. The results of the immunophenotypic and BCR/ABL Q-RT-PCR monitoring of MRD are reported in Table 1. *Conclusions.* This updated analysis demonstrates that in adult Ph$^+$ ALL dasatinib monotherapy induces a rapid CHR in all patients so far treated without important toxicities and no fatalities. The hematological response is coupled to a very marked and rapid debulking of the neoplastic clone documented at the MRD level. The optimal post-induction treatment strategy remains to be defined.

Table 1.

Day	Immunophenotype					Q-RT-PCR (copy number)		
	Evaluable patients	<3%- >1%	<1%- >0.01%	<0.01%	0%	Evaluable patients	>1x10^2	<1x10^2
+22	27	4	14	8	1	27	12	15
+43	26	1	7	14	2	26	5	21
+57	22	1	3	12	6	25	2	23
+84	21	0	5	12	4	25	5	20

0923

DASATINIB IN CHILDREN AND ADOLESCENTS WITH RELAPSED OR REFRACTORY LEUKEMIA: PRELIMINARY RESULTS OF THE CA180018 PHASE I/II STUDY FROM THE ITCC CONSORTIUM

C.M. Zwaan,[1] P. Kearns,[2] M.L. Den Boerd,[1] H.B. Beverloo,[1] V.H.J. Van der Velden,[1] J.M.A. Van Tornout,[3] D. Derreumaux,[3] T. Lehrnbecher,[4] F. Mechinaud,[5] C. Rizzari,[6] A. Baruchel,[7] R. Pieters[1]

[1]Erasmus MC/Sophia Children's Hospital, ROTTERDAM, Netherlands; [2]The Institute of Child Health, University of Birmingham, BIRMINGHAM, UK; [3]Bristol-Myers Squibb, WALLINGFORD, USA; [4]Johann Wolfgang Goethe University, FRANKFURT, Germany; [5]CHU Nantes-Hôpital des Enfants, NANTES, France; [6]University of Milano - Bicocca, MONZA, Italy; [7]Hôpital Saint-Louis, PARIS, France

Background. Relapsed/refractory leukemia in childhood carries a grave prognosis. Dasatinib is a potent oral kinase inhibitor of BCR-ABL, KIT, and SRC kinases. Recently, approval was granted for adult CML and Philadelphia chromosome-positive (Ph$^+$) ALL, resistant to prior therapy. *Aims.* To establish a safe and effective dose of dasatinib in children/adolescents with various leukemia sub-types. *Methods.* The CA180018 trial is being conducted via the ITCC-Consortium in 6 countries (12 centers). It involves a phase I/II dose-finding study in patients aged 1-21 years with imatinib-resistant or -intolerant CML, relapsed/refractory Ph$^+$ALL or Ph$^-$ALL, or AML in second/subsequent relapse. The starting dose was 60 mg/m^2 QD. Intra-patient dose escalation was allowed for lack of response and dose-escalations were safety/efficacy dependent. *Results.* Current data reflect the first 36 patients (median age 10.4 years, range 1-21) treated from 27MAR2006 through 09NOV2007, including six CP-CML, one accelerated-phase (AP)-CML, one lymphoid-blast-phase (LBP)-CML, nine Ph$^+$ALL, six Ph$^-$ALL, and 13 Ph$^-$AML patients. Prior therapy included chemotherapy(n=35), imatinib(n=17), and stem cell transplant(n=20). Intra-patient dose escalation occurred in 21 patients. At data cut-off, seven patients remained on study. *Safety.* Dasatinib was well tolerated up to the current 120 mg/m^2 dose. Cytopenias were infrequent and non-hematologic toxicities were mostly mild-to-moderate in severity. Drug-related toxicities were observed in 19 patients including two dose-limiting toxicities: one grade 4 anaphylaxis 5 hours after the first dose and one grade 3 upper-GI bleed on Day 6 of dasatinib dosing (120 mg/m^2). One escalation-limiting toxicity (pneumonia) was observed after escalation to 80

mg/m^2. One patient had a grade 3 pleural effusion containing leukemia blasts. *Responses*. 4/6 patients with CP-CML responded: two patients achieved a complete hematological response (CHR) and a complete cytogenetic response (CCyR); one patient achieved a CCyR with a molecular response (MR); one patient with molecular relapse only achieved a MR. One patient with AP-CML achieved both a CHR and CCyR. Among ten Ph(+)ALL/LBP-CML patients, seven started at 60 mg/m^2: 2/7 patients had a CHR, cleared their CSF, and had a CCyR; 1/7 patient with an isolated CNS relapse only cleared the CSF. Three patients started at 80 mg/m^2; one patient achieved a CHR, a CCyR, and cleared the CSF; another achieved a CHR and a CCyR; the third achieved a partial CyR by FISH only. Of 6 Ph$^-$ALL patients, three started at 60 mg/m^2, one at 80 mg/m^2, and two at 100 mg/m^2. Of the 13 Ph$^-$AML patients, two started at 60 mg/m^2, five at 80 mg/m^2, five at 100 mg/m^2, and one at 120 mg/m^2. A temporary significant decrease was achieved in PB blast count in one Ph$^-$ALL patient and in PB and BM blast count in two Ph$^-$AML patients (one with AML7 and one with Down's syndrome). No patients with FLT3- or KIT-mutated AML have been enrolled to-date. A maximum tolerated dose has not been established. *Summary.* These interim data demonstrate a favorable safety profile for dasatinib with efficacy in patients with Ph$^+$ leukemia. The temporary drops in BM/PB blast count in Ph$^-$ disease resemble the activity of FLT3 inhibitors. Higher dasatinib dose levels are being explored in Ph$^-$ patients. Updated data will be presented.

0924

IMPACT OF ALLOGENEIC STEM CELL TRANSPLANTATION (SCT) ON MINIMAL RESIDUAL DISEASE (MRD) AND BCR-ABL KINASE DOMAIN MUTATIONS IN PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH+ALL)

H. Pfeifer, S. Wystub, B. Wassmann, P. Brück, N. Goekbuget, L. Wunderle, J. Chromik, H. Serve, D. Hoelzer, O.G. Ottmann

Universitätsklinik Frankfurt, FRANKFURT, Germany

Background. BCR-ABL Tyrosine Kinase Domain (TKD) mutations are found in the vast majority of Ph+ALL patients who relapse during treatment with ABL kinase inhibitors such as imatinib (IM). In a previous retrospective analysis of mostly elderly and therefore non-transplanted patients, we demonstrated that the TKD mutations present at relapse are already detectable at low-level in 30-40% of newly diagnosed, IM-naïve patients. In younger Ph+ALL patients, neither the frequency of pre-exisiting mutations, the impact of combined treatment with IM and intensive chemotherapy or of allogeneic SCT on TKD mutations have been established. *Aims.* To determine the impact of imatinib plus chemotherapy followed by allogeneic SCT on MRD and BCR-ABL mutations in pts. with Ph+ALL. *Patients and Methods.* Bone marrow (BM) and/or peripheral blood (PB) samples were collected pre-treatment, during imatinib based combination therapy prior to SCT and serially after SCT from a total of 50 pts. with newly diagnosed Ph+ALL (median age: 45 yrs.) who were enrolled in prospective GMALL trials of IM and underwent SCT. MRD and mutational status were analysed by quantitative rtPCR of bcr-abl transcripts and by denaturing high-performance liquid chromatography (D-HPLC) plus cDNA sequencing, respectively. PCR negativity was always confirmed by nested rtPCR. Patients with undetectable bcr-abl transcripts were considered to not harbour a mutation at the time of analysis. *Results.* In this cohort of younger patients, the frequency of TKD mutations pre-IM was 18% (6/34), lower than previously observed in elderly patients (median age 68 years). Of the 32 pts. evaluable within 4 weeks prior to SCT, 12 pts. were MRD negative and 20 MRD pos., 8 (40%) of whom had a detectable mutation, for an overall mutation frequency of 25% pre-SCT. Shortly after SCT, 46 of 50 transplanted patients were MRD negative, including 7 of the 8 patients with a pre-transplant mutation. Two of the 4 pts. who remained MRD pos. after SCT initially displayed unmutated and 2 pts. mutated BCR-ABL (T315I, P-loop). Three of these 4 MRD positive patients converted to PCR negativity, including the patient with the T315I mutation, who however subsequently relapsed with the T315I mutation. In eleven of the 12 patients in whom a mutation was detected either at diagnosis and/or before SCT, no mutation was detected during serial analysis after SCT. Three of the 46 patients who were MRD negative shortly after SCT subsequently converted to PCR positivity, all of whom harbored a TKD mutation (T315I, E355G, Y253F). *Conclusions.* Bcr-abl mutations are detected prior to SCT in a clinically significant proportion (25%) of younger adult patients with Ph+ALL treated with imatinib and intensive chemotherapy. Allogeneic SCT appears to result in long-term elimination of mutant clones in the majority of patients. However, patients remain at risk of relapsing with previously undetectable TKD mutations, some of whom are considered responsive to second generation ABL TK inhibitors.

Myelodysplastic syndromes, myeloproliferative disorders and chronic myeloid leukemia

0925

5-AZACYTIDINE FOR THE TREATMENT OF LOW/INTERMEDIATE-1 IPSS RISK MYELODYSPLASTIC SYNDROMES : RESULTS IN 63 PATIENTS FROM THE ITALIAN PATIENT NAMED PROGRAM

P. Musto,[1] L. Maurillo,[2] A. Spagnoli,[3] A. Gozzini,[3] F. Rivellini,[3] C. Tatarelli,[3] M. Lunghi,[3] C. Fili,[3] E. Orciuolo,[3] L. Ciuffreda,[3] E. Vigna,[3] P. Della Cioppa,[3] D. Ferrero,[3] S. Palmieri,[3] G. Palumbo,[3] N. Di Renzo,[3] E. Oliva,[3] G. Sanpaolo,[3] D. Pastore,[3] A. Tonso,[3] A. Santagostino,[3] O. Villani,[3] F. D'Auria,[3] A. D'Arco,[3] G. Gaidano,[3] S. Galimberti,[3] D. Russo,[3] A. Venditti,[3] M.A. Aloe-Spiriti,[3] G. Leone,[3] V. Santini[3]

[1]CROB, Centro di Riferimento Oncologico della Basilicata, RIONERO IN VULTURE (PZ); [2]S. Eugenio Hospital, Tor Vergata University, ROME; [3]On behalf of ad hoc Italian Study Group, AZACYTIDINE IN MDS AND AML, Italy

Background. 5-azacytidine (AZA) was demonstrated to significantly prolong overall survival in higher-risk pts with myelodysplastic syndromes (MDS) in a large, international, randomized, phase III trial (AZA-001). However, efficacy and safety of AZA in lower risk MDS usually has not been considered in multicenter studies and a specific separate analysis is lacking. *Methods.* Among a total of 218 MDS patients treated with AZA outside of clinical trials, on the basis of a national patient named program in 31 different Italian Institutions since 2005, we evaluated 63 patients scored as low/int-1 IPSS risk MDS. Median age was 69 years (range 34-85), male female ratio 36/27. According to WHO classification, 14 were RA/RARS, 3 pts had 5q- syndromes, 16 RCMD, 19 RAEB-1, 4 RAEB-2, 1 CMMoL, 6 MDS unclassified. Median time from diagnosis was 16 months (range 1-120). Fifty-three pts (84%) were transfusion-dependent, fifty (79%) had received a prior treatment, mostly with erythropoiesis stimulating agents. AZA was administered as single agent in 51 pts (81%), while in remaining subjects the drug was variously combined with growth factors, valproic acid or other agents. Forty pts (63%) received a standard AZA dose of 75 mg/d/sqm s.c., twenty-two (35%) a fixed dose of 100 mg/d s.c.. Single cycle treatment duration was 7 days in 36 pts (57%), <7 days in 25 pts (40%), 7 days in 2 pts (3%), The median number of monthly cycles was 4.5 (range 1-11), and 39 pts (62%) completed at least 4 cycles. *Results.* The most relevant toxicity observed (grade 3-4) was represented by myelosuppression (21%) and infections (7%). According to 2006-updated IWG criteria, overall response rate in 47 evaluable pts was 41% (49% in 31 pts who had completed at least 4 cycles). In particular, complete response, partial response and hematological improvement occurred in 19%, 11% and 11% of pts (23%, 16% and 10% of those who were treated with at least 4 cycles), respectively. Stabilization of disease was obtained in 36% of pts, while progression or failure were observed in 23% Response duration ranged from 1 to +14 months. There were no significant differences in response rate according to dose and schedule employed, although a slight trend in favour of 75 mg/sqm *vs* 100 mg fixed dose was observed (57 *vs* 38%, respectively). *Conclusions.* These data are indicating AZA as an effective treatment for the subset of patients with low/int-1 IPSS risk MDS, resistant or not suitable for treatment with growth factors or IMIDs .

0926

IMMUNOSUPPRESSION FOR PATIENTS WITH LOW AND INTERMEDIATE RISK MYELODYSPLASTIC SYNDROME: A PROSPECTIVE RANDOMIZED MULTICENTER TRIAL COMPARING ANTITHYMOCYTE GLOBULIN + CYCLOSPORINE WITH BEST SUPPORTIVE CARE: SAKK 33/99

R. Passweg,[1] M. Simcock,[2] A. Giagounidis,[3] C. Aul,[3] C. Dobbelstein,[3] M. Stadler,[3] G. Ossenkoppele,[4] W. Hofmann,[3] K. Schilling,[3] A. Tichelli,[5] A. Ganser[3]

[1]Geneva University Hospitals, GENEVA, Switzerland; [2]SAKK and German MDS Study Group, HANNOVER, Germany; [3]German MDS Study Group, HANNOVER, Germany; [4]HOVON, AMSTERDAM, Netherlands; [5]SAKK, BERNE, Switzerland

Immunosuppressive treatment has been reported to improve cytopenia in some patients with myelodysplastic syndrome (MDS). The combined use of anti-thymocyte globulin (ATG) and cyclosporine (CSA) has been shown to be most effective in patients with immune mediated marrow failure. This trial was designed to assess the impact of immunosuppression on hematopoiesis, transfusion requirements, transformation and

survival in MDS patients. Eighty-eight transfusion dependent patients were randomized to receive ATG+CSA (15 mg/kg of horse ATG (Lymphoglobuline, Genzyme) for 5 days and oral CSA for 180 days) or best supportive care (BSC), stratified by treatment center and IPSS risk score between November 2000 and October 2006. The primary endpoint was best response at 6 months. Patients in the BSC arm crossed-over to ATG+CSA after 6 months or earlier in the event of progression: Information collected after cross-over was not evaluated outside of time-to-event analyses. Eligible patients had an ECOG performance status ≤2 along with a transfusion dependency of <2 years duration. Patients with MDS types CMMoL and RAEBt or treatment related MDS were not included in the trial. Statistical analyses consisted of comparisons of response rates and durations between treatment arms and time-to-event analyses including overall-survival (read as the time from trial randomization to all-cause mortality) and transformation-free survival (event defined as death, transformation to higher grade MDS or leukaemia). Also evaluated was medical resource utilization and hematological and non-hematological toxicities. Five patients in the ATG+CSA arm did not receive treatment for various reasons whilst 14 patients in the BSC arm crossed-over on to ATG+CSA treatment. At 6 months there were 13 (31%) patients with a hematologic response (CR+PR) vs. 29 without in the ATG+CSA arm, compared to 5 (12%) with vs. 35 without in the BSC arm (p=0.04, adjusted for the interim analysis): Response duration in the ATG arm was 1.4 (0.6-2.8) years. For various reasons no information was available for the remaining 6 patients. Transformation-free survival probability estimates at 2 years were 39% (21-56%) for patients receiving ATG+CSA and 43% (25-61%) for patients receiving BSC (log-rank p-value: 0.50). In total 33 deaths occurred; 17/45 patients randomized to receiving ATG+CSA and 16/43 patients receiving BSC. Overall survival probability estimates at 2 years were 46% (27-63%) for patients receiving ATG+CSA and 60% (40-75%) for patients receiving BSC (log-rank p-value: 0.85). The vast majority of adverse events occurred early after randomization, with particularly high WHO hematological toxicity gradings in both treatment arms. There were 23 SAEs; 16 in the ATG+CSA arm and 7 in the BSC arm (0.04). Reported SAEs were heterogeneous and included the most common types of infectious and inflammatory complications. Conclusions: In this open label randomized phase III trial of patients with transfusion dependent low and intermediate risk MDS, treatment with ATG + CSA appears to be associated with hematologic response in a subset of patients without apparent impact on transformation-free- and overall- survival.

Table 1.

	ATG+CSA	BSC
Patients: n	45	43
Age: median years (range)	62 (23-75)	65 (24-76)
Sex: n male (%)	25 (56)	35 (81)
IPSS score: (low/int-1/int-2/high/na	(8/24/7/1/4)	(8/25/5/0/5)
MDS type: n (RA/RAS/RAEB-I/RAEB-II/hypoplastic	(21/6/9/0/9)	(18/8/11/2/4)

0927

IMATINIB DISCONTINUATION AFTER IMATINIB/INTERFERON ALPHA COMBINATION THERAPY IS ASSOCIATED WITH CONTINUOUS RESPONSES IN THE MAJORITY OF PATIENTS

A. Hochhaus,[1] A. Neubauer,[2] M.C. Mueller,[1] S. Napieralski,[2] P. Erben,[1] T. Bostel,[1] R. Hehlmann,[1] A. Burchert[2]

[1]III.Med.Klinik, MANNHEIM;[2]Dept Hematol, Oncol, Immunol, MARBURG, Germany

Background. Most patients with chronic myelogenous leukemia (CML) relapse after discontinuation of imatinib (IM) due to residual BCR-ABL positive progenitor cells which are able to repopulate the bone marrow. Thus, current recommendations suggest an indefinite IM therapy even in complete molecular responders. However, in view of potential long term adverse effects there is a concern of lifelong tyrosine kinase inhibition. Hence, strategies to circumvent permanent kinase inhibitor therapy would be of substantial clinical value. Interferon alpha (IFN), in contrast to IM, is associated with an antileukemic immune response to control CML, and after stopping IFN in patients showing long-lasting complete cytogenetic remission response has been maintained in a significant proportion of cases. *Aims.* We sought to determine efficacy and tolerability of an IFN maintenance immunotherapy after IM/IFN induction in newly diagnosed chronic phase CML patients. *Methods.* Twenty patients (14 m, 6 f; median age 45, range 24-74 years) have been investigated. Hasford score revealed low (n=13), intermediate (n=6), and high risk (n=1) diseases. IM therapy had been administered for 2.4 years (0.2-4.9), combined with PEG-IFN alpha 2a (n=17) or IFN alpha 2a (n=3). Maintenance therapy consisted of PEG-IFN (n=16) or IFN (n=4). Dose was adjusted according to response and tolerability and ranged between 135 µg PEG-IFN every three weeks to 180 µg PEG-IFN once weekly week, or alternatively between 2 to 5*3 Mill IU IFN/week. IM was stopped due to side effects (n=5) or after the patient's individual request and informed consent (n=15). *Results.* At the time of imatinib discontinuation, 19 patients were in complete cytogenetic remission and one patient did not show any cytogenetic response. Major molecular response was determined in peripheral blood leukocytes of 16 patients, including three patient with undetectable BCR-ABL transcripts. After a median observation time of 1.3 years (range 0.4-3.3), 15 patients showed major molecular response, four of them were complete. Improvement of molecular response was observed in two and stable response levels in 13 patients. By six-weekly assessments of BCR-ABL expression gradual molecular relapse was observed in five patients. At the time of IM discontinuation and during IFN maintenance therapy myeloblastin (proteinase-3, PR3) mRNA expression was determined and compared to glucose-6-phosphate dehydrogenase transcripts as internal standard. During IFN monotherapy, median ratios PR3/G6PD increased from 0.06% (range, 0.02-3.5) to 0.12% (0.03-1.4; p=0.03). IFN response was associated with the detection of autoreactive PR3 specific T-lymphocytes during IFN maintenance therapy determined by a tetramer assay in 6/9 HLA A2-positive patients, suggesting that PR3-specific cytotoxic T lymphocytes contribute to the IFN-mediated antileukemic immunity. IFN maintenance therapy was well tolerated. Non-hematologic side effects (maximum grade I; including fatigue, flu-like symptoms, liver toxicity, and diarrhea) were noted in eight patients. *Summary and Conclusions.* We report a high rate of improved or continuous molecular remissions in 15 of 20 patients (75%) on IFN monotherapy after prior induction with IM/IFN. This suggests a beneficial role for IFN in the maintenance therapy after IM-mediated debulking and may impact future CML therapy.

0928

PROLONGED SURVIVAL IN HIGHER-RISK MYELODYSPLASTIC SYNDROME (MDS) PATIENTS (PTS) WITH -7/DEL(7Q) TREATED WITH AZACITIDINE (AZA)

G.J. Mufti,[1] G. Garcia-Manero,[2] N. Hovarth,[3] Z. Lim,[4] B. Quesnel,[5] G. Leone,[6] J. Bennett,[7] G. Sanz,[8] D. McKenzie,[9] J. Backstrom,[9] C.L. Beach[9]

[1]Kings College Hospital, LONDON, UK; [2]MD Anderson Cancer Center, HOUSTON, USA; [3]IMVS, ADELAIDE, Australia; [4]King's College London, LONDON, UK; [5]Service des Maladies du Sang, LILLE, France; [6]Policlinico Gemelli - Divisione Di Ematologia, ROMA, Italy; [7]University of Rochester, ROCHESTER, UK; [8]Institute Name Hospital Universitario La Fe, VALENCIA, Spain; [9]Pharmion Corporation, OVERLAND PARK, USA

Background. -7/del(7q) is a common cytogenetic abnormality in MDS patients (5%-10%), is frequently refractory to intensive therapies, and is associated with poor prognosis, both in isolation or as part of a complex karyotype. A recent small retrospective analysis suggested that azacitidine has promising activity in -7/del(7q) patients with MDS (Lim *et al.* Blood 2007;110:1449). *Aims.* To confirm the overall survival (OS) results observed in this small retrospective analysis, a subgroup analysis was conducted in -7/del(7q) pts from AZA-001, a large phase III, randomized trial of AZA versus conventional care regimens (CCR) in higher-risk MDS (Fenaux, Blood 2007;110:Abstract 817). *Methods.* AZA-001 enrolled pts with higher-risk MDS (FAB: RAEB, RAEB-T, CMML and IPSS: Int-2 or High). Pts were randomized to AZA (75 mg/m^2/d x 7d, every 28 days) or CCR. CCR comprised 3 treatments: BSC only; low-dose ara-C (20 mg/m^2/d x 14d, every 28 days); or intensive chemotherapy (7+3 regimen). No erythropoiesis-stimulating agents were allowed. The retrospective analysis analyzed outcomes in MDS pts with -7/del(7q), who received AZA at an identical dose and schedule. Median OS in AZA-001 was calculated using Kaplan-Meier *Methods.* All patients in AZA-001 and the retrospective analysis gave informed consent. *Results.* In the retrospective analysis, 31 pts were included with a median age of 61 years. Thirteen (42%) pts had -7/del(7q) alone and 18 (58%) as part of a complex karyotype (-7/del[7q]+). Five of 31 pts stopped AZA after 1 cycle and were excluded from further analyses. The remaining 26 patients received a median of 6 cycles of AZA (range: 2-28). Median OS of the entire cohort in the retrospective analysis was 19.8 months (95%CI: 16.3-23.3). Median survival in pts with -7/del(7q) alone was 24.8 months (95%CI: 17.8-31.8) and 17.3 months (95%CI: 8.3-26.3) in those with -7/del[7q]+, p=0.10). In the AZA-001 trial, 57 (30 AZA, 27 CCR) pts had -7/del(7q): 35% in isolation and 65% with -7/del[7q]+. Median patient age was 69 years and baseline characteristics were balanced across the AZA and CCR arms. AZA was administered for a median of 6.5 cycles (range: 1-28). In AZA-001, median KM OS was significantly prolonged with AZA *vs* CCR

(13.1 (95% CI: 9.9-24.5) *vs* 4.6 (95% CI: 3.5-6.7) months, respectively, stratified log-rank *p*=0.002, Table 1). The hazard ratio was 0.33 (95% CI: 0.16-0.68) indicating a 67% reduced risk of death for all -7/del(7q) pts in the AZA arm. At 2 years, a 4-fold OS advantage was observed in the AZA arm, with 33% of pts (10/30) alive vs. 8% (2/27) in the CCR arm (p=0.03). In the AZA group, OS was 18.4 months in patients with -7/del(7q) alone and 8.3 months in those with -7/del[7q]+ compared with 10.3 and 4.2 months in the CCR group, respectively. *Summary and conclusions.* Results from this large multicenter, international, randomized, controlled trial (AZA-001) confirm previous retrospective findings of the potent activity of AZA in high-risk MDS pts with chromosome 7 abnormalities and support its use as standard of care in these pts.

Table 1. Median OS (months) in Pts with - 7/del(7q): Results of a Controlled Trial (AZA-001) and of a retrospective analysis.

	AZA-001 Study						Retrospective Analysis		
	AZA Arm,			CCR Arm			AZA		
	Total	-7/del (7q)	-7/del [7q]+	Total	-7/del (7q)	-7/del [7q]+	Total	-7/del (7q)	-7/del [7q]+
Median OS (months)	13.1*	18.4	8.3	4.6*	10.3	4.2	19.8	24.8	17.3
P value	0.002*						0.10†		

*AZA Total vs CCR Total in AZA-001
†-7del(7q) only vs -7del[7q]+ in the retrospective analysis

0929

SOCS2: INHIBITOR OF JAK2V617F-MEDIATED SIGNAL TRANSDUCTION

H. Quentmeier,[1] R. Geffers,[2] E. Jost,[3] R.A.F. MacLeod,[1] S. Nagel,[1] S. Röhrs,[1] M. Scherr,[4] H.G. Drexler[1]

[1]DSMZ, BRAUNSCHWEIG; [2]Helmholtz Zentrum für Infektionsforschung, BRAUNSCHWEIG; [3]Universitätsklinikum Aachen, AACHEN;[4]Medizinische Hochschule Hannover, HANNOVER, Germany

Background. The Janus kinase 2 V617F (JAK2V617F) activating mutation has been described in myeloproliferative disorders (MPD) and rare cases of myelodysplastic syndromes (MDS). We identified 4 MPD/MDS-derived cell lines carrying the JAK2V617F mutation (JAK2mu). In apparent contradiction to the commonly held view that JAK2V617F expression leads to cytokine-independency, 2/4 JAK2mu cell lines were cytokine-dependent. *Aims.* We set out to elucidate which mechanism was responsible for cytokine-dependency of JAK2mu cell lines. *Methods.* Expression array analysis was performed to find genes that were differentially expressed in the cytokine-dependent and -independent JAK2mu cell lines. FISH analyses using chromosome painting probes and BAC clones were carried out to verify the amplification status of JAK2 and SOCS2. Ploidy status and expression levels of SOCS2 were analyzed by quantitative PCR. Methylation analysis of the CpG-rich SOCS2 5´region was performed using the methylation-specific restriction enzyme HhaI and quantitative PCR. Activation of the JAK2/STAT5 pathway was assessed by Western blot analysis. *Results.* Expression array analysis, verified by quantitative PCR, showed that cytokine-independency was associated with low, cytokine-dependency with high expression levels of the JAK/STAT pathway inhibitor SOCS2. SOCS proteins are antagonists of the JAK pathway. SOCS2 deletion and SOCS promoter methylation have been described in MPD and SOCS2 downregulation has been suggested as a mechanism complementary to the JAK2V617F mutation in the pathogenesis of this disease. In accordance, we found hypermethylation of the SOCS2 5´CpG-rich region, associated with inaccessibility of transcription factors to the promoter, in 1/2 cytokine-independent cell lines. Furthermore, knockdown of SOCS2 in the cytokine-dependent JAK2mu cell line MB-02 induced constitutive STAT phosphorylation confirming that SOCS2 operates as a negative regulator for JAK2V617F, as reported for the wild-type kinase. *Summary and conclusions.* Silencing of SOCS genes may be necessary to fully activate the JAK2V617F/STAT5 pathway. Methylation of SOCS1 has been reported for in primary MPD cases. In this line of evidence was our finding that JAK2mu cell lines with low-level expression of SOCS2 - associated with promoter methylation - were cytokine-independent, while SOCS2-high cell lines were growth factor-dependent. Knockdown experiments showed that SOCS2 impedes the activity of JAK2V617F. In conclusion, our data confirm that downregulation of SOCS2 expression by gene methylation might be an important second step in the genesis of cytokine-independent MPD clones.

Chronic myeloid leukemia - Biology

0930

IDENTIFICATION OF CANDIDATE GENES SUSTAINING BCR-ABL ONCOGENIC SIGNALLING AND CML PROGRESSION THROUGH A GENETIC TOOL BASED ON BCR-ABL TRANSGENIC DROSOPHILA MELANOGASTER

F. Messa,[1] F. Arruga,[1] E. Bracco,[1] I. Iacobucci,[2] C. Panuzzo,[1] E. Messa,[1] A. Rotolo,[1] T. Kalebic,[3] M. Baccarani,[2] F. Feiguin,[4] R. Bernardoni,[2] G. Martinelli,[2] G. Saglio,[1] D. Cilloni[1]

[1]University of Turin, TURIN, Italy; [2]University of Bologna, BOLOGNA, Italy; [3]Novartis Oncology, EAST HANOVER, USA; [4]Fondazione Cavalieri Ottolenghi for Neurosciences, TURIN, Italy

Background. Although the role of Bcr-Abl in the pathogenesis of Chronic Myeloid Leukaemia is well established, the mechanisms responsible for CML progression are largely unknown. *Aims.* To perform a genetic screening to identify new pathways leading to imatinib resistance and progression. *Methods.* for this purpose we developed and validated a model based on transgenic Bcr-Abl Drosophila melanogaster (Dm). This approach has the potential to identify genetic pathways that cause or influence the disease, and does not require a priori knowledge of the function of the disease gene. We generated two different stable transgenic flies expressing both human p210Bcr-Abl forms (either w.t. or the mutated form T315I) in a tissue specific manner, in particular, the activation of BCR-ABL led to a particular phenotype in the fly eyes, or alternatively in the hemocytes or in the wings. We conducted an extensive genetic screening using both Drosphila transgenic lines overexpressing human Bcr-Abl forms (Bcr-Abl/w.t. and Bcr-Abl/T315I) by making use of P-elements. This technique offers the possibility to randomly insert P-elements into the genome, thus disrupting genomic loci either in correspondence of coding sequences or regulatory elements and altering the gene function. The heterogeneous mutagenized fly population was carefully screened on the basis of the flies phenotype. The candidate genes were then identified by Southern Blot, ligase and inverted PCR. In addition, a drug screening was performed by feeding flies and using the eye phenotype as readout system. *Results.* Bcr-Abl expression results into a glazed phenotype correlated with the amount of p210 protein detected by western blot carried out on adult fly heads. The P-element induced mutagenesis generated a heterogeneous progeny whose phenotype is influenced by the affected gene. We selected a first group represented by flies displaying a more aggressive phenotype since they harbour mutated genes encoding for proteins which inhibit Bcr-Abl activity thus functioning as Bcr-Abl negative regulator. A second group was represented by flies displaying a mild phenotype. In the latter case the phenotype rescue is most likely due to a mutation occurred at a level of a gene encoding for a protein sustaining the oncogenic signalling or eventually involved in disease progression. Among the genes identified PI3K loss of function results into a phenotype improvement supporting the tool effectiveness. All the data obtained with the use of fly model were confirmed in both cell lines and in primary cells via the overexpression and/or silencing of the genes identified with the Drosophila genetic-screening. Finally we have set up a rapid method for drug testing based on Bcr-Abl and T315I/Bcr-Abl phenotype rescue induced by Bcr-Abl inhibitors. To validate this tool we used Imatinib, Nilotinib, Dasatinib, SKI-606, MK-0457 resulting in a phenotype improvement and in failure to detect phosphorylated Bcr-Abl by Western blot. *Conclusions.* Using Dm we identified a number of genes probably involved in CML progression and IM resistance. Finally, we set up an easy and rapid tool for drug libraries screening which will allow to identify molecules able to silence T315I/ Bcr-Abl tyrosine kinase activity.

0931

MULTIDRUG RESISTANCE GENE (MDR1) POLYMORPHISMS ARE ASSOCIATED WITH MAJOR MOLECULAR RESPONSE TO STANDARD-DOSE IMATINIB IN CHRONIC MYELOID LEUKEMIA

S. Dulucq,[1] S. Bouchet,[2] B. Turcq,[3] E. Lippert,[3] G. Etienne,[4] J. Reiffers,[5] M. Molimard,[2] F.X. Mahon[3]

[1]Université INSERM, BORDEAUX; [2]Department of Clinical Pharmacology and Toxicology, BORDEAUX; [3]INSERM U876, BORDEAUX; [4]INSERM U876 and Bergonie Institute, BORDEAUX; [5]Bergonie Institute, BORDEAUX, France

Background. Despite the excellent efficacy of imatinib in chronic

myeloid leukemia (CML) the response in patients is heterogeneous which may in part be caused by pharmacogenetic variability. Imatinib has been reported to be a substrate of the P-Glycoprotein pump. In the current study, we focused on ABCB1 (MDR1) genotype. We analysed the three most relevant single nucleotide polymorphisms (SNPs) of MDR1 in 90 CML patients treated by standard-dose imatinib (i.e 400 mg) on front line treatment (n=42) or on second line after interferon (n=48). *Aims.* We have tested the hypothesis that these SNPs may have an impact on imatinib treatment response by modulating the through imatinib plasma levels (Cmin) which have been recently reported to be associated with the major molecular response (MMR). *Methods.* To assess molecular responses, total RNA was extracted from peripheral blood cells and BCR-ABL transcript levels were quantify using real-time (RT-PCR). MMR was defined by the Bcr-Abl level 3 log reduction after 12 months of treatment. The cDNA was used to genotype the MDR1 polymorphisms: C1236T, G2677T/A and C3435T by PCR-restriction fragment length polymorphism (PCR-RFLP). For imatinib plasma quantification, blood samples were collected between 21 and 27 hours after last drug administration and the levels were determined using high-performance liquid chromatography-tandem mass spectrometry. A chi-square test was used to evaluate the association between MDR1 polymorphisms and chemotherapy response or Cmin. *Results.* The 3 variants appeared in partial linkage disequilibrium and were organised in 7 haplotypes. Regarding the polymorphisms separately, there was a significant difference in genotype frequencies at loci 1236 and 2677 between patients with or without MMR. The rate of MMR was correlated with the number of allele at locus 1236. Indeed, MMR was higher for patients heterozygous and even higher for patients homozygous for the 1236T allele when compared to patients with other genotype groups (63.8% for CT/TT *vs* 40.7% for CC, p=0.04 and 85% for TT *vs* 47.7% for CC/CT, p=0.003) suggesting a gene dosage effect for this SNP. For the G2677T/A polymorphism, a better MMR rate was observed for patients with genotype TT or TA than for other groups of genotype (GG/GT/GA p=0.018) (Figure 1). Regarding haplotypes, the rate of MMR after 12 months was found lower for haplotype 1 (1236C-2677G) patients as compared to the others (44.6% *vs* 81%; p=0.003) and, higher for haplotype 4 (1236T-2677T) patients as compared to the others (61.5% *vs* 41.2% p=0.05). Moreover, the haplotype 4 could participate to the modulation of the Cmin (59.6% of haplotype 4 patients with Cmin>1000 ng/mL *vs* 36.4% for other patients; p=0.03). *Conclusions.* We defined 2 haplotypes which are predictive of the MMR achievement after 12 months of imatinib treatment. One of the haplotypes was found statistically linked to the plasma imatinib concentration. In conclusion, genotyping of MDR1 polymorphisms is easy to perform that could be useful to early identify among CML patients the best responders to imatinib.

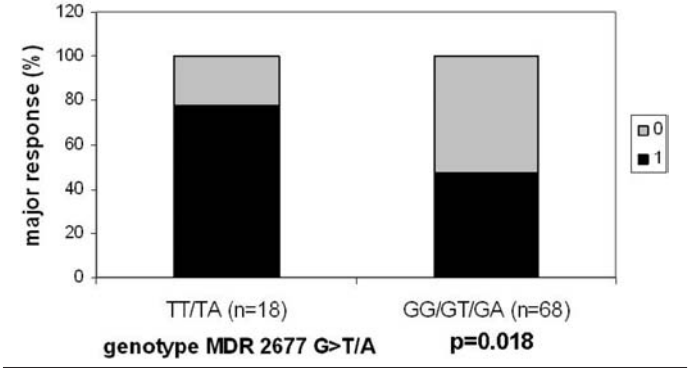


Figure 1. Proportion of patients with a MMR after 12 months of imatinib according to genotypes of *MDR-1* G2677T/A polymorphism. Black: MMR after 12 months of imatinib; Grey: no MMR after 12 months of imatinib.

0932

IMATINIB RESPONSE PREDICTION IN EARLY AND LATE CHRONIC PHASE CML BY EXPRESSION PROFILING

S. Schmidt,[1] D. Fong,[1] D. Wolf,[1] T. Lion,[2] A. Petzer,[3] G. Gastl[1]

[1]Medical University Innsbruck, INNSBRUCK; [2]Children´s Cancer Research Institute, VIENNA; [3]Krankenhaus der Barmherzigen Schwestern, LINZ, Austria

Background. Response prediction by expression profiling has proven useful in several hematological malignancies and might guide therapeutic decisions in the era of targeted therapy. However, for imatinib treatment in CML response prediction data are conflicting most likely because previous retrospective studies relied on various technical platforms using rather small patient populations. *Aims.* This profiling study aimed to establish a robust gene set for imatinib response prediction. Ideally, the resulting gene set should consist of few enough genes to be cost effectively and routinely used with inexpensive technologies like real time PCR. *Methods.* Blood samples from at total of 135 CML patients with early or late chronic phase disease were collected prior to and six weeks after treatment with either 400 mg/day for 12 months or 800 mg/day for 6 months followed by 6 months of 400 mg imatinib therapy. Cytogenetic response and molecular response were monitored. Whole blood samples preserved by PAX gene technology were further processed and evaluated centrally at the expression profiling core facility at Innsbruck Medical University. For expression profiling samples were subjected to hemoglobin mRNA reduction before target preparation for hybridization to Affymetrix hGU133 Plus 2 genechips detecting ~47000 transcripts thereby allowing a whole genome profiling approach. For response prediction we used both the linear discriminatory analysis (LDA) and a random Forest (RF) decision tree algorithm provided in Bioconductor software packages for the open source statistical language *R*. A 1000- or 100-fold crossvalidation was performed using a robust leave-10-out setting for LDA and RF respectively. Additionally imatinib response was analysed according to molecular response at 12 months and where available based on intra-patient comparisons of samples prior to and after 6 weeks of treatment. For condensing the resulting gene sets we applied recursive feature elimination (RFE). *Results.* Preliminary results presented at ASH 2007 and based on 75% of patients revealed a set of 145 genes which allow a mean correct prediction of 76% (±14%) in samples prior to treatment and a slightly better accuracy in samples after 6 weeks of treatment [79% (±14%)]. The resulting positive predictive value is 0.809. Here, we will present the final analysis and will complement the cytogenetic response based analysis by a molecular response based analysis and focus on intra-patient subgroup analyses. *Conclusions.* In this -so far largest- prospective multicenter gene expression profiling study we identified a robust imatinib response predicting gene set using a whole genome profiling approach in early or late chronic phase CML.

0933

DASATINIB EFFICACY AFTER IMATINIB FAILURE BY DOSING SCHEDULE AND BASELINE BCR-ABL MUTATION STATUS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP)

A. Hochhaus,[1] M.C. Müller,[2] J. Cortes,[3] D.W. Kim,[4] Y. Matloub,[5] L. Ploughman,[5] T. Hughes[6]

[1]Medizinische Klinik, Medizinische Fakultät Mannheim der Universität Heidelberg, MANNHEIM, Germany; [2]Universität Heidelberg, MANNHEIM, Germany; [3]MD Anderson Cancer Center, HOUSTON, USA; [4]St Mary's Hospital, The Catholic University of Korea, SEOUL, South-Korea; [5]Bristol-Myers Squibb, WALLINGFORD, USA; [6]Institute of Medical and Vetinary Science, ADELAIDE, Australia

Background. In patients with CML, mutations of the BCR-ABL fusion gene are the leading cause of imatinib resistance and treatment failure. Dasatinib is active against all imatinib-resistant mutations except T315I, and has marked efficacy in patients with CML-CP who are resistant or intolerant to imatinib. The label for dasatinib was recently changed such that the recommended schedule for CML-CP is now 100 mg QD, reflecting the results of a randomized, phase-III, dose-optimization study (CA180-034). In this study, 100 mg QD exhibited similar efficacy and increased tolerability compared with three other dosing schedules.

Table 1.

	MCyR: n/M (%) patients			
	100 mg QD (N=147)	70 mg BID (N=146)	140 mg QD (N=139)	50mg BID (N=149)
Mutation type				
M244V	3/6	1/5	4/7	2/2
G250E	3/5	3/10	3/9	2/7
E255K/V	1/3	2/2	1/2	2/8
T315I	0/5	0/1	1/4	0/5
M351T	1/7	4/8	5/8	8/10
F359C/I/V	5/8	6/9	2/3	7/10
H396P/R	4/5	1/2	2/4	2/5
Others	11/16	6/14	11/16	5/16
ANY	26/49 (53)	21/46 (46)	29/51 (57)	26/61 (43)

Aims. To conduct an analysis of efficacy of different dasatinib dosing regimens administered in CA180-034 study with regard to commonly occurring individual baseline BCR-ABL mutations. *Methods.* Patients were randomized to dasatinib 100 or 140 mg/d, and to a QD versus BID schedule, using a 2x2 factorial design. Peripheral blood cell mRNA was collected and analyzed for BCR-ABL point mutations by RT-PCR and sequencing. *Results.* A total of 670 patients were randomized, 581 of whom were eligible for this subanalysis, having baseline mutation status and one-year cytogenetic response data available. There were no significant differences between overall major cytogenetic response (MCyR) rates for the four schedules: 62% (100 mg QD), 58% (70 mg BID), 63% (140 mg QD), and 56% (50 mg BID). MCyRs observed in patients with the most frequent (observed in ≥15 patients) baseline mutations are shown in the Table 1. In patients with BCR-ABL mutations within the P-loop region (residues 248-256), MCyR rates were 63% (100 mg QD), 53% (70 mg BID), 35% (140 mg QD), and 29% (50 mg BID). *Conclusions.* Dasatinib 100 mg QD leads to MCyRs across individual baseline BCR-ABL mutations except T315I, including those occurring in the P-loop region. The MCyR rate appears similar with the other three dosing schedules.

0934

DASATINIB LONG-TERM EFFICACY IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) WITH RESISTANCE OR INTOLERANCE TO IMATINIB: A TWO-YEAR UPDATE OF THE START-C STUDY

F. Cervantes,[1] M. Baccarani,[2] J. Lipton,[3] Y. Matloub,[4] R. Sinha,[4] R.M. Stone,[5] M. Mauro[6]

[1]Hospital Clinic I Provincial, BARCELONA, Spain; [2]S. Orsola-Malpighi Hospital, University of Bologna, BOLOGNA, Italy; [3]Princess Margaret Hospital, TORONTO, Canada; [4]Bristol-Myers Squibb, WALLINGFORD, USA; [5]Dana-Farber Cancer Institute, BOSTON, USA; [6]Oregon Health & Science University, PORTLAND, USA

Background. Dasatinib is 325-fold more potent than imatinib and 16-fold more potent than nilotinib against BCR-ABL *in vitro*. The approval of dasatinib for the second-line treatment of CML-CP was based on the eight-month data from START-C, a global, 75-center, phase-II study in patients with CML-CP resistant or intolerant to imatinib. Extended two-year follow-up data from this study are now available. *Aims.* The primary objective of this study was to evaluate the major cytogenetic response (MCyR) rate for dasatinib as second-line treatment in patients with CML-CP resistant or intolerant to imatinib. *Methods.* 387 patients with CML-CP who were resistant (n=288) or intolerant (n=99) to imatinib were treated with dasatinib at a starting dose of 70 mg BID. Dose reductions (to 40 or 50 mg BID) in response to toxicity or escalations (to 90 mg BID) following lack of response were permitted. Data are presented for a minimum follow-up of 24 months. *Results.* Median time from diagnosis of CML was 61 months (range 3-251 months). Best response rates for prior imatinib therapy were 82% for complete hematologic response (CHR), 37% for major cytogenetic response (MCyR), and 19% for complete cytogenetic response (CCyR). After 24 months, the MCyR rate to dasatinib was 62% (95% CI 57-67%); MCyRs were long lasting with 88% of patients maintaining their response. In imatinib-resistant patients or patients with baseline BCR-ABL mutations overall, the MCyR rates were 55% and 63%, respectively. Responses were observed in patients with all BCR-ABL mutations, except T315I, and were durable. Overall, CHR was noted in 91% of patients, CCyR in 53%, and major molecular response (MMR) in 47%. In imatinib-intolerant patients, rates of CCyR (all patients evaluable) and MMR (n=94 evaluable) were both 78%. The rate of progression-free survival (PFS) at 24 months was 80% (75% in imatinib-resistant and 94% in imatinib-intolerant patients). Overall survival at 24 months was 94% (92% in imatinib-resistant and 100% in imatinib-intolerant patients). The most common grade 3-4 non-hematologic toxicities were pleural effusion (9%), dyspnea (6%), bleeding (4%), diarrhea (3%), and fatigue (3%). Grade 3-4 neutropenia and thrombocytopenia occurred in 50% and 49% of patients, respectively. There was little evidence of cross intolerance, since only 3% of imatinib-intolerant patients experienced similar grade 3-4 toxicities on dasatinib, and all were able to resume treatment following dose reduction. The appearance of new, higher-grade toxicity between 12 and 24 months was uncommon. *Conclusions.* In patients with CML-CP who are resistant or intolerant to imatinib, dasatinib is associated with a high rate of cytogenetic responses, which are mostly durable, resulting in a 24-month PFS of 80%. Dasatinib was well tolerated in this patient population.

Epigenetics, transcription and signalling

0935

CEBPA PROMOTER HYPERMETHYLATION IN IMMATURE ACUTE MYELOID/T-LYMPHOID LEUKEMIA IS ASSOCIATED WITH WIDESPREAD EPIGENETIC CHANGES

B.J. Wouters,[1] M.E. Figueroa,[2] Y. Li,[2] A.W. Langerak,[1] P.J.M. Valk,[1] B. Löwenberg,[1] J.M. Greally,[2] R. Delwel,[1] A. Melnick[2]

[1]Erasmus University Medical Center, ROTTERDAM, Netherlands; [2]Albert Einstein College of Medicine, BRONX, NY, USA

Background. Genome-wide gene expression profiling has demonstrated that various molecular abnormalities in acute myeloid leukemia (AML) are associated with distinct expression profiles. AMLs with mutations in the myeloid transcription factor CCAAT/enhancer binding protein-alpha (CEBPA) were found to carry discriminative expression signatures as well. Previously, a subgroup of immature myeloid/T-lymphoid cases without CEBPA gene aberrations was found to express a similar signature. Instead of mutations, CpG hypermethylation of the CEBPA proximal promoter was a frequent characteristic of those cases, which associated with silencing of CEBPA expression (Wouters *et al.*, Blood 2007). It has remained unclear whether CEBPA silencing is an isolated event in these leukemias or whether they carry an overall methylator phenotype that discriminates them from other AMLs and from T-cell acute lymphoblastic leukemia (T-ALL). *Aims.* To determine the global DNA methylation profiles of CEBPA silenced leukemias and to compare those to profiles obtained from AMLs with CEBPA mutations as well as from T-ALL samples. *Methods.* HpaII tiny fragment enrichment by ligation mediated PCR (HELP), a quantitative genome-wide method to assess DNA methylation, was carried out using DNA microarrays covering >25000 fragments located at gene promoters and imprinted regions. Eight leukemias with the CEBPA silenced phenotype, 9 AMLs with CEBPA mutations and 9 adult T-ALL samples of various maturation stages were studied. Gene expression profiles were obtained using Affymetrix HGU133plus2.0 microarrays. *Results*. Principal component analysis (PCA) of HELP data from CEBPA mutant and CEBPA silenced leukemias demonstrated that CEBPA silencing was associated with an overall methylation profile that was highly distinct from AMLs with CEBPA mutations. Using a supervised approach based on a moderated t-test, 398 genes were found to be differentially methylated between the two groups, of which almost all (n=389) were hypermethylated in those with silenced CEBPA, including CEBPA itself. Most, but not all, of these hypermethylated regions were located in CpG islands. Although the CEBPA promoter was also methylated in the majority of T-ALL samples studied, PCA separated those T-ALLs from CEBPA silenced leukemias based on global methylation as well as expression data, while a supervised analysis identified 199 genes to be differentially methylated. *Conclusions.* We conclude that CEBPA promoter hypermethylation is not an isolated event in the leukemias studied here, but part of a more widespread epigenetic modulation. Their methylation signature discriminates them from AMLs with CEBPA mutations as well as from a selection of T-ALL samples, supporting the hypothesis that they represent a distinct subgroup of immature leukemias. We suggest that the global hypermethylation profile of these leukemias renders them potential candidates for investigative treatment with DNA methyltransferase inhibitors.

0936

LEUKEMIC TRANSFORMATION BY PLZF/RARA IS MEDIATED BY THE RECRUITMENT OF THE PRC1 POLYCOMB GROUP COMPLEX

E. Duprez,[1] A. Boukarabila,[2] J. Saurin,[3] E. Batshé,[4] N. Mosadegh,[2] P. Otte,[5] J. Pradel,[6] C. Muchardt,[4] H. Sieweke,[2] A. Duprez[2]

[1]Centre d'Immunologie de Marseille, MARSEILLE CEDEX 09; [2]CIML-UMR610[2], MARSEILLE; [3]IBDML-UMR6216, MARSEILLE; [4]URA2578 CNRS Institut Pasteur, PARIS; [5]University of Amsterdam, AMSTERDAM, Netherlands; [6]IBDML, MARSEILLE CEDEX 09, France

The PLZF/RARA fusion protein forms high molecular weight complexes and binds to several nuclear corepressor complexes, which can be liberated in the response to ATRA, but fails to re-induce blast differentiation. In mammals, PLZF is involved in the stable and heritable repression of Hox genes at the HoxD locus during mouse development through the recruitment of the Polycomb group (PcG) protein Bmi-1 and its associated complex, PRC1. It is this normal liaison between PLZF

and the Bmi-1 PcG member that may provide insight into the repressive capacities of PLZF/RARA. We hypothesised that PLZF/RARA was capable of recruiting the PRC1 complex to RAR-responsive genes leading to stable and heritable PcG-dependent silencing of those genes. Using GST pulldown and coimmunoprecipitation assays we found that PLZF/RARA directly interacts with Bmi-1 and through PcG complex reconstitution experiments showed that PLZF/RARA forms a stable component of the PRC1 complex. Through *in vitro* chromatin reconstitution and recruitment assays, we further showed that PLZF/RARA recruits the PRC1 complex to chromatin when RAREs are present which renders the chromatin refractory to chromatin remodelling events. To compare the recruitment of PcG complexes to endogenous RA response elements (RAREs) by PLZF/RARA and PML/RARA fusion proteins we performed ChIP analysis. We demonstrated that expression of either PML/RARA or PLZF/RARA leads to PRC2 enrichment, identified by EZH2 and its trimethylated lysines 27 of histone H3 modification. However, we found that Bmi-1 and Ring1, two major components of PRC1, were specifically enriched upon expression of PLZF/RARA and not PML/RARA. This enrichment was still detectable upon ATRA treatment, offering an interpretation to the resistance linked to the PLZF/RARA leukemic phenotype. To assess whether Bmi-1 is required for transformation of haematopoietic progenitors by PLZF/RARA we used replating assays and compared the replating capacity of wild type and Bmi-1-/- BM cells when transduced by PLZF/RARA or PML/RARA. Our Data showed that Bmi-1 is necessary for transformation of myeloid progenitors by PLZF/RARA but not by the related PML/RARA oncogenic fusion, supporting our model that PRC1 plays a critical role in PLZF/RARA-mediated transformation. Based on these data, we propose a new mechanism involving the PRC1 Polycomb group complex in PLZF/RARA-mediated oncogenesis.

0937

SITE-SPECIFIC UBIQUITINATION OF THE G-CSF RECEPTOR AT EARLY ENDOSOMES DETERMINES ITS LYSOSOMAL SORTING AND SIGNAL DOWNREGULATION

A. Wolfler, M Irandoust, J Gits, O Roovers, I.P. Touw

Erasmus Medical Center, ROTTERDAM, Netherlands

Background. Ubiquitination of lysine residues in the cytoplasmic domain of cytokine receptors directs intracellular receptor trafficking and control of signal duration. We recently identified suppressor of cytokine signaling 3 (SOCS3) with its ubiquitin-ligase activity to be involved in lysosomal sorting of the activated granulocyte colony-stimulating factor receptor (G-CSFR). However, little is known about the mode as well as the kinetics of ubiquitination and whether specific lysines, eg. present in conserved motifs, are involved in G-CSFR sorting. *Aims.* To define cellular and molecular determinants regulating SOCS-dependent G-CSFR ubiquitination and to unravel the role of ubiquitination in a mutant G-CSFR, which is associated with development of acute myeloid leukemia (AML) in patients with severe congenital neutropenia (SCN). *Results.* Four out of five conserved cytoplasmic lysines were robustly ubiquitinated after G-CSFR activation, but lysosomal sorting and signal downregulation of the G-CSFR strictly depended on ubiquitination of a juxtamembrane lysine residue on position 632 (K632). Within this distinct region shifting of the position of the lysine was tolerated without losing its function, arguing against a specific motif present in the juxtamembrane domain. However, an imperative membrane-proximal location of a functional lysine was demonstrated by the finding that neither fusion of this domain to the C-terminus of a lysine-deficient (K5R) G-CSFR nor fusion of ubiquitin to the C-terminus of K5R could restore lysosomal sorting and signal downregulation. Strikingly, an endocytosis-deficient G-CSFR mutant lacking a critical internalization motif was severely hampered in ubiquitination indicating that the activated G-CSFR is not ubiquitinated at the plasma cell membrane but after receptor endocytosis in endosomes. Accordingly, G-CSFR ubiquitination became detectable only 15 min after activation, coinciding with the localization of the G-CSFR in early endosomes. Finally, an internalization-deficient G-CSFR deletion mutant, frequently found in patients with AML with a history of SCN, was also hardly ubiquitinated. *Summary*. These results establish that receptor endocytosis is a prerequisite for site-specific ubiquitination of the juxtamembrane K632 residue, which subsequently exclusively directs lysosomal sorting and signal downregulation of the activated G-CSFR. Because other, inactive lysines were also ubiquitinated, specificity of K632 likely stems from its interaction with downstream effector proteins, rather than from preferential lysine selection by the SOCS3 ubiquitin ligase complex. These findings thus identify abnormal G-CSFR ubiquitination as a possible major event in the pathobiology of AML in patients with SCN.

0938

THE ANTIOXIDANT PROTEIN PEROXIREDOXIN 4 DYNAMICALLY INTERACTS WITH THE CYTOPLASMIC DOMAIN OF G-CSF RECEPTOR IN EARLY ENDOSOMES AND ATTENUATES G-CSF-INDUCED PROLIFERATION IN MYELOID PROGENITOR CELLS

K. Palande, O. Roovers, J. Gits, I.P. Touw

Erasmus Medical Centre, ROTTERDAM, Netherlands

Background. Reactive Oxygen Species (ROS) are involved in signal transduction by regulating the activity of redox sensitive enzymes such as protein tyrosine phosphatases (PTPases). Peroxiredoxins (Prdx's) I-VI are thiol-containing antioxidant proteins that are able to reduce ROS. Localization of PrdxIII and V is confined to mitochondria and peroxisomes while others are cytoplasmic. PrdxII has been implicated in downmodulation of ROS production upon activation of PDGF receptor (Choi, Nature 2005). ROS production upon activation of granulocyte colony-stimulating factor receptor (G-CSFR) has recently been reported (Zhu, Blood 2006). Strikingly, activation of a truncation mutant of G-CSFR (d715-G-CSFR) found in SCN/AML patients resulted in significantly higher ROS levels compared to wild type G-CSFR, implying a negative role of the G-CSFR C-terminus in ROS production. *Aims.* To investigate whether Prdx proteins bind to the C-terminus of the G-CSFR and to determine how this affects the signaling properties of G-CSFR. *Methods.* The Mammalian protein-protein interaction trap (MAPPIT) assay was used to investigate Prdx interactions with G-CSFR. Confocal laser scanning microscopy (CLSM) was used to study the temporal and spatial kinetics of the interaction between Prdx and G-CSFR during ligand-induced endosomal trafficking. Fusion constructs of G-CSFR with catalytically active or inactive Prdx were generated to determine the effects of Prdx in a G-CSFR microdomain-specific setting. These constructs were transduced in 32D cells as well as primary bone marrow cells from G-CSFR deficient mice to determine the effects of Prdx on G-CSF-induced activation of signaling substrates, proliferation and differentiation. *Results.* Of the peroxiredoxins tested (PrdxI, II, IV and VI), only PrdxIV interacted with the G-CSFR in the MAPPIT assay. A region between amino acids 686 and 735 and the most C-terminal 20 amino acids of the G-CSFR were involved in PrdxIV binding. G-CSFR co-immunoprecipitated with endogenous PrdxIV in a transient manner and CLSM showed that this temporal interaction occurred in early endosomes. Experiments in 32D cells expressing wt-G-CSFR, d795-G-CSFR, d795-G-CSFR-PrdxIV and d795-G-CSFR-PrdxIVmut showed that cells expressing the d795-G-CSFR-PrdxIV (active PrdxIV) proliferated modestly in response to G-CSF compared to d795-G-CSFR-PrdxIVmut (inactive PrdxIV), which were hyperproliferative, similar to the d795-G-CSFR expressing cells. Activation of STAT5, STAT3 and ERK was also reduced in cells expressing the fusion of d795-G-CSFR with active PrdxIV compared to PrdxIVmut. In colony assays, bone marrow cells expressing d795-G-CSFR-PrdxIV gave rise to about 3-times lower colony numbers compared to cells expressing the d795-G-CSFR-PrdxIVmut and colonies also contained significantly fewer cell numbers. *Summary and Conclusions.* PrdxIV interacts with the C-terminus of G-CSFR, providing an explanation why the C-terminal truncation mutant of the G-CSFR (d715G-CSFR) produce increased ROS levels upon G-CSF stimulation. The interaction between PrdxIV and G-CSFR is controlled both temporally and spatially. Continuous presence of active PrdxIV at the C-terminus of the G-CSFR drastically attenuates signaling via the G-CSFR. Whether activation of redox sensitive PTPases plays a role in signal attenuation by PrdxIV remains to be investigated. Our results suggest a new mechanism for down regulation of transmembrane receptor-mediated signaling by antioxidants, involving inhibition of ROS production within the G-CSFR signaling microdomain formed in the early endosomes.

0939

SMALL MOLECULE XIAP INHIBITORS PRIME CHILDHOOD ACUTE LEUKEMIA CELLS FOR TRAIL-INDUCED APOPTOSIS AND OVERCOME BCL-2-MEDIATED RESISTANCE

S. Fulda,[1] M. Fakler,[1] S. Löder,[1] M. Vogler,[1] I. Jeremias,[2] K.M. Debatin[1]

[1]University Children's Hospital, ULM; [2]GSF, MUNICH, Germany

Defects in apoptosis signaling contribute to poor outcome in pediatric acute lymphoblastic leukemia (ALL) calling for novel strategies that counter apoptosis resistance. Here, we demonstrate for the first time that small molecule inhibitors of the antiapoptotic protein XIAP prime childhood acute leukemia cells for TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis. XIAP inhibitors at subtoxic concentrations, but not a structurally related control compound, synergize with TRAIL to trigger apoptosis and to inhibit clonogenic survival of acute leukemia cells. In contrast, they do not affect viability of normal peripheral blood mononuclear cells, suggesting some tumor selectivity. Analysis of signaling pathways reveals that XIAP inhibitors enhance TRAIL-induced activation of caspases, loss of mitochondrial membrane potential and cytochrome c release in a caspase-dependent manner, indicating that they promote a caspase-dependent feedback mitochondrial amplification loop. Intriguingly, XIAP inhibitors even overcome Bcl-2-mediated resistance to TRAIL by switching type II leukemia cells that depend on the mitochondrial contribution to the death receptor pathway to type I cells in which TRAIL-induced apoptosis proceeds irrespective of high Bcl-2 levels. Most importantly, XIAP inhibitors sensitize leukemic blasts from children with ALL for TRAIL-induced apoptosis and also kill ALL cells as single agents. Thus, targeting XIAP by small molecule inhibitors presents a promising novel approach to increase the antitumor activity of TRAIL in childhood acute leukemia, which warrants further investigation.

Acute myeloid leukemia - Biology II

0940

A KIT-INDEPENDENT ONCOGENIC PATHWAY IN NEOPLASTIC MAST CELLS THAT INVOLVES BTK AND IS DISRUPTED BY THE KIT/BTK-TARGETING DRUG DASATINIB

K.V. Gleixner,[1,2] M. Mayerhofer,[1] U. Rix,[3] G. Hörmann,[2] A. Gruze,[4] M.T. Krauth,[2] W.F. Pickl,[4] C. Sillaber,[2] G. Superti-Furga,[3] P. Valent[2]

[1]Medical University of Vienna, VIENNA; [2]Department of Internal Medicine I, Division of Hematology & Hemostaseology, VIENNA; [3]Center for Molecular Medicine of the Austrian Academy of Sciences, VIENNA; [4]Institute of Immunology, Medical University of Vienna, VIENNA, Austria

Systemic mastocytosis (SM) is a myeloid neoplasm characterized by an accumulation of neoplastic mast cells (MC) in internal organs. Aggressive SM (ASM) and MC leukemia (MCL) are advanced disease variants that usually are drug-resistant and have an unfavorable prognosis. In most SM patients, the D816V-mutated ´oncogenic´ variant of KIT is detectable. However, the mutant is also detectable in patients with indolent SM exhibiting a normal life-expectancy, and therefore is not considered to represent a fully transforming oncoprotein. This assumption is also supported by studies in Ba/F3 cells with doxycycline-induced expression of KIT D816V. More recently, it has been hypothesized that in addition to KIT, other pro-oncogenic molecules and signaling pathways may play a role in malignant transformation/progression in SM. We here describe a novel KIT D816V-independent oncogenic pathway in neoplastic MC that involves Lyn and Bruton´s tyrosine kinase (Btk). Western blotting and immunostaining revealed that neoplastic MC display the Btk- and Lyn protein. Both molecules were found to be constitutively phosphorylated in primary neoplastic MC and in the MC leukemia cell line HMC-1. Lyn/Btk-activation was not only detectable in KIT D816V-positive HMC-1.2 cells, but also in the KIT D816V-negative HMC-1.1 subclone. In studies employing Ba/F3 cells with doxycycline-inducible expression of KIT, we were able to show that KIT D816V induces activation of STAT5 and Akt, but does not induce activation of Btk. Correspondingly, pharmacologic deactivation/dephosphorylation of KIT in HMC-1 cells by midostaurin (PKC412) (Novartis, Basel, Switzerland) was not accompanied by a decrease in phosphorylation of Lyn or Btk. To study the function of Btk in neoplastic MC, a Btk-specific siRNA was applied. This Btk siRNA was found to reduce the proliferation and survival in HMC-1 cells, and to cooperate with midostaurin in producing growth inhibition. In consecutive experiments, we identified the Src/Abl kinase-targeting drug dasatinib (BMS, Princeton, NJ) as a potent inhibitor of Lyn/Btk activation in neoplastic MC. In particular, dasatinib (1 µM) was found to block Lyn and Btk activity in HMC-1.1 cells as well as in HMC-1.2 cells, and corresponding results were obtained with primary neoplastic MC. Finally, as assessed by a chemical proteomics approach, we were able to show that dasatinib directly binds to Btk and Lyn in neoplastic MC. In summary, our data show that a KIT-independent Lyn/Btk-driven signaling pathway contributes to growth and survival of neoplastic MC, and possibly to disease progression in SM. We also show that dasatinib as a potent inhibitor of the Lyn/Btk pathway, which may have clinical implications and may explain some of the synergistic effects obtained with combinations of dasatinib and other KIT-targeting drugs in neoplastic MC

0941

INSERTION OF FLT3 INTERNAL TANDEM DUPLICATION IN THE BETA-1-SHEET OF THE TYROSINE KINASE DOMAIN-1 IS ASSOCIATED WITH CHEMO-RESISTANCE AND INFERIOR CLINICAL OUTCOME

S. Kayser,[1] R.F. Schlenk,[1] F. Breitenbücher,[2] M. Porebski,[1] K. Wittke,[1] J. Du,[1] S. Groner,[1] D. Späth,[1] J. Krauter,[3] A. Ganser,[3] H. Döhner,[1] T. Fischer,[2] K. Döhner[1]

[1]University of Ulm, ULM; [2]University of Mainz, MAINZ; [3]Hannover Medical School, HANNOVER, Germany

Background. Activating FLT3 internal tandem duplication mutations (FLT3-ITDs) occur in approximately 30% of acute myeloid leukemia (AML) patients. Expression of an FLT3-ITD receptor results in autophosphorylation of FLT3 and subsequent activation of downstream signaling. Clinically, FLT3-ITDs are associated with a worse clinical outcome; previous explorative analyses suggest that not only FLT3-ITD per se but

also the mutant/wild-type allelic ratio and/or the length of the FLT3-ITD provide prognostic information. *Aims.* To determine ITD insertion sites and length in FLT3-ITD mutated AML and to correlate these findings with clinical outcome. *Methods.* In 226 patients, DNA-based amplification of the FLT3-ITD mutation was followed by DHPLC-based separation of FLT3 mutant and wild-type fragments. Single mutated fragments were collected by a fragment collector, reamplified and sequenced. Patients [16 to 60 years of age] were entered on 3 consecutive AMLSG treatment trials [AML HD93, AML HD98A, AMLSG 07-04] all including intensive therapy. *Results.* Thirty-three (14.6%) of the 226 patients had more than one ITD (two ITDs n=28, three ITDs n=3, four ITDs n=2). In total, 266 ITDs were analyzed with 177 being simple duplications (66.5%). The median length was 45 nucleotides (range 15-156). For further correlations we grouped ITD integration sites according to the functional regions of FLT3: JM-domain (JMD) [amino acid (AA) 572-603, patients n =133, ITDs n=140], hinge region of JMD [AA 604-609, patients n=42, ITDs n=45], beta-1-sheet of the tyrosine kinase domain-1 (TKD1) [AA 610-615, patients n=65, ITDs n=69], and the remaining region of TKD1 [AA >615, patients n=12, ITDs n=12]. Interestingly, ITD length was strongly correlated to functional regions with shortest ITDs being present in the JMD and longest in the TKD1 (p<0.001). Clinical data were available in 222 patients showing no differences in patient characteristics (age, WBC etc.); frequencies of cooperating class II mutations (NPM1-mut n=130, CEBPA-mut n=12, MLL-PTD n=17) were equally distributed among the functionally categorized groups. The logistic regression model on induction success (IS) revealed ITD integration sites in the beta-1-sheet (OR 0.43, 95%-CI 0.20-0.91) and logarithm of WBC count (OR 0.32, 95%-CI 0.16-0.65) as significant unfavorable variables. ITD insertion in the beta-1-sheet was also significant in the Cox regression analysis on overall survival (OS) (OR 1.5, 95%-CI 1.0-2.2). In univariable analyses, event free survival (EFS) and OS were significantly inferior in patients with ITD in the beta-1-sheet (p=0.006 and p=0.006). Of note, the proportions of patients receiving an allogeneic transplantation were comparable in both groups (55% and 53%, respectively). In multivariable analyses, neither length of ITD nor mutant/wild-type allelic ratio had an impact on IS and OS. *Conclusions*. FLT3-ITD insertion sites located in the beta-1-sheet of the TKD1 seems to be highly associated with induction failure and inferior EFS and OS. Therefore, not only FLT3-ITD mutation status but also ITD integration site should be prospectively analyzed in future clinical trials, in particular in the context of treatment with FLT3-tyrosine kinase inhibitors.

0942

AUTOCRINE IGF-1/IGF-1R SIGNALING IS RESPONSIBLE FOR PI3K/AKT CONSTITUTIVE ACTIVATION IN PRIMARY AML BLASTS

J. Tamburini,[1] N. Chapuis,[1] V. Bardet,[1] L. Willems,[1] S. Park,[1] A. Green,[1] P. Cornillet-Lefebvre,[2] P. Mayeux,[1] C. Lacombe,[1] D. Bouscary[1]

[1]Institut Cochin, PARIS; [2]Centre Hospitalo-Universitaire, REIMS, France

Background. The PI3K/Akt axis is involved in Acute Myeloid Leukemia (AML) blast cell proliferation. PI3K is activated downstream membrane receptors with tyrosine kinase activity (RTK), including Insulin-like Growth Factor 1 Receptor (IGF-1R), and is responsible for Akt activation. We previously reported that 50% of AML patients had constitutive Akt (S473) activating phosphorylation ($PI3K^+$ patients). Furthermore, we demonstrated that IGF-1R activates PI3K/Akt following mTORC1 inhibition through an IGF-1 autocrine loop. Patients, materials and *Methods.* Bone marrow samples were obtained from 10 newly diagnosed AML patients (all treated in the AML2001 chemotherapy trial from the GOELAMS). All samples had constitutive PI3K activity, detected after 4 hours of cytokine and serum starvation, by Western blot using anti-phospho-Akt (S473) antibody. *Results.* We first demonstrated that AML blasts expressed IGF-1R (using IGF-1R immunoprecipitation and Western Blot); and that IGF-1R was functional, as IGF-1 stimulation induced Akt (S473) phosphorylation and IGF-1R (Y1135/1136) phosphorylation. Furthermore, IGF-1 autocrine production was detected in all samples using immunofluorescence. An IGF-1R kinase inhibitor, NVP-AEW541, inhibited PI3K signaling at concentrations ranging from 1µM to 5µM. However, this inhibition was not specific of IGF-1R, as NVP-AEW541 fully reversed SCF- and FLT-3-induced Akt (S473) phosphorylation. Therefore, we used alpha-IR3, a mouse monoclonal antibody directed against the alpha-subunit of IGF-1R, to specifically inhibit IGF-1R signaling. We confirmed that alpha-IR3 inhibited both basal and IGF-1 induced IGF-1R (Y1135/1136) phosphorylation, and that alpha-IR3 did not reverse SCF- and FLT-3-induced Akt (S473) phosphorylation. In 7/10 $PI3K^+$ AML samples tested, alpha-IR3 fully inhibited Akt activation (decrease of 88% [±6%] of P-Akt S473 signal intensity), without affecting mTORC1 and ERK pathways. We then confirmed that alpha-IR3 inhibited IGF-1R signaling dependent on IGF-1 autocrine production, as alpha-IR3 did not reduce constitutive Akt phosphorylation in MOLM-14 AML cells that does not produce IGF-1. *Conclusions.* We demonstrate that constitutive PI3K/Akt activation is due to an IGF-1/IGF-1R autocrine loop in at least 70% of AML whereas PI3K/Akt constitutive activation may be driven by other RTKs in the remaining 30% of patients. IGF-1R mediated PI3K/Akt constitutive activation may represent a major determinant of cell proliferation in PI3K+ patients, and IGF-1R inhibition should be considered in clinical developments in this AML sub-group.

0943

PROGNOSTIC IMPACT OF WT1 MUTATIONS IN THE CONTEXT OF OTHER MOLECULAR MARKERS IN CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA (AML): A STUDY OF THE GERMAN-AUSTRIAN AML STUDY GROUP (AMLSG)

V.I. Gaidzik,[1] S. Moschny,[1] A. Becker,[1] S. Groner,[1] D. Spaeth,[1] J. Krauter,[2] B. Schlegelberger,[3] A. Ganser,[2] H. Doehner,[1] R.F. Schlenk,[1] K. Doehner[1]

[1]University Hospital of Ulm, ULM; [2]Hematology, Haemostaseology and Oncology, Hannover Medical School, 30625 HANNOVER; [3]Inst. of Cell and Molecular Pathology, Hannover Medical School, 30625 HANNOVER, Germany

Background. Recently, mutations in the Wilms' tumor 1 gene (WT1) have been detected in about 10% to 15% of cytogenetically normal (CN) acute myeloid leukemia (AML) and several studies are currently investigating the prognostic impact of WT1 mutations in this cytogenetic subgroup. *Aims.* To evaluate the incidence and the prognostic relevance of WT1 mutations in CN-AML in the context of NPM1, FLT3-ITD (internal tandem duplication)/TKD (tyrosine kinase domain mutations at codon 835), CEBPA, MLL-PTD (partial tandem duplication) and NRAS gene mutations. *Methods.* Bone marrow or peripheral blood samples from 602 younger adult patients (pts) (16 to 60 years) with CN-AML (n=482 *de novo*, n=66 secondary, n=13 therapy-related, n=41 not applicable) who had been entered on three consecutive AMLSG treatment trials (AML HD93, AML HD98A, AMLSG 07-04) were studied. WT1 gene mutation screening was performed using standard PCR-based direct sequencing of exons 1 to 10; mutation status of the other AML-associated genes was performed as previously described. *Results.* WT1 mutations (WT1mut) were identified in 72/602 (12%) CN-AMLs, predominantly clustering in exon 7 (51/72) and exon 9 (12/72), but also occurring in exons 1 (1/72), 2 (3/72), 3 (3/72), and 8 (4/72). Most of them were frameshift mutations as a result of insertions or deletions; 64 pts had heterozygous and 8 pts had homozygous mutations; two patients exhibited two mutations. Patients with the WT1mut genotype were younger (p=0.009), had higher LDH serum levels (p=0.01) and higher blood blast counts (p=0.009) at diagnosis. Correlation of WT1mut to the FLT3-ITD/TKD, NPM1, CEBPA, MLL-PTD and NRAS mutation status revealed mutant WT1 to be significantly associated with FLT3-ITD (p=0.0008) and CEBPAmut (p=0.001). In a multivariable logistic regression model on induction success, the genotype WT1mut was not significant (p=0.6). In subset analysis according to the FLT3-ITD mutation status, the combined WT1mut/FLT3-ITDpos genotype had a fairly low complete remission (CR) rate (62%) and a high rate of refractory disease (RD) (32%), whereas the WT1mut pts with the genotypes FLT3-ITD-neg/NPM1mut or CEBPAmut had a high CR rate (96%) and no RD. In univariable analyses for overall survival (OS), relapse-free survival (RFS) and event-free survival (EFS), there was no significant difference between the WT1wt and the WT1mut group. Cox proportional hazard models for OS, EFS and RFS also revealed no significant impact for the WT1mut genotype (p=0.8, p=0.5, and p=0.3, respectively). In exploratory subset analyses WT1mut had no impact on RFS in patients with the NPM1mut or CEBPAmut/FLT3-ITDneg genotypes whereas patients with the WT1mut/FLT3-ITDpos genotype had a significantly inferior RFS compared to WT1wt pts (p=0.02). *Conclusions.* In our study on a large series of molecularly well characterized CN-AMLs, WT1 mutations occurred in 12% of the pts and were significantly associated with the genotypes FLT3-ITDpos and CEBPAmut. In both univariable and multivariable analyses WT1 mutations did not impact on the prognosis. However, exploratory subset analyses suggest that the WT1mut/FLT3-ITDpos genotype is associated with a higher rate of RD and inferior RFS.

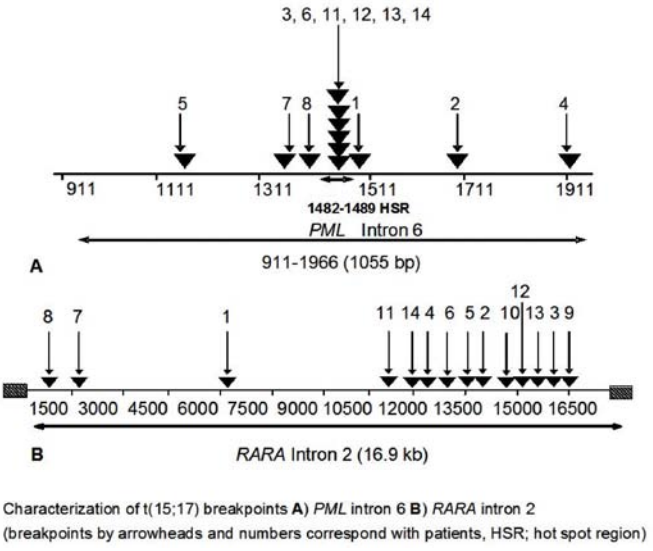
0944

MOLECULAR ANALYSIS OF T(15;17) GENOMIC BREAKPOINTS IN SECONDARY ACUTE PROMYELOCYTIC LEUKEMIA ARISING AFTER TREATMENT OF MULTIPLE SCLEROSIS

S.K. Hasan,[1] A.N. Mays,[2] T. Ottone,[1] A. Ledda,[3] G. La Nasa,[3] C. Cattaneo,[4] E. Borlenghi,[4] L. Melillo,[5] E. Montefusco,[6] J. Cervera,[7] C. Stephen,[8] G. Satchi,[9] A. Lennard,[10] J.A. Byl,[11] N. Osheroff,[11] S. Amadori,[1] C.A. Felix,[12] M.T. Voso,[13] W.R. Sperr,[14] J. Esteve,[15] M.A. Sanz,[7] D. Grimwade,[2] F. Lo-Coco[1]

[1]University Tor Vergata, ROME, Italy; [2]King's College London, LONDON, UK; [3]Ospedale R. Binaghi, CAGLIARI, Italy; [4]Ematologia, Spedali Civili, BRESCIA, Italy; [5]Hospital, S Giovanni Rotondo, ROME, Italy; [6]S. Andrea Hospital, University La Sapienza, ROME, Italy; [7]University Hospital La Fe, VALENCIA, Spain; [8]Pilgrim Hospital, Boston, LINCOLNSHIRE, UK; [9]Whiston Hospital, WHISTON, UK; [10]Royal Victoria Infirmary, NEWCASTLE, UK; [11]Vanderbilt University School of Medicine, NASHVILLE, USA; [12]The Children's Hospital of Philadelphia, PHILADELPHIA, USA; [13]Universita' Cattolica del Sacro Cuore, ROME, Italy; [14]Medical University of Vienna, VIENNA, Austria; [15]Hospital Clínic, Barcelona, BARCELONA, Spain

Background. There is increasing awareness of acute myeloid leukemia, particularly acute promyelocytic leukemia (APL) arising following mitoxantrone treatment of malignant and non-malignant conditions. However, the mechanism by which secondary leukemias with balanced chromosomal translocations such as the t(15;17) in APL develop remains controversial. *Aims.* To further investigate the role of agents targeting topoisomerase II in the development of therapy-related APL we focused on characterizing t(15;17) translocation breakpoints in cases arising in patients without a history of cancer, receiving mitoxantrone as an immunosuppressive agent for multiple sclerosis (MS). *Patients and Methods.* Fourteen patients with APL developing on a background of MS, including 12 exposed to a median of 120 mg mitoxantrone (range 30-234 mg) were studied. The median latency period between first exposure to mitoxantrone and presentation with APL was 28 months (range 4-60 mos). A long-range nested PCR strategy followed by direct sequencing was adopted to identify PML and RARA breakpoints at the DNA level. I*n vitro* functional assays were used to investigate whether observed translocation breakpoints were preferential sites of mitoxantrone-induced topoisomerase II-dependent DNA damage. *Results.* We observed a marked bias in PML breakpoint distribution in sAPL arising following mitoxantrone exposure, with 11 of 12 cases falling within PML intron 6 (bcr1), as compared to 303 of 488 cases of *de novo* APL treated in the PETHEMA trials (p=0.028 Yates corrected chi square). In six cases, the PML breakpoint was found to fall within the *hotspot* region, previously identified in APL cases arising following treatment with this agent for breast cancer and corresponding to a preferential site of mitoxantrone induced DNA cleavage by DNA topoisomerase II at PML nucleotide 1484 (Figure 1A).



Characterization of t(15;17) breakpoints **A)** *PML* intron 6 **B)** *RARA* intron 2 (breakpoints by arrowheads and numbers correspond with patients, HSR; hot spot region)

Figure 1.

With respect to RARA, in the 14 analyzed patients, breakpoints were scattered over the 16.9 kb long intron 2 without clustering in any small restricted region (Figure 1B). However, the breakpoint location in one of the mitoxantrone-related cases was identical to that of a previously reported case with sAPL arising following breast cancer treatment involving the same agent. Analysis of PML and RARA genomic breakpoints in functional assays in 3 cases, including the putative novel *hotspot* in RARA intron 2, confirmed each to be preferential sites of topoisomerase II induced DNA cleavage in the presence of mitoxantrone. Sequencing analyses of the reciprocal RARA-PML fusion revealed a balanced translocation in 8/12 analysed cases. Microhomologies at the breakpoint junctions were indicative of DNA repair by the non-homologous end-joining pathway. *Summary and conclusions.* This study lends further support to the presence of preferential sites of DNA damage induced by mitoxantrone within PML intron 6, and suggests the existence of a further *hotspot* at the distal end of RARA intron 2. The susceptibility of these regions of the PML and RARA loci to topoisomerase II induced cleavage by mitoxantrone may underlie the propensity to develop this particular subtype of AML following exposure to this agent. Further studies are warranted to investigate whether MS patients have a particular predisposition to the development of sAPL.

Platelets and thrombocytopenia

0945

EVALUATING THE LONG-TERM EFFICACY OF ROMIPLOSTIM (AMG 531) IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA (ITP) DURING AN OPEN-LABEL EXTENSION STUDY

C. Newland,[1] M.A. Sanz,[2] E. Bourgeois,[3] C. Grande,[4] P.A.W. te Boekhorst,[5] E. Vellenga,[6] Y. Shi,[7] D. Berger,[7] D.J. Kuter[8]

[1]The Royal London Hospital, LONDON, UK; [2]Hospital La Fe, VALENCIA, Spain; [3]CHRU Claude Huriez, LILLE CEDEX, France; [4]Hospital 12 de Octubre, MADRID, Spain; [5]Erasmus MC, ROTTERDAM, Netherlands; [6]Universitair Medisch Centrum Groningen, GRONINGEN, Netherlands; [7]Amgen Inc., THOUSAND OAKS, USA; [8]Massachusetts General Hospital, BOSTON, USA

Background. Chronic ITP is characterized by low platelet counts due to increased platelet destruction and suboptimal platelet production. Romiplostim is an investigational Fc-peptide fusion protein (peptibody) that increases platelet production by a mechanism similar to that of endogenous TPO. *Aims.* This ongoing open-label extension study is designed to evaluate the long-term safety and efficacy of romiplostim in adult patients with chronic ITP. *Methods.* Eligible patients had completed a prior romiplostim study and had platelet counts ≤50×10^9/L. Patients previously randomized to romiplostim initiated romiplostim at their previous dose unless more than 24 weeks had elapsed since their last dose of romiplostim. These patients and patients previously randomized to placebo initiated romiplostim at 1 µg/kg per week. Romiplostim was administered subcutaneously once weekly with dose adjustments to maintain a platelet count between 50 and 250×10^9/L. *Results.* As of July 13 2007, 143 patients had enrolled and 142 had been treated with romiplostim. Sixty-seven percent were female and 60% had previously undergone a splenectomy. The median duration of treatment was 65 weeks (range 1-156 weeks). The median baseline platelet count was 17×10^9/L (range 1-50×10^9/L). Twenty nine (20%) patients discontinued the study, 10 (7%) due to adverse events. A platelet response (platelet count >50×10^9/L and double baseline) was achieved by 30% (42/138) of patients after the first dose, by 51% (71/138) of patients after the third dose, and by 87% (124/142) of patients overall. Platelet counts were increased from baseline by ≥20×10^9/L more than half of the time in 86% of patients and more than four-fifths of the time in 57% of patients. In an additional ad hoc analysis of response durability, platelet counts >50×10^9/L were maintained for ≥10, ≥25, and ≥52 consecutive weeks in 78% (102/131), 54% (66/122), and 35% (29/84) of patients, respectively. Of patients receiving concurrent ITP medications at baseline, 84% (27/32) discontinued or reduced their dose by >25%. The proportion of patients using rescue medications decreased from 23% (33/142) during Weeks 1-12 to 15% (18/124) during Weeks 24-36, remaining between 12-18% through Weeks 130-132. Adverse events were reported in 95% of patients, with most mild to moderate in severity and transient in duration. The most common were headache (37%), nasopharyngitis (32%), and contusion, fatigue and epistaxis (each 30%). Treatment-related adverse events were reported in 36% (51/142) of patients. Serious adverse events were reported in 31% (44/142) of patients and were considered related to treatment in 9% (13/142) of patients. Bone marrow reticulin was present in samples from 8 patients with no evidence of progression to collagen fibrosis or chronic idiopathic myelofibrosis. Thrombotic/thromboembolic events were reported in 7 (5%) patients; 6 of whom had pre-existing risk factors for thrombosis. Neutralizing antibodies to romiplostim were reported in one patient at study discontinuation and were absent upon retesting 4 months later. *Summary and conclusions.* Romiplostim was well-tolerated in this population of adults with chronic ITP and produced rapid and sustained increases in platelet counts that allowed most to discontinue or reduce concurrent ITP medications.

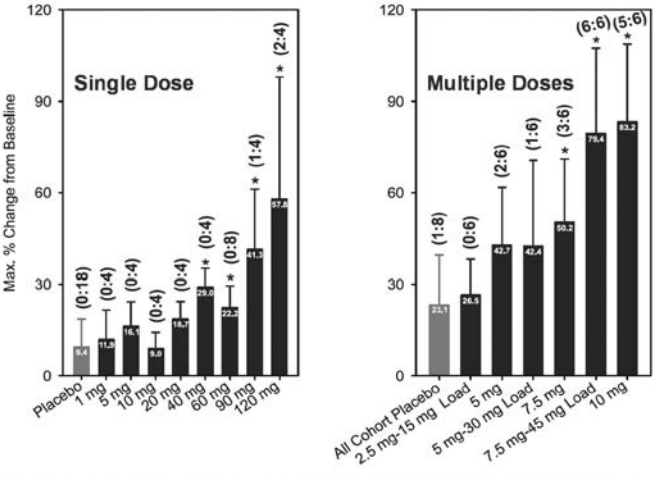
0946

SINGLE AND MULTIPLE ORAL DOSES OF LGD-4665, A SMALL MOLECULE THROMBOPOIETIN RECEPTOR AGONIST, INCREASE PLATELET COUNTS IN HEALTHY MALE SUBJECTS AND DEMONSTRATE POTENTIAL TO TREAT THROMBOCYTOPENIC PATIENTS

E. Dziewanowska,[1] J. Zhang,[1] P. Kapil,[1] G. Greenberg,[1] M. Matsumoto,[1] J. Schindler,[1] J. Berg,[2] B. Newberry[2]

[1]Ligand Pharmaceuticals, LA JOLLA, CALIFORNIA; [2]CEDRA Clinical Research, SAN ANTONIO, USA

Background. LGD-4665, an oral thromobopoietin receptor agonist, is being developed as a new generation small molecule thrombopoietin (TPO) mimetic. It is a highly selective and potent agonist of the TPO receptor and therefore induces differentiation and proliferation of megakaryocytes. The pharmacologic characteristics indicate potential therapeutic use in thrombocytopenic patients with a variety of clinical etiologies. *Aim.* Demonstrate activity, safety, dose responses and optimal dose regimens for single and multiple administration of LGD-4665 to healthy male subjects. *Methods.* Two single-center, randomized, placebo-controlled, double-blind, escalating dose group studies were conducted to assess the pharmacokinetics, pharmacodynamics and safety of LGD-4665 in healthy male subjects after single and multiple doses. The single doses were tested sequentially at 1, 5, 10, 20, 40, 60, 90 and 120 mg levels. In the multiple dose phase subjects received up to 10 mg daily for 14 days, with or without loading doses. Clinical assessments were conducted up to 21 days after last dose. Dose escalation stopped when the mean platelet increase exceeded 50%. All subjects provided informed consent before receiving study drug. *Results.* A dose-proportional increase in systemic exposure was seen in Cmax and AUC after single and multiple doses of LGD-4665. The half-life of LGD-4665 was approximately 90 hours. Following single and multiple dose administration, statistically-significant ($p < 0.05$) percentage increase of platelet count from baseline compared to placebo were observed at the single dose levels ≥ 40 mg (from 29% at 40 mg to 58% at 120 mg) and at multiple dose levels ≥ 7.5 mg daily (up to 83% at 10 mg daily with all subjects responding seen in Figure 1).



*Showing statistical difference comparing to Placebo (2-sided P < 0.05 and adjusted for multiplicity). Numbers between parenthesis indicate number of subjects responded by exceeding 50% increase from baseline over total subject per group.

Figure 1. Platelet counts peak Increase after single and multiple LGD-4665 administration.

The onset of platelet increase occurred within 3 to 4 days when a one-day 45 mg loading dose was followed by 7.5 mg daily. Peak platelet increase was observed between D12 up to D16 in single dose and D18 up to D21 in multiple doses which reflected the long half-life of LGD-4665. These pharmacologic characteristics of LGD-4665 will allow for flexible dosing frequencies as daily or weekly in future clinical trials. The molecule was well tolerated with no serious adverse events or Grade 3/4 AEs observed. The most frequent AEs were upper respiratory infections, gastrointestinal complaints and headaches and were mild, transient and seldom considered LGD-4665 related. There were no clinically significant and LGD-4665-related changes in vital signs, laboratory abnormalities or early withdrawals. *Conclusions.* LGD-4665, a specific and potent oral TPO receptor agonist, increased the platelet counts of normal volunteers in a

dose-dependent manner when administered in either single or multiple once-daily doses. These findings are consistent with its mechanism of action. Pharmacokinetics in humans suggests that it may become either a once-daily or once-weekly oral drug for the treatment of thrombocytopenia of various etiologies. These studies also demonstrated that LGD-4665 was well-tolerated with an excellent safety profile. Multiple phase-II studies in thrombocytopenic patients due to ITP, Hep-C and MDS with daily and weekly dosing regimens, as well as additional Phase-I pharmacology studies in healthy volunteers are underway.

0947

RECRUITMENT OF T-CELLS INTO BONE MARROW OF ITP PATIENTS POSSIBLY DUE TO ELEVATED EXPRESSION VLA-4 AND CX3CR1

B. Olsson,[1] B. Ridell,[2] L. Carlsson,[1] S. Jacobsson,[2] H. Wadenvik[2]

[1]University of Gothenburg, GOTHENBURG, Sweden; [2]Sahlgrenska University Hospital, GOTHENBURG, Sweden

Background. In idiopathic thrombocytopenic purpura (ITP), platelets are destroyed in the spleen, liver and bone marrow by autoantibodies and cytotoxic T-cells. *Aim.* To investigate mRNA and surface expression of genes involved in T-cell homing and to investigate the number of T-cells in bone marrow, an organ involved in platelet destruction. *Material and Methods.* Genes involved in homing of T-cells were investigated by DNA microarray analysis of pooled RNA isolated from T-cells from 5 patients with ITP (plc<50×10^9 cells/L) and 5 controls. Surface expression of proteins involved in activation and homing and the number of regulatory T-cells (CD4$^+$CD25$^+$) were analyzed in peripheral blood and bone marrow from 6 ITP patients and 8 controls by flow cytometry using FACSCanto and the FACSDiva software. The number of T-cells, B-cells and monocytes/macrophages in blood and bone marrow was analyzed by flow cytometry in 5 ITP patients and 6 controls. The number of T-cells in bone marrow of ITP patients was also analyzed in 17 newly diagnosed and untreated ITP patients and 7 healthy controls by immunohistochemistry on bone marrow biopsies.

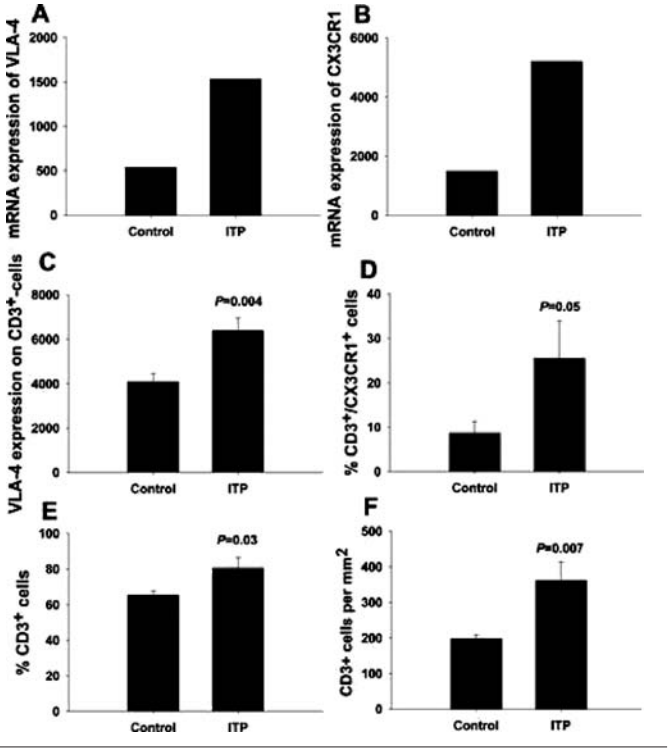


Figure 1. A-B. Microarray expression of VLA-4 and CX3CR1. C. Mean fluorescence intensity of VLA-4 on T-cells by flow cytometry. D. Percent CD3$^+$/CX3CR1$^+$ cells of all T-cells. E. Percent CD3$^+$ cells of lymphocytes in bone marrow. F. The number of CD3$^+$ cells in bone marrow by immunohistochemistry of bone marrow biopsies.

Results. By DNA microarray analysis of peripheral blood T-cells we found that the mRNA expression of the integrin VLA-4 and the chemokine receptors CX3CR1 and CXCR4 was increased in ITP patients compared with controls. In peripheral blood, there was no difference in the surface expression of CX3CR1 or CXCR4 on T-cells. However, the mean fluorescence intensity of VLA-4 on peripheral blood T-cells was increased in ITP compared with controls (p=0.05). Furthermore, in bone marrow, we found an increased percentage of CD3$^+$CX3CR1$^+$ cells (p=0.05) and increased mean fluorescence intensity of VLA-4 on T-cells from ITP patients compared with controls (p=0.004). The surface expression of CXCR4 on T-cells was not different between ITP patients and controls. Furthermore, the surface expression of Fas (p=0.05) and was increased and the number of regulatory T-cells decreased (p=0.004) in T-cells from ITP patients compared with controls. Flow cytometric analysis of T-cells, B-cells and monocytes/macrophages in blood showed that there was no difference in the number of cells between ITP patients and control whereas in bone marrow ITP patients had increased number of T-cells (p=0.03). The increased number of T-cells in bone marrow of ITP patients was confirmed by immunohistochemistry of bone marrow biopsies (p=0.007). *Conclusions.* ITP is associated with accumulation and activation of T-cells in the bone marrow. Recruitment of T-cells into the target organ, e.g. bone marrow, is plausible and may be facilitated through increased VLA-4 and CX3CR1 expression. These molecules might serve as new treatment targets in ITP.

0948

CLINICAL-MOLECULAR PREDICTORS OF THROMBOCYTOPENIA AND RISK OF BLEEDING IN PATIENTS WITH VON WILLEBRAND DISEASE TYPE 2B: A PROSPECTIVE STUDY IN A COHORT OF 67 PATIENTS

A.B. Federici,[1] L. Baronciani,[1] G. Castaman,[2] P.M. Mannucci,[1] M.T. Canciani,[1] P. Bucciarelli,[1] A. Pecci,[3] P.L. Lenting,[4] P.G. De Groot[4]

[1]University of Milan, MILANO, Italy; [2]S. Bortolo Hospital, VICENZA, Italy; [3]University of Pavia, PAVIA, Italy; [4]University of Utrecht, UTRECHT, Netherlands

Background. Type 2B von Willebrand disease (VWD2B) is caused by an abnormal von Willebrand factor (VWF) with increased affinity for the platelet receptor glycoprotein 1balpha. This usually results in moderate-severe thrombocytopenia. Despite the extensive information reported in the literature on VWD2B, no large prospective studies about the role of thrombocytopenia on the clinical outcomes have been performed so far. *Aims, Design of the study, Patients and Methods.* To determine the prevalence and clinical-molecular predictors of thrombocytopenia as well as the risk of bleeding associated with VWD2B we have enrolled 67 VWD2B patients from 38 unrelated families in a 2-year prospective study. At enrolment, patients with phenotypic diagnosis of VWD2B and identified mutation in exon 28 of the VWF gene were also exposed to detailed questionnaire useful to calculate the bleeding severity score (BSS). Platelet counts with mean platelet volume and morphologic evaluations of blood smear were associated with the occurrence of physiological (pregnancy) or pathological (infections, surgeries) stress situations or DDAVP administration. Active VWF was tested in plasma using the AuVWFa11-based immunosorbent assay, which allows quantification of the VWF-GpIbα-binding conformation *in vivo*. Bleeding-free survival according to BSS [<4 (reference), 4-8, >8] and to different platelets levels [≥140×10^9/L (reference), <140×10^9/L] was calculated with the Kaplan-Meier method. *Results.* Thrombocytopenia was found in 20 patients (30%) at baseline and in 38 (57%) after stress situations. These patients carried eight different VWF mutations: H1268D, R1306W, R1308C, I1309V, V1316M, P1337L, R1341Q/W and showed loss of the high molecular weight multimers in plasma. Platelet counts were always normal in 16 (24%) patients from 5 families with P1266L/Q or R1308L mutations and normal multimers in plasma. The activated VWF measured by AuVWFa11 nanobody was higher than normal in all but those 16 cases, with values 2 to 6-fold higher than controls: values >1 correlated always with thrombocytopenia. Patients with lower platelet counts at baseline showed an incidence of bleeding 10 times higher than those with normal platelet counts. Bleeding-free survival calculated with the Kaplan-Meier method was significantly different in the three groups of patients with BSS <4, 4-8 and >8 (log rank test: p=0.003) and in the two groups with platelet counts higher or lower than 140,000/uL (log rank test: p<0.0001). The adjusted hazard ratio (HR, 95% CI) was about four times higher in patient who had BSS >8 [HR=3.78 (1.00-14.79)] and platelet counts <140,000/uL [HR=3.65 (1.53-8.70)], compared to the reference group. *Conclusions*. Not all VWD2B patients show thrombocytopenia at baseline and platelet counts can decrease only after physiological and pathological stress situations. There is a group of VWD2B who never show low platelet counts. Activated VWF as tested by AuVWFa11 is useful to predict thrombocytopenia in VWD2B: this is clinically important because patients with low platelet counts show greater risk of bleeding.

0949

LONG-TERM SAFETY AND EFFICACY OF ORAL ELTROMBOPAG FOR THE TREATMENT OF IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

J.B. Bussel,[1] G. Cheng,[2] M.N. Saleh,[3] B. Meddeb,[4] N. Stone,[5] B. Mayer,[5] M. Arning,[5] M. Aivado[5]

[1]Weill-Cornell Medical College of Cornell University, NEW YORK, NY, USA; [2]Prince of Wales Hospital, SHATIN, NT, Hong Kong; [3]Georgia Cancer Specialists, ATLANTA, GA, USA; [4]Hopital La Rabta, TUNIS, Tunisia; [5]GlaxoSmithKline, COLLEGEVILLE, PA, USA

Background. Eltrombopag is a new oral non-peptide thrombopoietin receptor agonist for the treatment of thrombocytopenia. In two 6-week, placebo-controlled studies of adult patients with chronic ITP, eltrombopag produced a substantial dose-dependent increase in platelet counts and reduction in bleeding. EXTEND is an ongoing study assessing the long-term safety and efficacy of oral eltrombopag. *Aims*. This trial was conducted to evaluate the long-term safety and efficacy of eltrombopag treatment in adult patients with chronic ITP. *Methods.* Patients with previously treated, chronic ITP who completed a prior eltrombopag study were eligible for this open-label, phase III extension study. Eltrombopag was initiated at 50 mg daily and then adjusted according to the platelet count with doses between 25 and 75 mg daily (or less frequently if necessary). Written, informed consent was obtained from all study participants. *Results*. To date, 109 patients (median age, 47 years; 64% female) received eltrombopag. At baseline, 37% were receiving concomitant ITP medication, 44% were splenectomized, 70% had platelet counts <30K/μL, 17% had 30K-≤50K/μL, and 14% had >50K/μL. Median duration in the study was 194 days (range 2-387 days). After starting eltrombopag treatment, 80% of patients achieved platelet counts >50K/μL at least once and 78% of these patients maintained platelets >50K/μL for >50% of their time in the study. A similar response to eltrombopag was observed regardless of whether patients were taking concomitant ITP medication at baseline or had been splenectomized. There was no evidence for loss of efficacy of eltrombopag during the study. 85 patients (78%) reported at least one AE on-therapy; the majority of which were mild to moderate. Headache (17%) was the most commonly reported AE. There were 2 deaths unrelated to eltrombopag and 18 grade 3/4 AEs. Four patients experienced 4 thromboembolic events (2 pulmonary embolism, 1 transient ischemic attack, 1 deep vein thrombosis). The platelet counts at the time of these events ranged from 27K-407K/μL. All 4 patients had risk factors for the development of thromboembolic events. *Conclusions*. Oral eltrombopag appears to be effective and well tolerated for long-term treatment in patients with previously treated chronic ITP.

Stem cell biology and microenvironment

0950

MICRORNA-223 INHIBITS E2F1 DURING GRANULOPOEIS AND IS DOWNREGULATED IN AML

J.A. Pulikkan,[1] V. Dengler,[1] A. Peerzada,[1] C. Müller Tidow,[2] S. Buhlander,[3] D. G Tenen,[4] G. Behre[1]

[1]LZG; AG BEHRE, HALLE, Germany; [2]University of Munster, MÜNSTER, Germany; [3]KLINIKUM GROSSHADER; LMU MUNICH, MUNICH, Germany; [4]Harvard Institutes of Medicine, BOSTON, USA

Recent studies show the significance of microRNAs in regulating gene expression. Several studies have shown the deregulation of microRNAs is associated with the development of many cancers including leukemia. MicroRNA-223 has been shown to be upregulated during granulopoiesis and to be regulated by the transcription factor CCAAT enhancer binding protein α (C/EBPα). In this study we show that C/EBPα induces microRNA-223 during granulopoiesis. Recent studies suggest that loss of function or expression of C/EBPα provides a platform on which acute myeloid leukemia (AML) develops. Based on these findings, we hypothesized that miR-223 could be downregulated in human AML. Our preliminary data show that miR-223 is downregulated in different subtypes of AML. We asked what are the critical targets of miR-223 during granulopoiesis. Computational analysis shows that E2F1 could be a putative target of miR-223. By luciferase assay using 3' UTR of E2F1, we confirmed that E2F1 is a potential target of miR-223. Our data shows that microRNA-223 downregulates E2F1 by translational repression as revealed by downregulation of E2F1 protein level after overexpressing microRNA-223. Silencing of microRNA-223 leads to upregulation of E2F1 protein level as analyzed by Western blot analysis. It has been shown that E2F1 is able to block granulocytic differentiation and E2F1 inhibition by C/EBPα is necessary for granulopoiesis. Recent studies suggest that disruption of E2F1 inhibition leads to leukemia, pointing out that E2F1 inhibition is a critical step in granulopoiesis. Our findings demonstrate that inhibition of E2F1 by miR-223 as a critical event during granulopoiesis.

0951

EARLY ROLE OF THE NON-CANONICAL NF-KB PATHWAY ON THE MYELOID DIFFERENTIATION OF CD34+ HEMATOPOIETIC STEM/ PROGENITOR CELLS

G. Andreotti De Molfetta

University of Sao Paulo, RIBEIRAO PRETO - SP, Brazil

Background. Efforts to understand the molecular mechanisms underlying lineage-specific hematopoietic differentiation into mature blood cells have focused mainly on late events that largely reflect the differentiated state of the cell. *Aims.* To better understand the early events of the gene expression that drive the myeloid differentiation process, transcriptional profiles of $CD34^+$ HSPC were generated before and after short culture in conditions that stimulate either myeloid or erythroid differentiation. *Methods.* Four independent samples of immune-magnetically sorted CD34 cells from adult bone marrow (BM) were pooled and submitted to each treatment: 12 or 40-hour culture with supplemented media favoring either myeloid or erythroid commitment. Cells aliquots obtained before and after the experiments were then subjected to RNA extraction and to methylcellulose cultures. *Results.* $CD34^+$ HSPC without previous stimulation generated about equal percentages of CFU-GM and BFU-E colonies, whereas upon erythroid stimulation the median percentages CFU-GM, BFU-E and CFU-Mix were, respectively, 38%, 61% and 1% for the 12h treatment and 17%, 83% and 0% for 40h treatment. Conversely, upon myeloid stimulation, respectively, 61%, 37% and 2% colonies were observed after 12h and 63%, 23% and 14% for 40h treatment. These results indicate that after 12 and 40 hours of treatment, the genetic program of those cells were shifted towards the desired phenotype. Serial analysis of gene expression (SAGE) was employed to generate five independent libraries. By comparing the differentially regulated transcripts between the control $CD34^+$ HSPC and the stimulated cells, we observed a set of genes that were initially up-regulated at 12h then down-regulated at 40h, exclusively after myeloid stimulus. Among those we found transcripts for NFKB2, RELB, IL1B, LTB, LTBR, TNFRSF4, TNF, TGFB1, and IKBKA. Also, the inhibitor NFKBIA (IKBA) was more expressed at 12h. All these transcripts code for signaling proteins of the NF-kB pathway. NFKB2 is a subunit of the NF-kB transcription factor that

together with RELB mediates the non-canonical NF-kB pathway. The up-regulation followed by a down-regulation indicates that the NF-kB pathway could be involved in the early commitment of CD34+ HSPC towards the myeloid lineage. To test this hypothesis, interference RNA against NFKB2 and control iRNA were transfected into BM CD34+ HSPC. Cells submitted to transfection with iRNA were stimulated towards the myeloid lineage and subjected to evaluation on methylcellulose cultures and qPCR. After inhibition of NFKB2, the percentage of CFU-GM and BFU-E colonies shifted from 40% and 57% for negative control cells, respectively, to 23% and 77% on siNFKB2 cells, a change that was accompanied by a reduction of the size of the CFU-GM colonies. *Conclusions.* The results are compatible with the coexistence of two levels of control driving the early myeloid differentiation: inhibition of the canonical NFKB pathway and a subsequently activation of the non-canonical NFKB pathway. Inhibition of the canonical pathway may be controlled by IKB proteins via NKFBIA binding to NFKB1-RelA complex. The activation of the non-canonical pathway, in turn, would occur in response to binding of LTB-LTBR or TNF-members, and IKK action via IKBKA protein. Financial support: FAPESP, CNPq, FINEP.

0952

BONE MARROW STROMA CELLS REGULATE TIEG1 EXPRESSION IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS; ROLE OF TGF-BETA SUPERFAMILY MEMBERS AND TIEG1 IN CHEMOTHERAPY ESCAPE

E. Munthe, G. Døsen-Dahl, M.K. Nygren, H. Stubberud, E. Rian

Rikshospitalet University Hospital, OSLO, Norway

Background. The bone marrow microenvironment regulates early B lymphopoiesis and protects leukemia cells against chemotherapy treatment. The microenvironment may thus serve as a sanctuary site for leukemic cells. Information regarding the factors that contribute to this process is sparse. *Aims.* Members of the TGF-beta superfamily are central regulators of cell fate and homeostasis, and participate in cell-cell communication in the bone marrow. We therefore explored the roles of TGF-beta and BMP-6 and the TGF-beta target gene, TIEG1 (KLF10), in the communication between stroma cells and acute lymphoblastic leukemia (ALL) cells and their escape from chemotherapy. *Methods.* TIEG1 mRNA levels in progenitor B cells from healthy donors and pre-B ALL cell lines were measured by real-time PCR. Proliferation was measured by thymidine incorporation and cell survival by PI exclusion. Overexpression or knockdown of TIEG1 by siRNA was achieved by transient transfection. Pre-B ALL cell lines were cocultured with immortalized mesenchymal stem cells (MSC-tert3) and the effect on proliferation and cell survival was measured. AraC was used to induce cell death. *Results.* We demonstrated TIEG1 mRNA in BM early B lymphocytes and pro-B cells, but expression dropped strongly at the pro- to pre-B transition. Moreover, we found that TIEG1 mRNA levels increased rapidly and transiently upon TGF-beta stimulation of pre-B ALL cells. Stimulation of pre-B ALL cells with TGF-beta or BMP-6, as well as overexpression of TIEG1 inhibited proliferation, while siRNA knockdown of TIEG1 increased the proliferation. Furthermore, interaction with stroma cells induced TIEG1 expression in pre-B ALL cells, inhibited their proliferation and protected the cells against chemotherapeutic treatment. Similarly, treatment with TGF-beta and BMP-6, as well as overexpression of TIEG1, protected ALL cells against chemotherapy induced cell death. *Conclusions.* These data suggest that TGF-beta and BMP-6 in the bone marrow microenvironment allow leukemia cells to escape therapy. Further, the data indicate that TIEG1 is involved in mediating this effect from the microenvironment onto the leukemia cells.

0953

EXPRESSION OF CEBP≥ DEFINES A DISTINCT CELL POPULATION WITHIN THE MURINE HEMATOPOIETIC STEM CELL AND MULTIPOTENT PROGENITOR COMPARTMENT

A. Wolfler, A.A. Danen-van Oorschot, M. Valkhof, P. van Strien, E.J. Rombouts, I.P. Touw

Erasmus Medical Center, ROTTERDAM, Netherlands

Background. Transcription factors control lineage commitment and differentiation in hematopoietic stem and progenitor cells. They are expressed in a cell-type-restricted pattern and activate lineage-specific genetic programs. However, contrary to earlier assumptions, it was recently shown that hematopoietic stem cells (HSCs) and multipotential progenitor cells (MPPs), also express lineage-specific transcription factors, mostly at low levels. Among these is the gene encoding CEBPα, which instructs granulocytic lineage commitment. It was suggested that the priming of genes affiliated with lineages in HSCs and MPPs would afford flexibility in cell fate decisions and a rapid response to environmental cues. However, an alternative possibility is that definitive lineage specification may already take place in the early hematopoietic compartment of Lin-Sca-1+c-Kit+ (LSK) cells encompassing HSCs and MPPs. A suitable model to address this question was not yet available. *Methods and Aims.* We generated a knock-in mouse model expressing cre recombinase under the regulation of the cebpa promoter and crossed these CEBPαcre/+ mice with R26 YFP reporter mice. This model enables us 1. to trace cebpa-driven Cre expression in single LSK cells and their ancestry by FACS and 2. to flow sort these cells to delineate a possible lineage-instructive function of CEBPα in the LSK compartment. *Results*. Using immunofluorescence analysis of cryosections from various organs of CEBPαcre/+ R26 YFP mice, we found that cebpa-driven Cre expression was grossly limited to the bone marrow, liver and lungs. This is consistent with the pattern described for cebpa, substantiating the validity of the model. FACS analysis of bone marrow cells of 8- to 10-week old mice revealed that the vast majority of granulocytes was positive for YFP (88%; range 85%-92%). Intriguingly, 21% (17-24%) of erythroid cells, 8% (7-10%) of T-cells and 4% (2-7%) of B-cells were also positive for YFP. Because cebpa expression is not found in these differentiated cell stages, these results are indicative of cebpa expression in earlier common progenitors or even HSCs. Indeed, within the LSK population we detected 33% (21-42%) of cells positive for YFP. Within the LSK compartment, the percentage of YFP-positive cells was higher among the CD34+ than the CD34--fraction (45% vs. 13%). In the myeloid progenitor compartment, we found an increase of YFP positive cells during the transition from common myeloid progenitors (35%) towards granulocyte/monocyte progenitors (>90%), which is consistent with the indispensable role of CEBPα during myeloid differentiation. In contrast, megakaryocyte/erythroid progenitors displayed a low percentage of cells with YFP expression (17%). *Summary.* Using a newly developed CEBPαcre mouse model, we found that 33% of LSK cells express cepba. Because a much lower fraction of lymphoid cells and erythroid cells is derived from cepba-Cre expressing cells, these results suggest that CEBPα directs lineage restriction already within the LSK population. Studies to reveal functional differences between CEBPα positive and negative LSK cells are currently performed. By crossing them with relevant floxed strains, the CEBPαcre mice also serve as a useful tool to study the consequences of gene induction or inactivation at an early stage of myeloid cell commitment.

Novel therapies, drug resistance and pharmacology

0954

MODULATING NOTCH1 WITH SIGNATURE-BASED SMALL MOLECULE LIBRARY SCREENING

G. Roti,[1] K. Ross,[2] C. Mecucci,[3] J. Aster,[4] K. Stegmaier[5]

[1]Dana Farber Cancer Insitute, BOSTON, USA; [2]The Broad Institute of Harvard University and Massachusetts Institute of Technol, CAMBRIDGE, USA; [3]Hematology, IBIT Foundation, Universita' degli Studi di Perugia, PERUGIA, Italy; [4]Department of Pathology, Brigham and Women's Hospital, BOSTON, USA; [5]Dana Farber Cancer Institute and Broad Institute, BOSTON, USA

Background. Transcription factor abnormalities are common in the acute leukemias, but with rare exceptions it has not been possible to specifically target this protein class. It has been difficult to identify modulators of transcription factor activity with traditional pharmacological approaches. We developed a new approach to address this challenge, gene expression-based high-throughput screening (GE-HTS), which uses gene expression signatures as surrogates for mutant protein activity. In this study, we apply GE-HTS to identify modulators of Notch1. Notch1 encodes a trans-membrane receptor transcription factor, activated by gamma-secretase, that regulates normal T cell development. Notch1 activating mutations are present in over 50% of T cell acute lymphoblastic leukemia (T-ALL). Initial of trials of gamma-secretase inhibitors (GSI) in T-ALL were halted due to toxicity, indicating that new ways of modulating Notch1 activity are needed. *Aims.* 1) Define a gene expression signature for Notch on versus off. 2) Adapt the Notch signature to the GE-HTS assay. 3) Perform GE-HTS for compounds inducing the Notch1 off signature. *Methods and Results.* A gene expression signature for the two different states of interest, Notch1 on versus off, was defined with microarray expression profiling of 7 different Notch1 mutant T-ALL cell lines treated in duplicate with vehicle versus a GSI, compound E. From a set of ~500 genes with differences of $p<0.01$ by 2-sided Student's t-test, 32 genes were selected to define the Notch1 off signature based on mean fold changes >1.5 between the Notch on versus off state. We next adapted this signature to our GE-HTS assay, which uses ligation-mediated amplification (LMA) and a Luminex bead-based detection system. Briefly, the 32-genes are amplified by LMA, yielding biotinylated PCR products containing molecular barcode sequences. These PCR products are hybridized in solution to beads dyed with unique fluorescent colors containing complementary barcode sequence. Following hybridization and staining with streptavidin-phycoerythrin (PE), the beads are analyzed by dual-color flow cytometry, in which the bead color identifies the gene of interest and PE intensity the quantity of transcript. We tested the 32-gene signature in the cell lines DND41, KOPTK1, and HPB ALL treated with vehicle versus Compound E at multiple time points. The 32-gene signature reproducibly distinguished vehicle versus GSI treated cells at 72-hours, with DND41 yielding the most robust distinctions. We also confirmed that shRNA knockdown of Notch1 in DND41 cells recapitulates the Notch off signature. We next addressed whether the Notch off 32-gene signature is specific for loss of Notch or merely a marker of cell growth inhibition. We treated DND41 cells with known T-ALL cytotoxics and measured the effects on the 32-gene signature. The majority of cytotoxics did not induce the Notch1 off signature. Screening of a 4500 small molecule collection of bioactives to identify novel compounds inducing the Notch1 off signature is now in progress. *Summary.* Therapeutically targeting transcription factors has been a challenge in the treatment of leukemia. GE-HTS may complement traditional screening approaches as one solution to this challenge. Our preliminary data suggests that GE-HTS is a feasible approach to identifying new modulators of mutant Notch1.

0955

SGI-1252: A POTENT SMALL MOLECULE JAK2 INHIBITOR

L. Warner,[1] E. Gourley,[1] X.H. Liu,[1] C. Olsen,[1] C. Parker,[2] J. Prchal,[2] H. Vankayalapati,[1] D. Bearss[1]

[1]SuperGen, Inc., SALT LAKE CITY; [2]University of Utah, SALT LAKE CITY, USA

Background. JAK2 is an intracellular protein tyrosine kinase whose dysregulation has been implicated in leukemia, lymphoma, and myeloproliferative disorders (MPD). Increased kinase activity of JAK2, caused by point mutation of the JH2 autoinhibitory region or formation of JAK2 fusion proteins, causes increased activation of downstream signaling pathways affecting cell differentiation, proliferation, migration, and apoptosis. *Aims.* Develop a potent and selective, orally available JAK2 inhibitor. *Methods.* Through the use of CLIMBTM, our proprietary drug discovery process, the published JAK2 crystal structure was used to build several models that were then used as a substrate for in silico docking of 2.3 million virtual small molecule compounds to generate a subset of leads based on calculated binding energies. These leads were further screened using a number of in silico physicochemical and ADMET prediction algorithms to determine *druggable* leads which were most likely to be successful in a biological context. Optimized analogs were synthesized to produce SGI-1252. *Results*. SGI-1252 exhibits low nanomolar IC50 activity against the JAK2 and JAK2 V617F mutant enzymes, with more than 100-fold selectivity for JAK2 over JAK3. Cancer cell lines expressing either the wild-type or mutant JAK2 enzyme, including HEL, AP-1060, K562, and MV-4-11, demonstrate sensitivity to this inhibitor, resulting in IC50 values in the nanomolar range. Consistent with the inhibition of the JAK2 enzyme, activity of downstream signaling partners are severely decreased. The phosphorylation level of STAT5, a downstream effector of JAK2 signaling, in treated HEL cell lysates was analyzed by western blot. These results showed that SGI-1252 caused an inhibition of STAT5 phosphorylation at an EC50 of 76.2 nM. Another downstream target of JAK2, Bcl-XL, was evaluated for gene expression levels via RT-PCR. In the presence of SGI-1252, Bcl-XL levels were reduced with an EC50 value of 778 nM. SGI-1252 has also been shown to be efficacious in preventing tumor growth *in vivo* in HEL and MV-4-11 mouse xenograft models. Additionally, cell viability of expanded cells from patients with MPD was effectively knocked down in the presence of SGI-1252, with IC50 values in the nanomolar range. SGI-1252 was developed to be orally bioavailable; this has been validated in pharmacokinetic studies. *Summary and Conclusions.* SuperGen's lead selective JAK2 inhibitor, SGI-1252, exhibits potent inhibition of wild-type and mutant JAK2 activity, translating into potent inhibition of cellular signaling pathways and cancer cell proliferation in non-clinical models.

0956

SELECTIVE JAK INHIBITION IS EFFICACIOUS AGAINST MULTIPLE MYELOMA CELLS AND REVERSES THE PROTECTIVE EFFECTS OF CYTOKINE AND STROMAL CELL SUPPORT

S. Fridman, J. Li, E. Caulder, M. Favata, D. Rodgers, P. Combs, C. Newton, R. Redman, M. Friedman, K. Vaddi

Incyte Corporation, WILMINGTON, USA

Background. Tyrosine kinase hyperactivity is common in hematological malignancies. Indeed, Janus kinase (JAK) activation is frequently observed in multiple myeloma (MM) and is associated with tumorigenesis and resistance to therapy. Pathways activated downstream of the JAKs include those with well understood roles in cancer biology such as MAPK, Akt, and the STATs. As such, inhibition of JAKs may be therapeutic for the treatment of various hematological diseases associated with JAK activation, such as MM - a hypothesis we are currently evaluating clinically. *Aims.* We sought to explore the potential of selective JAK inhibition in controlling the growth and survival of MM cells. Further, we hypothesized that pharmacological inhibition of JAKs may revert the resistance afforded by growth of MM cells in the supportive environment of cytokines or bone marrow stromal cells (BMSC). *Methods.* Three structurally unique JAK inhibitors (INCB016562, INCB018424, and JAK Inhibitor I) were utilized to treat a number of MM cell lines (e.g. MM1.S and INA.6) grown under conditions mimicking the supportive natural environment. The impact of JAK inhibition on various signaling pathways, proliferation, survival, and response to chemotherapy was assessed. In addition, human tumor xenograft experiments were conducted to investigate the efficacy and tolerability of chronic oral JAK inhibition. *Results.* INCB016562, INCB018424, and JAK Inhibitor I (Calbiochem®) reduced STAT phosphorylation, proliferation, and survival of the IL-6 dependent INA-6 cells at concentrations < 500 nM. In combination with drugs such as bortezomib these inhibitors reversed the protective effects of BMSC on the growth of MM cells; e.g. 20% growth inhibition with bortezomib alone compared to 80% inhibition when combined with JAK inhibitor. Similarly, the JAK inhibitor INCB018424 improved the therapeutic response to dexamethasone in MM1.S cells when grown in the protective environment of IL-6 or BMSC. In vivo, each of the three JAK inhibitors inhibited the growth of INA-6.Tu1 xenografts and induced tumor regressions. Near complete (>90%) suppression of phosphorylated STAT3 was observed after a single oral dose of JAK inhibitor and this was accompanied by reduced tumor cell proliferation and increased apoptosis. As was observed *in vitro*, combining oral JAK inhibitors with therapeutics including dexamethasone, mel-

phalan, or bortezomib markedly improved tumor growth control without additional toxicity. *Summary.* Aberrant JAK/STAT activation has been observed consistently in MM. Here we demonstrate that potent and selective JAK inhibitors ablate STAT phosphorylation in MM cell lines. The sequela of reduced JAK/STAT activity include reduced MM tumor cell proliferation and survival and improved responsiveness to a variety of therapeutics *in vitro* and *in vivo*. These data provide a strong scientific rationale for the ongoing clinical trial evaluating INCB018424, a selective JAK inhibitor, as treatment for MM.

0957

FOLATE GENE VARIANTS AFFECT METHOTREXATE-RELATED TOXICITY IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

A. Ongaro,[1] M. Della Porta,[2] C. Ambrosio,[3] A. Caruso,[1] F.F. Masieri,[1] F. Di Raimondo,[4] D. Gemmati[5]

[1]Department of Morphology and Embryology, University of Ferrara, FERRARA; [2]Medical School, Division of Haematology, Policlinico San Matteo, University of P, PAVIA; [3]Division of Hematology, University of Ferrara, FERRARA; [4]Division of Hematology, University of Catania, CATANIA; [5]Center Study Haemostasis and Thrombosis, University of Ferrara, FERRARA, Italy

Background. Methotrexate (MTX), an antifolate, is an important component of maintenance therapy of acute lymphoblastic leukaemia (ALL). The major MTX activity is the impairment of folate metabolism with consequent antiproliferative effects. MTX exerts a competitive inhibition of dihydrofolate reductase (DHFR), the enzyme which reduces the dihydrofolate (DHF) into tetrahydrofolate (THF), resulting in the lack of folate pool and impairment of purine and pyrimidine synthesis. Another crucial folate metabolism enzyme is the 5,10-methylenetetrahydrofolate reductase (MTHFR). It catalyzes the conversion of 5,10-methyleneTHF into 5-methylTHF, a major circulating form of folate. MTHFR gene variants, which reduce the enzyme activity, have been involved in the risk and in the outcome of patients affected by haematological malignancies. Specifically, MTHFR C677T variant reduces adult ALL susceptibility and increases drug-related toxicity in non-Hodgkin lymphoma when folate antagonists are used.[1] Recently, a new 19 bp deletion in intron 1 of DHFR gene has been described[2] and the deleted allele has been associated with increased gene expression. Since MTHFR and DHFR are key players in folate metabolism, differences in their activity or gene expression, due to gene variants, might modulate therapy-associated toxicity to antifolate chemotherapeutic agents. *Aims.* To investigate the impact of MTHFR C677T and DHFR 19 bp deletion on therapy-related toxicity in adult ALL patients. *Methods.* We analyzed 93 ALL patients (46 male, 47 female; age at onset 40 ≥16 years), treated with MTX in maintenance therapy (15 mg/m^2 weekly for 2 years). Haematological (anaemia, thrombocytic, lymphocytic), and non-haematological (mucositis and hepatic) toxicities were graded according to WHO criteria. Patients were genotyped by polymerase chain reaction -restriction fragment length polymorphism analysis. Genotypes stratified by MTHFR and DHFR variants, were scored by WHO toxicity and the associations were statistically analyzed. *Results.* When we compared patients without toxicity with patients with any grade of toxicity, the MTHFR 677TT genotype was significantly associated with an increased risk of hepatic (OR 4.46, 95%CI 1.05-19.02; p 0.03) and lymphocytic (OR 3.50, 95%CI 0.84-14.56; p 0.07) toxicity, respect to wild-type genotype. By considering the same comparison, patients carrying the 19 bp deleted allele of DHFR gene (heterozygous + homozygous genotypes) were associated with an increased risk of hepatic (OR 2.71, 95%CI 0.99-7.44; p 0.04) and thrombocytic (OR 5.20, 95%CI 1.08-25.16; p 0.02) toxicity. Notably, the combined analysis for MTHFR and DHFR gene variants showed that patients with a polymorphic allele in both genes had a further increased risk of hepatic toxicity (OR 6.00, 95%CI 1.14-31.53; p 0.05) compared with the risks for each polymorphism. *Conclusions.* Our data suggest that specific polymorphisms (MTHFR C677T and DHFR 19bp deletion) of the folate cycle play a critical role in ALL therapy-related toxicity, possibly by interference with MTX action. Genotyping of folate pathway gene variants might be useful to reduce chemotherapy toxicity by means of dose adjustments or the choice of alternative treatments.

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0958

OVEREXPRESSION OF THE IL3/IL5/GM-CSFRB AS POTENTIAL MECHANISM LEADING TO SURVIVAL FACTOR ACTIVATION IN RESPONSE TO ABL-INHIBITOR TREATMENT

N. Härtel, T. Klag, A. Hochhaus, P. La Rosée

Medizinische Fakultät Mannheim, Universität Heidelberg, MANNHEIM, Germany

Background. Targeted treatment of chronic myelogenous leukemia using the small molecule imatinib has dramatically improved patient outcome. However, residual disease can be detected in the majority of patients treated with imatinib monotherapy. Mechanisms of primary molecular resistance are most likely multifactorial. A consistent finding is compensatory activation of survival pathways in response to imatinib treatment which may explain the reduced apoptotic sensitivity of a subpopulation of leukemic cells. *Aims and Methods.* In order to study mechanisms leading to survival factor activation, we applied the IL3-dependent murine hematopoietic cell line Ba/F3 and its IL3-independent derivative Ba/F3p210 to signaling studies by Western blot after *in vitro* exposure to clinically approved ABL-inhibitors. This model recapitulates cytokine-rescue of imatinib-treated cells which represents a proposed resistance mechanism as demonstrated for immature CML stem cells. *Results.* The presence of IL3 in imatinib treated cells prevents inhibition of BCR-ABL-dependent signaling as shown by unaffected activation of the MAPK Erk1/2 after 6 and 24 hrs. Dose increase of imatinib up to 25µM does not overcome stustained MAPK-activation. The imatinib-derivative nilotinib, known to be 20fold more potent compared to imatinib shows a tendency of dose-dependent MAPK-suppression. However, pMAPK is still detectable even at dose escalation beyond the clinically relevant dose range. Asking, whether inhibition of BCR-ABL by imatinib or nilotinib is essential for paradoxical MAPK-activation, we applied a mutant cell line Ba/F3p210T315I , which expresses kinase active BCR-ABL not amenable to inhibition by ABL-inhibitors. Exposure to imatinib and nilotinib revealed MAPK-activation exeeding the pMAPK-levels observed in unmutated cell lines. This indicates off target activity of the inhibitors governing paradoxical MAPK-activation. Significantly enhanced MAPK-activation in BCR-ABLT315I-expressing cells cultured in the absence of IL3 furthermore suggests a positive feedback for the off-target activity induced by BCR-ABL. When BCR-ABLT315I-expressing cells were exposed to the multikinase-inhibitor dasatinib, pMAPK-levels remained unchanged suggesting that multikinase-inhibition prevents signal transduction leading to MAPK-activation. A potential role of Src-kinases in stimulating the MAPK-cascaded was excluded by combined treatment with nilotinib and PP2, a selective Src-inhibitor. However, we identified overexpression of the common cytokine receptor subunit b (IL3/IL5/GM-CSF) being overexpressed in Ba/F3p210 cells providing a first hint to mechanisms regulating pradoxical MAPK-activation in response to ABL-inhibitors. *Conclusions.* In conclusion, our data support evidence of compensatory cytokine dependent survival factor activation as a potential target to optimize ABL-inhibitor based treatment of CML. Overexpression of a cytokine receptor in response to imatinib provides a rationale for the development of receptor-directed treatment strategies.

Publication only

0959

INCIDENCE OF BACTERIAL AND FUNGAL INFECTIONS IN NEWLY DIAGNOSED ACUTE MYELOID LEUKAEMIA PATIENTS TREATED WITH INDUCTION REGIMENS INCLUDING FLUDARABINE: RETROSPECTIVE ANALYSIS OF 224 CASES

M. Malagola,[1] A. Peli,[2] D. Damiani,[2] A. Candoni,[2] M. Tiribelli,[2] G. Martinelli,[2] P.P. Piccaluga,[2] S. Paolini,[2] F. De Rosa,[2] F. Lauria,[2] B Monica,[2] G. Marco,[2] I. Pierri,[2] A. Zaccaria,[3] E. Zuffa,[3] P. Mazza,[3] G. Priccolo,[3] L. Gugliotta,[3] A. Bonini,[3] G. Visani,[3] C. Skert,[2] C. Bergonzi,[2] A.M. Roccaro,[4] C. Filì,[2] R. Fanin,[2] M. Baccarani,[2] D. Russo[2]

[1]Chair of Haematology, Brescia, BRESCIA, Italy; [2]Chair of Haematology, BRESCIA, Italy; [3]Unit of Haematology, RAVENNA, Italy; [4]Dana Farber Cancer Institute, BOSTON, USA

Background. Infections are the major cause of morbidity and mortality in patients with acute myeloid leukaemia (AML). They primarily occur during the first course of induction chemotherapy and may increase the risk of leukaemia relapse, due to a significant delay in consolidation therapy. The intensification of induction chemotherapy and the use of non-conventional drugs such as Fludarabine are considered responsible of the increased risk of infections. *Aims and Methods.* In this study, we retrospectively analyzed the infections occurred in 224 newly diagnosed AML patients, aged at least 65 years, consecutively treated between 1997 and 2002 with an induction regimen including fludarabine (Fluda), arabinosyl cytosine, and idarubicin, with or without etoposide (FLAI/FLAIE), in the context of three multicentric prospective trials (AML97, AML99, AML02). *Results.* The complete remission rate, and the haematological and extra-haematological toxicities were comparable in the three consecutive clinical trials. During the induction phase, 146 (65%) patients experienced fever of undetermined origin (FUO), 30 (13%) and 47 (21%) patients had Gram-negative and -positive sepsis, respectively, and 10 (4%) patients developed a probable/proven invasive fungal infection (IFI). These data were comparable in the three clinical trials. We then collected the data of the incidence of infections during the first consolidation course. Seventy-five (35%) patients had FUO, 43 (20%) and 40 (19%) patients had Gram-negative and -positive sepsis, respectively, and 5 (2%) patients developed a probable/proven IFI. Summary and Conclusions: Interestingly, the overall incidence of microbiologically documented infections during induction was 39% and the incidence of probable/proven IFIs during the induction/consolidation program was 7%. These data, although retrospectively collected, suggest that Fluda-based chemotherapy is not associated with an increased incidence of infections, in particular IFIs, compared to conventional non-Fluda-based regimens commonly used for AML induction.

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INTENSIVE INDUCTION CHEMOTHERAPY WITH HIGH-DOSE IDARUBICIN, COMBINED WITH HIGH-DOSE ARACYTIN AND AMIFOSTINE, IN OLDER AML PATIENTS: LONG-TERM RESULTS IN 78 PATIENTS IN A SINGLE CENTRE STUDY

A. Poloni,[1] S. Trappolini,[1] B. Costantini,[1] D. Capelli,[1] E. Troiani,[1] G. Mancini,[1] G. Discepoli,[2] M. Montanari,[1] G. Gini,[1] I. Scortechini,[1] P. Leoni,[1] A. Olivieri[1]

[1]Clinica di Ematologia, ANCONA; [2]Istituto di Citogenetica, ANCONA, Italy

Background and Aims. A phase II study was conducted to investigate the effects of a new regimen based on the combination of high-dose (HD) idarubicin and HD-cytarabine (Ara-C), with amifostine, in untreated elderly patients with acute myeloid leukemia (AML). The main end points were overall response rate (ORR) and toxicity; secondary end points were leukaemia-free survival (LFS), overall survival (OS) and feasibility of peripheral blood stem cell (PBSC) collection. *Methods.* From June 1999 to December 2007 one-hundred twenty-three patients with non-M3 AML, median age 71 years (range 55-89), were observed in our institution. All patients were preliminary evaluated according to a simplified Multidimensional Geriatric Assesment, so we were able to separate fit patients from unfit or frail patients. Seventy-eight fit patients were enrolled in this protocol. Twenty-seven patients (35%) had AML secondary to myelodysplastic syndrome. Patients achieving complete remission (CR) were intended to receive a consolidation followed by PBSC collection and autologous stem cell transplantation (ASCT). *Results.* Overall, 57 patients (73%) achieved complete remission (CR). There were 5 induction deaths (6%), while 16 patients were refractory (21%). The main extrahematological toxicity was represented by grade III to IV infections in 65% of patients. Hematological toxicity was acceptable with 16 days (range, 9-29 days) to reach >500×10^6/L absolute neutrophil count and 16 days (range, 3-39 days) to achieve an unsupported platelet count >20,000×10^6/L. Median duration of hospitalization was 30 days (range, 15-69). Forthy-eight patients remained in CR and received intensive consolidation therapy; 20 patients were able to mobilize a sufficient number of $CD34^+$ cells and undergo ASCT. *Conclusions.* According to the intention to treat criteria all patients were analyzed for outcomes. Six-year OS and LFS was 24% and 30% respectively, with median follow-up of 29 months (range, 4-73). Patients with unfavorable cytogenetic and those with secondary AML had poorer OS; about 40% of patients could mobilize a sufficient amount of PBSC for autologous stem cell transplantation.

0961

THE INCIDENCE AND SURVIVAL OF ACUTE DE NOVO LEUKEMIAS IN ESTONIA AND IN A WELL-DEFINED REGION OF WESTERN SWEDEN DURING 1997-2001: A SURVEY OF PATIENTS AGED ≥65 YEARS

K. Palk,[1] E. Luik,[2] M. Varik,[1] I. Viigimaa,[1] K. Vaht,[1] H. Everaus,[2] L. Wennström,[3] D. Stockelberg,[3] S. Safai-Kutti,[3] E. Holmberg,[3] J. Kutti[3]

[1]North Estonia Regional Hospital, TALLINN, Estonia; [2]Tartu University Clinics, TARTU, Estonia; [3]Sahlgrenska University Hospital, GÖTEBORG, Sweden

Estonia regained its independence in 1991 after having been occupied by the Soviet Union for 5 decades. In view of political/socio-economic differences in between Estonia and a neighbouring country, a well-defined Region of Western Sweden, in a recent survey (J Intern Med 2004; 256: 79-85) we retrospectively compared the incidence and survival of *de novo* acute leukaemia (AL) patients aged ≥65 years over three 5-year periods (1982-1996) in these two countries. Estonia and the so-called Western Swedish Health Care Region are well comparable area-wise (45,000 km^2 and 27,000, respectively) as to population (1.38 million inhabitants and 1.65, respectively). The age standardized incidence rates regarding the total number of *de novo* AL was 5.31 per 100,000 inhabitants/year for Estonia and 7.99 for Sweden, this difference being statistically significant. However, this difference was merely attributable to incidence rates as regards acute myeloblastic leukemias (AML); the differences were negligible as regards acute lymphoblastic leukemias (ALL) and non-classifiable, undifferentiated or biphenotypic leukemias (uAL). The relative survival for the total material of *de novo* AL patients was significantly higher for Swedish when compared with Estonian patients (p<0.001). Thus, the relative survival for the total material of patients aged ≥65 years in Estonia at 1 year was 8.5% and at 3 years 3.5%, respectively. The corresponding figures for the Swedish patients were considerably higher, 22.7% and 7.7%, respectively. This difference, however, applied only for patients with AML (p<0.001), whereas the results for patients with ALL and uAL were equally dismal. In view of the poor outcome for Estonian patients we decided to prospectively compare the results for incidence and outcome of *de novo* AL between the two countries over forthcoming 5-year periods. Herein we report on the results of the first 5-year period comprising 1997-2001. All hospital records were carefully reviewed and patients only with *de novo* AL were identified. The current report deals only with patients aged ≥65 years. The total number of *de novo* AL in the Estonian population comprising all patients ≥65 years was 75 (30 males and 45 females), the corresponding figures for the Swedish population being 165 (88 males and 77 females). As regards Estonia the subgroups were: AML n=67, ALL n=6, and uAL n=2. The corresponding figures for Sweden were: AML n=146, ALL n=11, and uAL n=8. In Estonia, the yearly age standardized incidence per 100,000 inhabitants for *de novo* AL was 7.18 (5.53-8.83); for Western Sweden the figure was 10.70 (8.80-12.50). The survival data for the two countries were also different. Thus, the relative survival for patients aged ≥65 years in Estonia at 1 year was 14.2% (7.3-23.4%) and at 3 years 3.2% (0.6-10.1%), respectively. The corresponding figures for the Swedish patients were higher, 25.8% (19.2-33.0%) and 6.6% (3.3-11.7%), respectively. As compared to our previous study (cf. above) the survival at 1 year had improved for the Estonian but not for the Swedish patients whereas the survival at 3 (and also at 5) years were equally dismal in both countries.

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CLONAL EVOLUTION STUDIED BY I-FISH IN B-CLL

A. Berkova,[1] L. Pavlistova,[1] J. Brezinova,[2] L. Babicka,[1] E. Malinova,[1] L. Grosova,[1] J. Tajtlova,[1] E. Cmunt,[3] J. Schwarz,[2] J. Karban,[3] M. Trneny,[3] Z. Zemanova,[1] K. Michalova[1]

[1]General Faculty Hospital and First Faculty of Medicine, Charles University, PRAGUE; [2]Institute of Hematology and Blood Transfusion, PRAGUE; [3]First Medical Department, General Faculty Hospital and First Faculty of Medicine, PRAGUE, Czech Republic

Background. The most frequent type of leukemia in the Western world - chronic lymphocytic leukemia (CLL), follows an extremely variable clinical course. As the majority of patients are diagnosed in early disease stages, the identification of markers having the prognostic power has been the focus of intensive investigation. Cytogenetic analyses and interphase fluorescence *in situ* hybridization (I-FISH), among other modern diagnostic methods, have attained importance for diagnosis and outcome prediction. In addition, the acquisition of new genomic aberrations during the disease course (clonal evolution) has been reported in several sequential studies and was mostly associated with shortened survival. *Aims.* Our goal was to assess the frequency of newly acquired cytogenetic abnormalities within the clonal evolution in patients followed-up at our institution, to compare molecular cytogenetic findings with IgVH mutation pattern, expression of CD38 and ZAP-70 and, consequently, to evaluate these cytogenetic changes in the context of clinical disease course as the previously published conclusions have been inconsistent. *Methods.* Between 1982 and 2006, 1161 patients with B-CLL were examined at the Center of Oncocytogenetics in Prague. 102 patients diagnosed according to standard criteria were enrolled in presented study evaluating clonal evolution by I-FISH. Genomic aberrations were analyzed at various time points during the disease course with a probe set allowing the detection of trisomy 12, del(13)(q14.3), del(11)(q22.3) - ATM gene deletion and del(17)(p13.1) - deletion of p53 gene. ZAP-70 and CD38 expression analyses were performed by flow cytometry and IgVH mutation analyses by sequencing. *Results.* Cytogenetic abnormalities were detected in 69 of the 102 patients at the time of the baseline analysis. During the follow-up 25 patients experienced clonal evolution with the following newly acquired aberrations: monoallelic del(13)(q14.3) in ten, biallelic del(13)(q14.3) in three, evolution from monoallelic to biallelic del(13)(q14.3) in three, del(11)(q22.3) in four, del(17)(p13.1) in three, trisomy 12 in one and del(13)(q14.3) together with del(17)(p13.1) in one case too. Among these 25 patients, 14 had unmutated and 7 mutated IgVH genes. Of these with mutated IgVH status, six patients acquired any variation of del(13)(q14.3) and one newly acquired del(11)(q22.3). The characterization of each patient with clonal evolution as well as statistical consideration will be shown. *Summary.* The patients with B-CLL should be sequentially cytogenetically examined as the clonal evolution is not uncommon in this disease. Although certain risk groups of patients are more likely to experience this evolution, we did not prove statistically significant relation to either unmutated IgVH genes or ZAP-70 and CD38 positivity. However, the occurence of high-risk deletions adversely affects the patients overall survival when detected at initial analysis or at the follow-up examination and especially deletion of gene p53 revealed at any time point of the disease course predicts the resistance to chemotherapy. We conclude that I-FISH analyses represent one of the possible views of genomic instability in leukemic cells and in combination with conventional banding analyses on stimulated CLL cells would be even more yielding in future studies.

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PROGNOSTIC SIGNIFICANCE OF COMBINED ANALYSIS OF IGVH MUTATIONAL STATUS AND GENETIC ABNORMALITIES DETECTED BY FISH ANALYSIS IN A STAGE CHRONIC LYMPHOCYTIC LEUKEMIA

M. Tarnani,[1] L. Laurenti,[1] S. De Matteis,[1] S. Marietti,[1] F. Sorà,[1] S. Gobessi,[2] G. La Torre,[1] S. Sica,[1] G. Leone,[1] D.G. Efremov[2]

[1]Policlinico A. Gemelli, ROMA; [2]ICGEB Monterotondo-outstation, ROMA, Italy

Background. The clinical course of chronic lymphocytic leukemia (CLL) is extremely variable. The Rai-Binet clinical staging system identify risk groups, correlated with survival. However, this system failed to predict clinical course for individual patients at early-stage disease and to identify patients with poor prognosis. The new biological parameters (IgVH mutational status, CD38, ZAP-70 and chromosomal abnormalities by FISH analysis) have emerged as the most useful tools in identifying aggressive CLL. *Aims.* We evaluated prognostic significance of combined analysis of IgVH mutational status and karyotype by FISH analysis, and their correlation with progression-free-survival (PFS). *Methods.* The biological parameters were studied at presentation. The cohort of patients (Binet stage A) was divided in 4 groups according to the simultaneous evaluation of IgVH genes mutational status and FISH analysis data. We considered the absence of chromosomal abnormalities, del13q and trisomy 12 as low risk karyotype, while del11q and del17p were considered high risk. The following groups were obtained: A group IgVH mutated/low risk, B group IgVH mutated/high risk, C group IgVH unmutated/low risk, D group IgVH unmutated/high risk. The univariate survival analysis, by Kaplan-Meier curves, was performed to assess differences in PFS among 4 groups. *Results.* We have studied 165 previously untreated stage A CLL patients: 102 were mutated, 63 unmutated; 144 shown low risk karyotype, 21 high risk. Ninety-eight (60%) patients were included in A group, 4 (2%) in B group, 46 (28%) in C group, and 17 (10%) in D group. The median follow-up was 60 months. Among 165 patients, 104 (63%) had stable disease, while 61 (37%) showed progressive disease. The median PFS was 40 months for patients with unmutated IgVH genes and not reached (at 211 months) for mutated cases ($p<0.0001$). There was a statistical difference also between low and high risk karyotype, with median PFS of 112 *vs* 36 months respectively ($p<0.0001$). The patients with mutated IgVH/low risk (A group) showed a significantly longer median PFS (not reached at 199 months) than mutated/high risk (B group) (43 months), unmutated/low risk (C group) (58 months) and unmutated/high risk (D group) (36 months) ($p<0.0001$). Analyzing the all possible combinations singularly, we noticed statistical differences between groups A and B ($p=0.038$), A and C ($p<0.0001$), A and D ($p<0.0001$). There wasn't any statistical difference between groups B and C ($p=0.9$), B and D ($p=0.5$), C and D ($p=0.3$). *Summaryand conclusion*s.The IgVH and Fish analysis were the best prognostic biological markers in our experience. The combination of two markers in a group of stage A CLL led to stratify the patient in four categories that help clinicians in the future management. Moreover group A patients (mutated IgVH/low risk fish analysis) showed significantly longer median PFS respect to B, C and D groups ($p>0.0001$) leading to consider as superstable 60% of stage A CLL patients who didn't achieve median PFS after 199 months of follow-up.

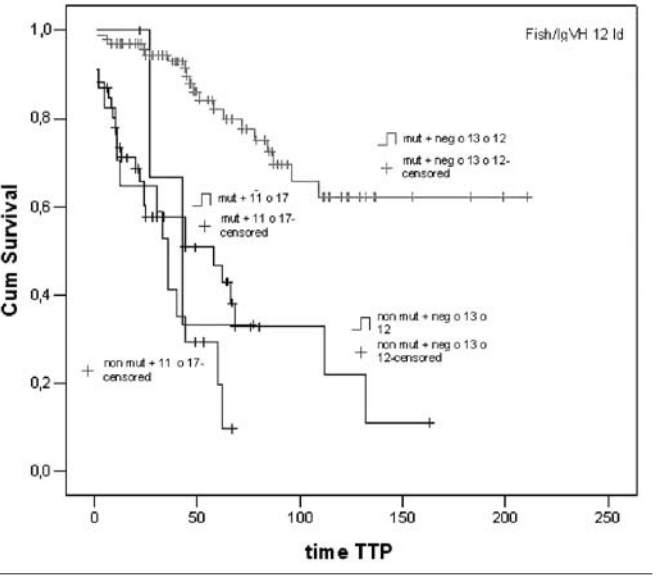

Figure 1. Survival functions.

0964

GENETIC SUSCEPTIBILITY TO B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA IS ASSOCIATED WITH CD38 GENE POLYMORPHISMS: EVIDENCE FROM TWO INDEPENDENT COHORTS OF POLISH CAUCASIANS

K. Jamroziak,[1] Z. Szemraj,[1] O. Grzybowska-Izydorczyk,[1] J. Szemraj,[1] B. Cebula,[1] M. Bieniasz,[1] K. Giannopoulos,[2] D. Jesionek-Kupnicka,[1] E. Balcerczak,[1] E. Wawrzyniak,[1] M. Kowal,[2] A. Kostyra,[3] M. Mirowski,[1] R. Kordek,[1] T. Robak[1]

[1]Medical University of Lodz, LODZ; [2]Medical University of Lublin, LUBLIN; [3]City Hospital of Torun, TORUN, Poland

There is growing evidence that CD38 molecule is not only a prognostic marker but also an important factor in the pathogenetic network underlying B-cell chronic lymphocytic leukemia (B-CLL). In this study we hypothesized that inherited genetic variants in CD38 gene may alter predisposition to B-CLL. Two CD38 gene single nucleotide polymorphisms (SNPs), including 184C>G SNP in intron 1 and 418C>T (Arg140Trp) SNP in exon 3, were assessed using PCR-based *Methods.* Genotyping was performed in a total of 460 Polish Caucasian B-CLL patients and 503 healthy controls, having comparable sex and age distributions to cases. Initial case-control study included 252 B-CLL patients and 249 healthy controls (Cohort I). Subsequently, the results were validated in an independent cohort (Cohort II) of 208 B-CLL patients from different hematological centers and 254 controls. The results of genotyping in both cohorts revealed that frequencies of variant alleles of both studied CD38 gene SNPs were highly increased in patients with B-CLL. Considering 184C>G SNP, relative risk of developing B-CLL in the carriers of variant 184G allele reached odds ratio (OR)=2.91 in the Cohort I (p<0.00001) and OR=3.28 in the Cohort II (p<0.00001). In regard to 418C>T (Arg140Trp) SNP, the carriers of 418T allele had the risk of B-CLL OR=6.57 in the Cohort I (p=0.014) and OR=9.94 in Cohort II (p=0.031) as compared with wild-type individuals. In conclusion, consistent results obtained from genotyping of two independent cohorts indicate that CD38 gene SNPs contribute to the genetic predisposition to B-CLL.

0965

MINIMAL RESIDUAL DISEASE (MRD) AND BCR-ABL ARE THE TWO MOST IMPORTANT RISK PREDICTORS OF POOR OUTCOME IN MALAYSIA-SINGAPORE (MA-SPORE) ALL 2003 TRIAL

D.W.K. Lum,[1] H. Ariffin,[2] Y.H. Chan,[1] H.P. Lin,[3] S.K.Y. Kham,[1] A.E.J. Yeoh[1]

[1]National University of Singapore, SINGAPORE, Singapore; [2]Department of Paediatrics, University of Malaya Medical Centre, KUALA LUMPUR, Malaysia; [3]Subang Jaya Medical Centre, KUALA LUMPUR, Malaysia

Background. Modern chemotherapy regimen has improved treatment outcome in childhood acute lymphoblastic leukaemia (ALL) up to 80% in developed countries. However, 20% of patients are resistant to therapy and eventually relapse. The Ma-Spore ALL study group employs a risk-directed therapy based on early response to therapy as determined by minimal residual disease (MRD) and cytogenetics to personalized therapy. What is clear is that there exists a distinct group of patients with very high risk disease who has event-free survival (EFS) of <30% despite intensive chemotherapy. Our aim is to single out these very high risk patients early during therapy who have poor chance of cure on conventional therapy but will benefit for experimental therapy. *Aims.* Hence, we sought to define patients with very high risk features who may need intervention using targeted or novel therapeutics in order to increase their chances of cure. *Methods.* A total of 324 children with ALL treated on Ma-Spore ALL 2003 study were analyzed. Age range from 0.14 to 15.29 years (mean age is 5.52), B-lineage = 298, T-lineage = 26, males = 178, females = 146. Patients were categorized into 4 subgroups based on MRD levels: MRD at day 33 (TP#1) ≥5×10^{-3} and MRD at week 12 (TP#2) ≥1×10^{-3} and presence of BCR-ABL by polymerase chain reaction (PCR). MRD quantifications were performed on LightCycler 1.0 real-time quantitative PCR (RQ-PCR), using immunoglobulin heavy chains (IgH) and T cell receptors (TCR) rearrangements as surrogate markers. MRD marker with minimum sensitivity of 10^{-3}. Measurable units such as deaths, induction failures and relapses were used as yardstick to determine treatment outcome. *Results*. A total of 45 events in the Ma-Spore ALL 2003 study: deaths = 16; induction failures = 10; relapses = 19. The events in each of the subgroup are shown in Table 1. Among the 4 subgroups, our results show that high MRD levels of ≥5×10^{-3} in the presence of BCR-ABL at TP#1 are better predictors of adverse outcome as compared to high MRD levels alone (ROC = 0.73, sensitivity = 61.1%, specificity = 89.9%, PPV = 47.8%, NPV = 93.9%, Table 1). However, there is no significant difference between high MRD levels at TP#2 in the presence or absence of BCR-ABL. *Summary and Conclusions.* Our results in the Ma-Spore ALL 2003 study clearly demonstrates that patients harbouring BCR-ABL and have high MRD levels of ≥5×10^{-3} are significantly associated with poor prognosis. Intensive chemotherapy using novel therapeutics such as imatinib may help to improve outcome in this very high risk group of patients.

Table 1. Evaluation of prognostic biomarkers using receiver operating characteristic (ROC) curve analysis.

Subgroups	n(E)	ROC	Sensitivity	Specificity	PPV	NPV
MRD TP#1	17*	0.705	48.6	91.2	44.7	92.3
MRD TP#1+BCR-ABL	22*	0.730	61.1	89.9	47.8	93.9
MRD TP#2	10*	0.646	31.3	97.9	66.7	91.2
MRD TP#2+BCR-ABL	16*	0.684	45.7	96.1	64.0	92.2
MRD TP#1	14 †	0.721	51.9	90.2	36.8	94.5
MRD TP#1+BCR-ABL	18 †	0.753	64.3	88.6	39.1	95.6
MRD TP#2	9 †	0.661	34.6	97.5	60.0	93.2
MRD TP#2+BCR-ABL	14 †	0.710	50.0	95.4	56.0	94.2

* E = Deaths, Induction failures, Relapses

† E = Induction failures, Relapses

PPV = Positive predictive value; NPV = Negative predictive value

0966

INVESTIGATIONS ON HEREDITARY MUTATIONS IN PATIENTS WITH LEUKEMIA AND LYMPHOMA: FIRST RESULTS

H.J. Janiszewska,[1] A.B. Bak,[2] A. Junkiert-Czarnecka,[2] M. Schab,[2] M. Pilarska,[2] M. Calbecka,[3] M. Kielbinski,[4] B. Jazwiec,[4] K. Kuliczkowski,[4] O. Haus[2]

[1]Collegium Medicum, Nicolaus Copernicus University, BYDGOSZCZ; [2]Department of Clinical Genetics, Collegium Medicum, N.Copernicus University, BYDGOSZCZ; [3]Department of Hematology, Municipal Hospital, TORUN; [4]Department of Hematology, Medical University, WROCLAW, Poland

Background. The most of blood cancer cases are sporadic, but the patients with a familial history of cancer are also observed, especially among those suffering from lymphoid malignancies. *Aims.* The aim of the study was to find the hereditary mutations in cancer risk associated genes: BRCA1(5382insC, 300T/G, 4153delA), CHEK2(1100delC, I157T, IVS2+1G→A), CDKN2A (p16)(A148T), NOD2(3020insC), and the polymorphic variant of CYP1B1(355T/T) in a cohort of consecutive patients with various blood malignancies. *Material and Methods.* Total number of 60 adult patients were included in the study as yet. 43 of them had myeloid malignancies (15-MDS, 7-CML, 6-AML, 15-other myeloid disorders) and 17 had lymphoid malignancies (8-NHL, 7-myeloma, 2-ALL). The mutations were examined in DNA isolated from peripheral blood cells by RFLP-PCR and ASO-PCR *Methods.* In mutation-positive cases the constitutional character of changes was verified by analyzing DNA isolated from buccal smears. *Results.* Carrying of the mutation was found in 22 (36.7%) patients. In 21 of them a single mutation and in one patient two mutations in different genes were disclosed. Among the group with myeloid malignancies 13 (30.2%) patients were found to carry a mutation: 4 out of 15 patients with MDS (CDKN2A - 1, BRCA1-300T/G-1, CHEK2-I157T-1, NOD2-1) one with CML (NOD2), one with AML (CDKN2A), and 7 out of 15 with other myeloid diseases, mostly myeloproliferative disorders (CDKN2A-3, CYP1B1-2, CHEK2-I157T-1, CHEK2-IVS2+1G→A-1). Among the group with lymphoid malignancies 9 (52.9%) patients were found to carry mutations. 4 out of 8 patients with NHL had single mutations (CYP1B1 - 2, CDKN2A - 1, NOD2 - 1) and one - mutation in two different genes: CHEK2(I157T) and CYP1B1. 3 out of 7 myeloma patients had single mutations: two - of CHEK2 gene (1100delC and I157T), and one of CYP1B1. One ALL patient had CDKN2A mutation. *Conclusions.* The results of our preliminary study show a great impact of constitutional genetic changes on leukemogenesis. This underlines the necessity to continue the investigations on the frequency of constitutinal mutations among leukemia patients and on the relationship between hereditary mutations and the familial risk of disease.

0967

DIFFERENT PATTERNS, DETECTED BY FISH, OF 5Q DELETIONS AND OTHER CHROMOSOME ABNORMALITIES IN ADULT FAMILIAL MYELODYSPLASTIC SYNDROME

A. Carrió, A. Valera, D. Costa, C. Gómez, J.LL. Aguilar, M. Aymerich, D. Colomer, B. Nomdedeu, E. Monserrat, E. Campo

Hospital Clínic, BARCELONA, Spain

The myelodysplastic syndromes (MDS) are a heterogeneous group of diseases characterized by a variable degree of marrow failure often accompanied by well characterized chromosome abnormalities, including different deletions size of 5q, 7q and 20q, monosomy 7 and trisomy 8, leading to risk for acute leukaemia transformation. MDS are unusual in adults younger than fifties and its pathogenesis is very complex and frequently unexplained. Familial occurrence of MSD has been reported in a number of families, and it is worth reporting familial cases because they can throw light on the pathogenetic mechanisms of MDS in general. We report an adult MDS family with 6 siblings (Figure 1), of which two presented myelodysplastic syndrome (MDS), diagnosis as refractory cytopenia with multilineage dysplasia (RCMD), at the ages of 37 and 49, respectively. Conventional cytogenetics showed complex karyotypes, at diagnoses, in both: 44,XX,del(5)(q13q33),-7,-9,der(15;21)(q10;q10),-21,+mar[9]/46,XX[10] in the propositus, and 46,XY,-3,del(5)(q13q35),+8,der(12)t(3;12)(q13;p13) [20] in the second one. The propositus developed acute leukaemia and after achieving a complete remission with ICE chemotherapy underwent an allogenic transplantation of peripheral blood progenitor cells from an unrelated donor. She relapsed and eventually died of sepsis eight months post-transplantation. The second one also underwent an allogenic stem cell transplantation from a sibling in a MDS condition and is alive. In order to know the status of the four remaing healthy siblings, we performed morphological and cytogenetics bone marrow analyses. Since among their complex karyotype del(5)(q31q33) was the only chromosomal abnormality common to both patients, we decided to investigate retrospectively and prospectively all the samples by FISH. We used a specific probe for 5q31 (EGR1 gene in red) with an internal control probe (D5S23,D5S721 in green) in 5(p15.2) (Vysis). Surprisingly, we found that in the propositus the deleted chromosome was an i(5)(p10;p10), and one of the healthy brothers showed 12% of deletion 5q. Our results show that both methods are important in diagnosis and follow up of MDS patients. Because using conventional and molecular cytogenetics together, we identified the deleted 5q in the propositus as an i(5)(p10;p10). We also detected that in a healthy brother 12% of deletion 5q was present, he remains without apparent disease and his follow-up is continuing. Finally, it can be speculated in this family an inherited mutator effect could be present, and be the cause of a karyotype instability, leading to MDS with an unstable 5 chromosome.

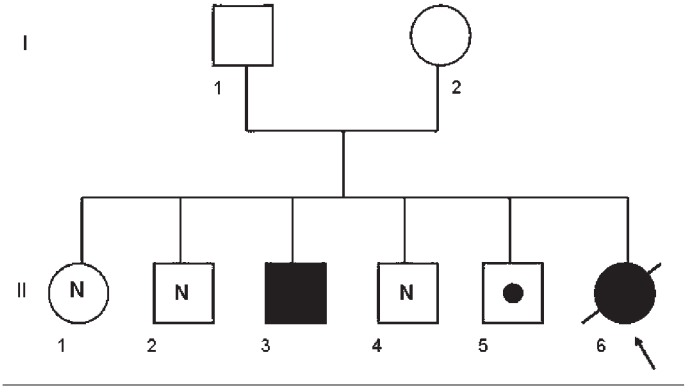

Figure 1. Pedigree of family affected with MDS. In black MSD patients and normal brother with del (5q) in white box with black point. THe arrow indicates the propositus and the slash the deceased patient.

0968

Withdrawn by the authors

0969

EFFECT OF METHOTREXATE ON GENE EXPRESSION OF JURKAT LEUKEMIC CELLS AND COMPARISON WITH THE METHYLATION INHIBITOR AGENT 5-AZA-2-DEOXYCYTIDINE. PRELIMINARY DATA

V. Danilatou,[1] G. Tsiliki,[1] V. Aris,[2] P. Soteropoulos,[2] D. Kafetzopoulos,[1] B. Kamen,[3] P. Cole[4]

[1]IMBB, Forth, HERAKLION CRETE, Greece; [2]Center for Applied Genomics, Public Health Research Institute, NEWARK, NJ, USA; [3]Cancer Institute of New Jersey and the Leukemia & Lymphoma Society, N. BRUNSWICK, NJ, USA; [4]Albert Einstein College of Medicine, BRONX, NY, USA

Background. Methotrexate (MTX), an antifolate used in the treatment of acute lymphoblastic leukemia, inhibits dihydrofolate reductase and depletes cells of reduced folates, essential in one-carbon transfer reactions, such as the final step in thymidine synthesis. Folate depletion, similarly, limits the availability of methyl donors for DNA methylation. Methylation of CpG islands inhibits gene expression, and aberrant patterns of methylation appear in cancer cells. *Aims.* We hypothesized that MTX might have an effect on DNA methylation. To identify putatively hypermethylated genes we compared the gene-expression profile of leukemic cell lines in the presence or absence of MTX. As a positive control, we used 5-aza-2-deoxycytidine (DAC), an inhibitor of DNA-methyltranferase-1 that is able to reactivate hyper-methylated silenced genes and has therapeutic use in hematologic malignancies. *Methods.* Jurkat cells, while growing exponentially, were exposed to 1 μM MTX or 1 μM DAC for three consecutive days. Freshly prepared DAC was added every 24 hours. RNA was extracted with Trizol and the Qiagen RNeasy kit. Gene-expression was analyzed by oligonucleotide microarrays [Affymetrix HG-U133 Plus 2.0 gene chip (54,675 probe sets)]. Statistical analysis was employed using multi-class SAM algorithm (FDR=0) resulting in the selection of 956 probe-sets that were further filtered to include only those with a $p<0.05$ and at least 2-fold difference relative to the control group.

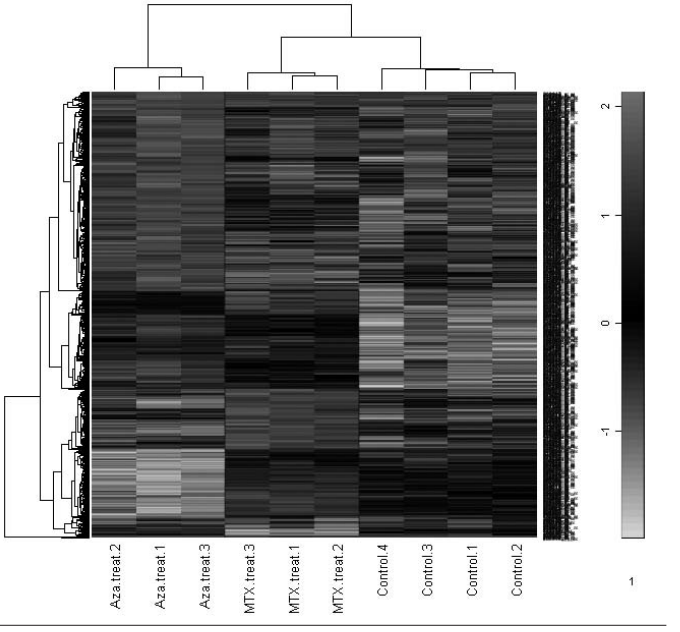

Figure 1. Clustering of 956 probe sets selected by SAM.

Results. The expression of 596 probe-sets was affected by DAC (505 up-regulated and 91 down-regulated) and 222 probe-sets by MTX (193 up-regulated and 29 down-regulated). Hierarchical clustering of the filtered data set showed a clear separation of the three groups of cells. As previously demonstrated in other cell lines, DAC up-regulated the expression of interferon-induced genes, tumor antigens, and spermatogenesis-involved genes. 63 probe-sets (51 genes) were up-regulated by both DAC and MTX. These genes were involved in apoptosis (CSTA, STK17A), transcription (ATF5, JUN, JDP2, ETS1, etc.), signal transduction (PIK3R1, JUN, CALM1), cell adhesion (SELL, ITGB1), metabolism, immune response and methylation processes. PIK3R is involved in many

important signaling pathways like erbB, mTOR, VEGF, JAK/STAT, and phosphatidylinositol pathways. CDH1, DAPK, MGMT, and RASF1 which are known to be hyper-methylated in Jurkat cells were not induced by either MTX or DAC. Nevertheless, CDH4, RASSF2 and RASSF4 were up-regulated by DAC but not MTX. We identified CpG islands in the promoter area (CpGFinder, SoftBerry) in 21 of the 51 genes that they were induced by MTX or DAC. Eight genes were down-regulated by both MTX and DAC. Histone 1 H1D involved in nucleosome assembly and genes involved in steroid biosynthesis were significantly down-regulated. *Conclusions*. We identified several genes implicated in important biological processes and signaling pathways that are induced by both MTX and DAC. A significant proportion of these genes did not contain CpG islands in their 5' regions, suggesting that DAC also activates genes through mechanisms other than inhibition of promoter methylation. Further methylation studies are needed to clarify whether MTX has any effects on DNA methylation. The identification of these interactions could reveal novel therapeutic molecular targets, markers of MTX resistance or effectiveness, and other effects of the drug.

0970

DETECTION OF METHYLATION STATUS CHANGES IN REFRACTORY ANEMIA WITH RINGED SIDEROBLASTS (RARS) BY MS-MLPA

A. Valencia, J. Cervera, E. Such, S. Oltra, E. Marco, I. Luna, M.L. Senent, M.A. Sanz, G.F. Sanz

Hospital Universitario La Fe, VALENCIA, Spain

Background. Epigenetic inactivation of tumour suppressor genes by promoter methylation is a common event in human cancers. In myelodysplastic syndromes (MDS) aberrant methylation of some tumour suppressor genes, such as p15INK4B, is a frequent event particularly in RAEB or RAEB-T. However, its involvement in low blast counts MDS subtypes is unknown. The most common method for identifying this alteration is the methylation specific PCR (MS-PCR). As the number of genes hypermethylated in cancer is increasing a methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) technique has been recently developed. This technique allows the detection of the methylation status of 25 sequences of a set of tumour suppressor genes frequently silenced by methylation in different tumours by multiplex PCR. *Objective.* To study the methylation status of 25 tumour suppressor genes in 37 patients with Refractory Anemia with Ringed Sideroblasts (RARS) by means of MS-MLPA. *Patients and Methods.* We selected 37 RARS bone marrow samples at the time of diagnosis [22M/15F, median age: 73 yr (range: 47-86)]. MLPA reagents were obtained from MRC-Holland (wwwmrc-holland.com). Bone marrow DNA from healthy donors was used as negative control. CpGenome™ Universal Methylated DNA (Chemicon, Millipore) was used as a positive control. Quantification of the methylation status was done by dividing the peak area with the combined areas of the control probes lacking the target sequence of the HhaI restricition enzyme. Finally, the relative peak area of each target probe from the digested sample was compared with those obtained from the undigested sample. Aberrant methylation was scored when the calculated methylation percentage was >10%. *Results.* Aberrant methylation was found in the following genes: RASSF1 (n=8, 22%), p15INK4B (n=7, 19%), CDH13 (n=3, 8%), FHIT (n=3, 5%) and RARβ-2 (n=2, 5%). The vast majority of patients showed no methylation (20/37; 54%) or just one methylated gene (12/37; 32%). By contrast a small proportion of the patients (5/37; 14%) showed two methylated genes. To validate these findings, MS-PCR was carried out to amplify CpG regions in p15INK4B and we confirmed the methylation status of p15INK4B by both methods in all the patients. The aberrant methylated genes found in the experiment belong to different molecular pathways involved in cell progression and differentiation: cell cycle (p15INK4B, RASSF1 and FHIT), cell adherence (CDH13 and IGSF4) and cellular differentiation (RARβ-2). All of these genes are found frequently inactivated by aberrant methylation in haematological malignancies. No significant correlation was found between methylation status of the different genes and the clinical or biological characteristics. *Conclusions.* MS-MLPA appears to be a new valid method for a multiplex detection of aberrant methylation patterns of CpG islands of several genes in just one assay. We found methylation of these tumour suppressor genes is uncommon in patients with RARS. However, 14% of the patients show abnormal methylation in two genes. Clinical relevance of these findings should be explored.

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0971

EXPRESSION PROFILES DURING ERYTHROPOIESIS REVEALED A SET OF NEW GENES INVOLVED IN THE MODIFICATION OF CELLS

A. Cunha, A.F. Brugnerotto, A.S.S. Duarte, G.G.L. Costa, S.T.O. Saad, F.F. Costa

State University of Campinas, CAMPINAS, Brazil

Erythroid differentiation is a dynamic and complex process in which a pluripotent stem cell undergoes a series of developmental changes that commit it to a specific lineage. These alterations involve changes in gene expression profiles. Extensive studies have led to a considerable understanding of the cellular and molecular control of hemoglobin production during red blood cell differentiation, however, a complete understanding of human erythropoiesis will require a robust description of the entire transcriptome of these cells during differentiation. From a global point of view of cell metabolic regulation, where genomic information could be complemented with gene expression, the use of methods that enable quantification of the entire transcriptome of the red blood cell during differentiation is of great importance. In this study, the gene expression profiles during differentiation of Human erythroid cells of a normal blood donor in a two-phase liquid culture (Fibach & Rachmilewitz, 1993) were evaluated using Serial Analysis of Gene Expression (SAGE). Global gene expression was evaluated in cells collected immediately before the addition of erythropoeitin (0 hour) and 192 and 336 hours after the addition of this hormone. We generated a total of 30512 tags at 0h, 30117 tags at 192h and 30189 tags at 336h, representing 12026, 11709 and 11337 unique tags, respectively. In the 0h library, a high expression of ferritin genes and CD74 antigen gene was observed. As expected, the expression of globin genes started during intermediate stages of differentiation (predominantly basophilic erythroblasts) and were the most expressed genes at the end of the culture (predominantly orthocromatic erythroblasts). Ribosomal genes were the most expressed genes at 192 hours, indicating an increase in protein synthesis. To identify the genes that were differentially expressed between the libraries, a p value <0.01 and fold ≥5 were considered as statistically significant. In the comparison of the 0h and 192h libraries, 179 differentially expressed transcripts were identified. From these genes, in addition to the globin genes, we found an up-regulation of several genes related to protein binding (LXN, GSTM3, and TRIP6), transcription factor (GATA-1), hydrolase activity (TPSAB1), ion transport (SLC12A9) and regulation of apoptosis (PRDX2). Comparing the 192h and 336h libraries, 103 differentially expressed transcripts were identified. The up-regulated genes were generally related to hemoglobin synthesis, such as ALAS2, involved in the biosynthesis of the heme group or related to intracellular transport such as MSCP and NUDT4 and cell differentiation such as GDF15. Some of these genes, such as SLC12A9, TRB3, EYA3 and TWIST2 are described for the first time during erythroid differentiation and its expression were evaluated by Real time PCR, confirming the results found in SAGE libraries. The results indicated that the global aspects of the transcriptome were similar during differentiation for the majority of the genes and that probably a relative small set of genes is involved in the modification of erythroid cells during differentiation. The results of this study amplify the previous published data (Komor *et al.,* 2005) and may contribute to the comprehension of erythroid differentiation and identification of new target genes involved in some erythroid diseases.

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EFFECTS OF IRON THERAPY ON CONGESTIVE HEART FAILURE PATIENTS - A META-ANALYSIS

M. Shah,[1] A. Shah[2]

[1]Bridgeport Hospital, MERIDEN; [2]Hartford Hospital, HARTFORD, USA

Background. Anemia is a common co-morbidity associated with congestive heart failure patients. There are few data which suggest role of Iron therapy with or without Epotein therapy in Congestive Heart Failure (CHF) patients with anemia. *Aims.* We performed a meta-analysis of studies investigating role of Iron therapy for CHF. *Methods.* The MEDLINE, EMBASE, CINAHL and the Cochrane data base were searched for published English-language clinical trials. The most frequently investigated combinations were Iron therapy with heart failure and anemia with heart failure. Total 523 articles were identified, out of which 26 were clinical trials. Only trials evaluating role of iron therapy in congestive heart failure patients, who had either *6-minutes walk* test, *Minnesota Heart Failure Living Questionare*, *NYHA* class, *Ejection Fraction*, *Mortality*

and *Hospitalization* as an end point, were selected. *Results.* In the 6 included trials, 247 patients were included. 36 patients received only intravenous iron, 149 patients received iron therapy plus subcutaneous erythropoietin. Mean age was 72 years, the mean Hb concentration was <12.5 g/dL, mean EF was 30.35%. For 2 studies, there were no control groups and the comparision was done pre and post-treatment groups. There was significant improvement in 6-minutes walk test, MHFL score, NYHA class and mean ejection fraction in patients receiving iron therapy. *Conclusions.* This meta-analysis suggests that Iron therapy is associated with significant improvement in clinical markers of CHF. These results should be interpreted cautiously as some of the included studies were retrospective or non-randomized, and long-term data are missing.

0973

MOLECULAR CHARACTERIZING OF HB COLUMBIA MISSOURI [ALPHA2 88(F9) ALA/VAL]

P. Paloma,[1] F.A. González,[1] S. Garzón,[2] M. Polo,[1] E. Anguita,[1] A. León,[2] A. Villegas[1]

[1]Hospital Clínico San Carlos, MADRID; [2]Hospital de Jerez, JEREZ, Spain

Background. Structural hemoglobinopathies are one of the causes for secondary erythrocytosis. *Aims.* The steps to characterize a alpha-chain hemoglobinopathy (Hb Columbia Missouri) that exhibits high oxygen affinity are described next. *Methods and Results.* In an ethnically Caucasian family of Spanish origin, 4 members were studied. Abnormal Hb was not segregated from HbA neither by routine electrophoretic studies nor ionic exchange HPLC, while that reversed phase HPLC, a peak for the alpha-chain, wider than the normal one, was noticed. This seems to suggest that an abnormal alpha-chain was present. The isopropanol test was negative and an increased oxygen affinity was found in the 4 cases (P50 17 to 21 mmHg). The possibility of an associated alpha-thalassemia deletion was ruled out by Southern blot study. A GCG>GTG mutation in the 2nd exon of codon 88 in the heterozygotic alpha2 gene that determines the replacement Ala/Val was disclosed by the molecular study. *Conclusions.* The Hb Columbia Missouri had been described earlier following a protean test of a Caucasian woman in the USA. But, the gene, where the mutation responsible for the amino acid replacement took place, had not been determined. We show that the Ala replacement by Val is due to a mutation in codon 88 of the alpha2 gene.

0974

THE FIRST STUDY OF HFE GENE MUTATIONS IN WESTERN ROMANIA REVEALS THE PRESENCE OF HEREDITARY HEMOCHROMATOSIS IN 50% OF PATIENTS WITH IRON OVERLOAD AND LIVER DISEASE

M. Neghina,[1] A. Anghel,[1] A. Popescu,[1] C. Samoila,[1] R. Neghina,[1] K. Thorstensen[2]

[1]Victor Babes University of Medicine and Pharmacy, TIMISOARA, Romania; [2]Department of Medical Biochemistry, St. Olavs Hospital, TRONDHEIM, Norway

Background. Hereditary hemochromatosis (HH) is an autosomal recessive disorder of iron metabolism affecting 0.4-0.7% of European populations. Approximately 80% of affected individuals are homozygous for the C282Y mutation, but the H63D and S65C mutations are also of interest. The clinical penetrance and frequency of complications of HH is a subject of controversy. *Aims.* The present study aims at assessing the presence of HFE mutations in patients with liver disease of diverse aetiologies and suspected iron overload. There is no data on the frequency of HFE gene mutations in Romanian population. *Methods*. A total of 21 patients, all residents in the western part of Romania and hospitalized with clinical suspicion of iron overload and liver diseases, were assayed for C282Y, H63D and S65C mutations of the HFE gene, as well as serum ferritin, transaminases, gamma-glutamyl transferase, HBV and HCV serum markers. Information was obtained on alcohol intake. Liver ultrasound was performed in 13 subjects and liver biopsy was performed in 12 subjects. *Results*. We found the following HFE genotypes: 4 C282Y homozygotes (19.0%), 1 compound heterozygote C282Y/ H63D (4.8%), 1 single heterozygote C282Y (4.8%), 2 single heterozygotes H63D (9.5%), 1 single heterozygote S65C (4.8%), and 12 wild-type cases (57.1%). We shall discuss the clinical patterns and laboratory data of the study group according to the HFE genotypes. *Summary.* Overall, the HFE genotype was in accordance with the suspicion of iron overload in 5 out of 21 cases (23.8%). All these individuals belonged to the group of 10 patients with the most prominent signs of iron overload (hyperferritinemia and/or hepatic iron score ≥1). They had significantly increased ferritin levels compared to those patients carrying no mutations (p=0.008), or those patients that were single heterozygous for one mutation or homozygous for the H63D mutation (p=0.014). We consider that the inclusion of iron studies during routine clinical visits coupled with the availability of HFE genotyping for family and population studies will facilitate the early detection of HH in Romania.

0975

IRON OVERLOAD IN SICKLE CELL ANEMIA

A. Koren, C. Levin

Ha'Emek Medical Center, AFULA, Israel

Sickle Cell Anemia (SCA) is a hemolytic anemia caused by a single mutation in position 6 of the β globin molecule. The clinical picture of SCA included vaso-occlusive crises, acute chest syndrome, CNS infarcts, hemolytic and aplastic crises, avascular necrosis of hip, splenic sequestration and others. SCA is autosomal recessive transmitted and beside the homozygous SS form, the combination with other abnormal hemoglobin's like β^+ or β^0 thalassemia, Hgb C or D can cause similar clinical presentation. In β Thalassemia Major Iron overload is a direct consequence of blood transfusions and intestinal Iron absorption, secondary to increased erythropoiesis. Recently it was proven that the intestinal Iron absorption is regulated by Hepcidine. In β thalassemia low Hepcidine levels allowed increased iron absorption despite high ferritin levels. The clinical symptoms of hemosiderosis include cardiomyopathy and endocrine dysfunction. Those complications are rare or absent in Sickle Cell Anemia patients even in those patients that received blood transfusions and with high ferritin levels. We studied the clinical symptoms related to Iron overload in a cohort of 60 sickle cell patients (28 patients with Sickle Cell Anemia (SCA) and 32 patients with Sickle cell β thalassemia (SβThal)). None of the patients was treated by Iron chelators. The mean age of the SCA patients is 16.2±10 ys *vs* 21.1±9.3 ys in the SβThal group. In the first 5 years of age the SCA patients received a mean of 57±88 cc/kg/yr of packed cells compared to 44 ±55 in SβThal patients. At older years the annual blood requirement decreased significantly probably due to the response to Hydroxyurea treatment. The ferritin levels and Transferrin Saturation are presented in Table 1. In spite ferritin levels above 2000 ng/mL, none of the patients developed cardiac symptoms and mild cardiomyopathy was detected only in one patient aged 7 ys. The mean final height in the group of patients older than 20 ys is in the 30 percentile, while two patients are below the 3rd percentile at this age. All the patients have normal thyroid and parathyroid function and Diabetes Mellitus is not present. The results of our data and other's, published in the last years, suggests that the regulation of Iron in SCA patients is different from Thalassemia patients. Then further studies should be done, including assessment of Non Transferrin Binding Iron in order to establish the management of SCA patients, principally in relation to Iron chelator treatment.

Table 1. Iron overload in sickle cell disease.

Age (ys)	Sickle Cell Anemia		Sickle Cell β Thalassemia	
	Ferritin (ng/dl)	Transferrin Saturation (%)	Ferritin (ng/dl)	Transferrin Saturation (%)
5	785 ±896	39 ±26	613 ±596	37 ±19
10	1310 ±1486	43 ±34	2334 ±2888	60 ±33
15	1364 ±1124	58 ±22	2892 ±2820	63 ±37
20	4762 ±8011	64 ±31	3725 ±2846	82 ±30

0976

HEMATOLOGICAL AND IRON METABOLISM FEATURES IN ADULTS - COMPARISON BETWEEN UNSPLENECTOMISED AND SPLENECTOMISED PATIENTS WITH HEREDITARY SPHEROCYTOSIS

S. Rocha

Faculdade de Farmácia/Instituto de Biologia Molecular e Celular (IBMC), PORTO, Portugal

Background. Hereditary Spherocytosis (HS) is the most common non-immune hemolytic anemia in individuals of northern European ancestry, ranging from an asymptomatic condition to a severe life-threatening anemia. HS is a red blood cell (RBC) membrane disorder presenting as screening hallmarks the presence of spherocytes in peripheral blood smears, the increase in osmotic fragility and in reticulocyte count. The common features of HS include mild anemia, jaundice and splenomegaly. Splenectomy, usually, corrects the anemic state. *Aims.* The aims of our work were the characterization of an adult population of unsplenectomised and splenectomised HS patients and the evaluation of the anemia improvement after spleen removal. *Methods.* In 50 Portuguese individuals of the northern region of Portugal (18 controls, 13 unsplenectomised HS patients, 19 splenectomised HS patients) we performed the basic hematological, biochemical and iron metabolism studies, by standard routine methods; we evaluated the levels of erythropoietin (EPO), soluble transferrin receptor (sTfR), pro-hepcidin, by ELISA; the reticulocyte production index (RPI) and the iron transferrin saturation (TS); the cryo-hemolysis and osmotic fragility tests were also performed. *Results*. As expected, the HS unsplenectomised patients presented clear markers of hemolytic anemia such as, decreased RBC count, hemoglobin concentration (Hb) and hematocrit (HCT) and statistically significant increased levels of reticulocytes, mean cell hemoglobin concentration (MCHC), RBC distribution width (RDW) and bilirrubin, when compared with controls; in the case of splenectomised patients, RBC count, Hb and HCT values were similar to controls, but reticulocytes, MCHC, RDW and bilirrubin were increased. Comparing the two HS populations, we found statistically significant differences for Hb, HCT and MCHC, which were lower in unsplenectomised patients. The markers of erythropoiesis, EPO and sTfR levels and RPI were significantly higher than controls in unsplenectomised patients; in splenectomised patients, EPO and sTfR showed similar findings to controls (sTfR was slightly increased), although RPI was statistically higher. Only EPO was statistically different when comparing the HS groups (higher in unsplenectomised patients). As for iron metabolism features, HS patients, in general, presented increased levels of iron, ferritin, folic acid, B12 vitamin, pro-hepcidin and TS and lower levels of transferrin, when compared with controls; no differences were found between the unsplenectomised and splenectomised HS patients. Cryo-hemolysis and osmotic fragility values, which are markers of RBC membrane fragility, were higher in both subsets of patients, particularly in splenectomised patients when compared to controls and unsplenectomised patients. *Summaryand Conclusions.* The removal of the spleen avoids premature RBC destruction and, usually, *cures* the anemia in HS patients, as was observed in our studied population. However, splenectomised patients did not fully revert to control levels in most of the studied features. This probably reflects that more fragile RBC are still prematurely removed and, therefore, an increased destruction/production of erythrocytes is still occurring. In fact, our data about the iron status appear to be representative of a body-iron accumulation.

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0977

MEAN NEUTROPHIL VOLUME: A NEW AUTOMATED HEMATOLOGIC PARAMETER FOR ACUTE INFECTION

A.E. Papakonstantinou,[1] A. Chondrothanasi,[2] E. Christodoulaki,[2] I. Grafakos,[2] A. Skourbouti,[2] P. Georgoutsou,[2] C. Manti[2]

[1]*Thriasion Hospital, ATHENS;* [2]*Thriassion Hospital, ELEUSINA, Greece*

*Introduction. R*eview of peripheral blood smears can yield important diagnostic information through the identification of the morphologic changes characteristically seen in reactive neutrophils during infection. Such changes include the presence of toxic granulation, toxic vacuolization, and Döhle bodies in the cytoplasm. Younger forms (left shift), such as bands and metamyelocytes, also can be identified. This approach, however, is labor-intensive and time-consuming because it requires manual examination. Furthermore, the results are subjective because they depend on human interpretation, and only a few hundred cells can be analyzed for any given sample. The Coulter LH 750 hematology analyzer has a ability to measure specific parameters of neutrophil populations like mean and standard deviation (SD) of cell volume (MVI,SDVI), conductivity (MCI,SDCI), and light scatter (MSI, SDSI). These so-called positional parameters (PP) can detect morphologic changes in neutrophil population and can be an additional indicator for diagnosing acute infection. *Aims*. To investigate the value of the neutrophil MVI, generated by VCS technology of the Coulter LH 750 hematology analyzer, as an additional predictor of acute infection. *Material and Methods.* Total white blood cell count, percentage of neutrophils, and positional parameters data from 64 patients with positive blood cultures for bacteria and from 54 age-matched healthy control subjects were prospectively analyzed. We then studied whether changes in MVI correlated with patients' WBC counts and neutrophil percentage. We subdivided the patients into 5 groups based on WBC count: group A, WBC less than 11000/μL, group B between 11000/μL and 15000/μL, group C greater than 15000/μL and on neutrophil percentage: group D neutrophil percentage less than 85% and finally group E neutrophil percentage greater than 85%. The PP was obtained by the Coulter LH 750 hematology analyzer (Beckman Coulter, Fullerton, CA). Comparisons between means were performed by analysis of variance. Comparison between 2 means was performed by using the Student t test. A P value less than 0,05 was considered significant. *Results.* Tables 1, 2.

Table 1.

	Control	Patients	P
Number	54	64	
MVI mean	146,17	159,56	<0.001
MCI "	145,85	145,47	0,749
MSI "	146,06	137,4	<0.001

Table 2.

Groups	Control 54	A 29	B 18	C 17	D 29	E 35
MVI	146.17	158,41	160	161,06	157,9	160,94
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Conclusions. 1) The MVI increases in acute infection, while MSI decreases; 2) Using an MVI cutoff of 150, as in bibliography, seems that MVI is a good predictor of acute infection; 3) The MVI increase correlated significantly with leukocytosis and neutrophilia but was observed even in patients with white blood cell counts less than 11000/ μL or with percentage of neutrophils less than 85%; 4) As a quantitive parameter, the MVI has potential for use as an additional indicator for diagnosing acute infection.

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THALIDOMIDE INCREASES THE NUMBER OF CFU-GM AND ALTERS THE CYTOKINE EXPRESSION PROFILE IN LONG TERM BONE MARROW CULTURES FROM PATIENTS WITH MYELODYSPLASTIC SYNDROMES

M. Lazarini, F. Traina, S.M.B.. Winnischofer, M.L.S. Queiroz, F.F. Costa, S.T.O. Saad

State University of Campinas, CAMPINAS, Brazil

Background. Thalidomide is a recently *re-discovered* drug exerting a series of biological activities on hematopoiesis, including immunomodulation, anti-apoptotic, anti-inflammatory and anti-angiogenic effects. However, its mechanism of action in myelodysplastic syndromes (MDS) is still not clarified. *Aims.* We used, for the first time, long term bone marrow cultures (LTBMCs) from MDS patients to analyze the activity of thalidomide on the cytokine expression profile, apoptosis and the ability of stromal cells to support hematopoiesis. *Methods.* LTBMCs were established from bone marrow samples of five patients with MDS; 4 RA and 1 RARS (FAB classification). The cultures were treated with 10μM of thalidomide or DMSO (0.01%) and maintained for 4 weeks. At weekly intervals, when new drug was added, the cultures were fed by demidepopulation and nonadherent cells were used for clonogenic assays in

methylcellulose. On the third week, cells were analyzed by FACS for detection of cell death (Annexin V and PI). After four weeks of culture, stromal cells were collected and the expressions of IL-6, IL-10, TNF-α, IL-1β and IFNγ were verified by Real Time PCR. *Results.* In LTBMCs from 4 patients, the number of CFU-GM in the cultures treated with thalidomide increased compared with the number of CFU-GM in the control cultures, and this result was more pronounced following the second week (increase of CFU-GM varies from 10% to 80%). There was no colony formation in the culture of one patient (RA). Regarding cell death, after 3 weeks of cell culture, thalidomide treated or untreated cells from 3 RA patients had a low apoptosis rate (approximately 10%), whereas cells from 2 patients (RARS and RA) showed a reduction in apoptosis (from 35% to 26% and from 34% to 19%, respectively). Thalidomide treatment resulted in increased expression of TNF-α (2.4 to 4.6 fold), IL-10 (3.0 to 4.3 fold) and IL-6 (1.8 to 2.1 fold) in stromal cells of 4 RA patients, but no modulation was observed in the RARS patient. IL-1β expression was not affected by thalidomide treatment and IFNγ expression was not detected in stromal cells. Conclusions: Our studies showed that thalidomide has no toxic effects on hematopoietic precursors of LTBMCs, being capable of reducing apoptosis in some cases. The increased numbers of CFU-GM observed in the cultures indicate an improvement in the clonogenic potential of the myeloid cells in LTBMCs. Increased expressions of TNF-α, IL-6 and IL-10 were found in stromal cells after thalidomide treatment. Interestingly, increased IL-10 levels in $CD34^+$ MDS cells treated with lenalidomide have been previously reported. Taken together, these results suggest that thalidomide is active in MDS by modifying both bone marrow hematopoietic cells and microenvironment cells, improving hematopoiesis. Given the biologic heterogeneity of MDS, no single treatment is effective for all patients with the disease. With more detailed knowledge of cytokine signaling cascades and of mechanism of action of different drugs, including thalidomide, the future treatment of this challenging disease may lie in combination therapies customized for relevant biologic effectors.

0979

RELAPSE IN ACUTE MYELOID LEUKAEMIA (AML) WITH INV16 OR T(16;16) MAY OFTEN BE SLOW , AS SHOWN BY SEQUENTIAL RQ-PCR ANALYSES

M. Venot,[1] A. Renneville,[2] C. Gardin,[1] C. Kelaidi,[1] G. Leroux,[3] V. Eclache,[3] C. Preudhomme,[2] P. Fenaux,[1] L. Ades[1]

[1]Assistance Publique Hopitaux de Paris, Hopital Avicenne, Universite Paris 13, BOBIGNY; [2]Laboratoire d'Hématologie A, INSERM U837-Institut de Recherche sur le Cancer, LILLE; [3]Hopital Avicenne - Hematologie Biologique, BOBIGNY, France

Background.. AML with inv 16 or t (16;16),are generally associated with good prognosis but relapses still occur in 25% of patients . In AML with reciprocal translocations, molecular CR, as assessed by *classical* RT-PCR methods or by quantitative methods (RQ-PCR), often precedes hematological CR but generally only by a few weeks, in particular for APL. 2 of the 3 relapses we observed in 9 AML with inv 16 or t(16;16)studied molecularly after CR achievement occurred slowly, and are presented here. *Patients and Methods*. Between 2005 and 2007, 9 AML with inv(16)(p13q22) or t(16;16) who had reached CR in French cooperative AML trials were prospectively monitored for CBFB-MYH11 rearrangement by RQ-PCR in bone marrow cells , performed according to the EAC procedure (results expressed as the ratio of CBFB-MYH1copies per /100 ABL copies in%, as we previously reported) (Guièze ASH 2007,abstr n°: 3496). *Results.* Median follow up after CR achievement was 18 months (range 2-33) and median number of RQ-PCR analyses/patient was 6 (range 1-9). 3 patients, aged 50, 58 and 60 years, resp, had hematological (hem) relapse, 12, 17 and 23 months after CR achievement. All 3 patients had achieved at least 3 log reduction of the fusion transcript level after chemotherapy. Hem relapse was preceded in the 3 cases by molecular (mol) relapse in the bone marrow, by respectively 1, 10 (pt n°2) and 6 (pt n°3) months . In the last 2 patients, 5 and 3 RQ-PCR determinations, resp, were positive before hem relapse, showing increasing number of abnormal transcripts in both cases, from 0.083 to 10.89% in pt n°2 (and 15% at the time of hem relapse) and from 0.16 to 12.2% in pt n° 3 (and 33% at the time of hem relapse). During the period of isolated mol relapse , CBC remained normal in pt n° 3 while cytopenias appeared after 9 months in pt n° 2; karyotype was normal (20 mitoses) 5 months before hem relapse in pt n° 2 ; successive marrow aspirates showed <5% blasts in both pts but abnormal eosinophils reappeared in the marrow after 5 months in pt n° 3 . The 3 relapsing patients achieved a second CR with chemotherapy and/or gentuzumab and were subsequently allotransplanted. All 3 pts were alive, 16+, 4+ and 8+months resp , after hem relapse. *Conclusions*. Our results suggest that relapses may often occur slowly in AML with inv16 or t(16;16), with several months between molecular and hematological relapse. Therapeutic intervention when mol relapse is confirmed, and before overt relapse, could be useful in such cases.

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INITIAL REPORT ON 3 PATIENTS IN AN INNOVATIVE PHASE I STUDY OF CONCOMITANT AND CONSECUTIVE TREATMENT WITH DASATINIB AND MK-0457 IN REFRACTORY PH+ CML AND ALL PATIENTS

C. Papayannidis,[1] I. Iacobucci,[1] S. Soverini,[1] S. Paolini,[1] F. De Rosa,[1] D. Cilloni,[2] F. Messa,[2] F. Pane,[3] V. Meneghini,[4] P. Giannoulia,[1] E. Ottaviani,[1] N. Testoni,[1] B. Lama,[1] M. Baccarani,[1] G. Martinelli[1]

[1]Department of Hematology/Oncology Seràgnoli, BOLOGNA ; [2]Division of Hematology, San Luigi Gonzaga Hospital, ORBASSANO, TURIN; [3]CEINGE, University of Naples Federico II, NAPLES; [4]Dept of Hematology, University of Verona, VERONA, Italy

Background. Although first and second generation BCR-ABL inhibitors have determined a relevant clinical benefit for patients affected by Philadelphia chromosome-positive leukemias, the emergence of mutations in the ABL kinase domain still represents the main mechanism of resistance to TK inhibitors. A new promising molecular target could be constituted by Aurora kinase, a family of serine/threonine kinases which plays a crucial role in the regulation of diverse cell cycle events. Their overexpression has been detected in a wide variety of solid tumors, as well as in Ph^+ CML and ALL cells. Few compounds have been pre-clinically screened for their activity against Aurora kinases, and many of these also showed inhibition of T315I-BCR-ABL with high affinity. MK-0457 is a small-molecule, novel pan-aurora kinase inhibitor with demonstrated activity against wild-type and mutated BCR-ABL, including the T315I mutation, as well as JAK2 and FLT3. *Aims.* In our Institution, an innovative Phase I clinical study of sequential and concomitant treatment with Dasatinib, previously administered for three months, and MK-0457 has been conducted. This combined activity suggests that MK-0457, in association with Dasatinib, would be able to suppress the emergence of T315I and other resistant clone, improving upon the response rate for Dasatinib in advanced and refractory CML and Ph^+ ALL patients, as well as the durability of response. The protocol investigated two different schedules of combined therapy, that have been rationally chosen according to the achievement of hematologic response after three months of previous Dasatinib monotherapy, administered at the standard dosage (70 mg twice daily). In details, patients who achieved and maintained a major hematologic response after three months of therapy with Dasatinib received a 6-hour biweekly infusion of MK-0457, whereas patients who failed to achieve a major hematologic response received a 5-days continuous infusion of MK-0457, administered every 4 weeks. The biological rationale is based on the different activity of the drug combination: the first schedule was demonstrated to suppress the emergence of Dasatinib-resistant clones, through a stronger inhibition of BCR-ABL, whereas the second one was showed to inhibit more potently Aurora Kinases activity. *Results.* To date, two patients with Ph+ ALL and one patient with CML in myeloid blast crisis, previously unsuccessfully treated with imatinib, were enrolled in the protocol, starting with Dasatinib 70 mg twice daily, for three months. The first two patients, both in hematologic response at the beginning of the study protocol, subsequently received the 6-hour biweekly schedule of treatment. They both maintained the haematological response and no haematological toxicity can be described. The third patient, enrolled in phase of progression disease, received the 5 days MK-0457 schedule of treatment. His peripheral blood count was consistent with a severe pancytopenia, which required frequent platelets and red blood cells transfusions. His bad clinical performance status was also compromised by a severe hemorrhagic pleural effusion, responsible for moderate dyspnoea and severe asthenia. After one cycle of therapy with MK-0457, the patient obtained a complete recovery of the pulmonary disease and, after a long period of severe neutropenia, a complete hematologic response. No adverse events related to the treatment have been documented. However, dose limiting toxicities in larger monotherapy studies have been observed. *Conclusions.* The sequential and concomitant administration of Dasatinib and MK-0457 represents a promising therapeutic strategy for refractory Ph^+ CML and ALL, showing a relevant haematological activity in a limited number of patients. Assessment of the benefit/risk profile for this combination remains to be determined.

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TREATMENT AND RESPONSE MONITORING IN ADVANCED PHASE CML AND PH+ALL IN CLINICAL PRACTICE IN EUROPE: THE UNIC STUDY

G. Ossenkoppele,[1] M. Michallet,[2] E. Morra,[3] J.L. Steegmann,[4] D. Marin,[5] G. Verhoef,[6] T. Kühr,[7] M. Björeman,[8] K. Pugner,[9] V. Halkin[9]

[1]VU University Medical Centre, AMSTERDAM, Netherlands; [2]Hôpital Edouard Herriot, LYON, France; [3]Ospedale di Niguarda Ca' Granda, MILAN, Italy; [4]Hospital de La Princesa, MADRID, Spain; [5]Hammersmith Hospital, LONDON, UK; [6]University Hospital Leuven, LEUVEN, Belgium; [7]Klinikum Kreuzschwestern Wels, WELS, Austria; [8]University Hospital Örebro, ÖREBRO, Sweden; [9]Bristol-Myers Squibb International, BRAINE L'ALLEUD, Belgium

Background. Chronic myeloid leukaemia (CML) is typically diagnosed in the chronic phase and can progress to an accelerated phase and ultimately to a blast crisis. Imatinib is widely used for the treatment of CML and Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph+ALL). However, development of imatinib resistance has been reported in >70% of patients with advanced phases of CML. *Aims.* The Unmet Needs in CML (UNIC) study aimed to address knowledge gaps on how CML/Ph+ALL patients are treated and monitored in clinical practice. Here, we present data for those patients with accelerated phase CML, blast crisis CML or Ph+ALL. *Methods.* UNIC was a cross-sectional study, with retrospective chart review of patients currently treated for CML or Ph+ALL. Patients were recruited from September 2006 to March 2007. The study was designed to estimate the proportion of patients ever treated with imatinib and those imatinib-treated patients who have experienced imatinib resistance and/or intolerance (primary objectives), as well as to determine disease management patterns. A registry was collected from eight European countries of, in total, 4139 potentially eligible patients - those aged ≥18 years and treated for CML/Ph+ALL at the participating centres (academic, non-academic, private clinic or other). Case Report Forms (CRFs) were completed for eligible patients until the recruitment target was reached. Data were collected at the most recent visit and retrospectively through clinical chart review. Patients were defined as imatinib resistant/intolerant if resistance/toxicity led to a change in, or discontinuation of, imatinib use, as reported in their medical chart. *Results.* CRFs were complete and analyzable for 1599 patients, of whom 26 (2%) had accelerated phase CML, 11 (1%) had blast crisis CML, and 48 (3%) had Ph+ALL. Imatinib exposure and response monitoring patterns in these patients are shown in the Table 1. *Summary and Conclusions*. This is the largest European observational study of CML/Ph+ALL patients to date. As expected, only a small number had accelerated phase CML, blast crisis CML or Ph+ALL, limiting interpretation of the data. Nearly all patients were exposed to imatinib therapy, at least half of whom experienced imatinib resistance and/or intolerance. Response appeared to be monitored less often in real life than is recommended by the ELN for chronic phase CML patients.

Table 1.

Patients, n/N (%)	Accelerated phase CML	Blast crisis CML	Ph+ALL
Imatinib treatment			
Ever treated with imatinib	25/26 (96)	11/11 (100)	46/48 (96)
Imatinib resistant	16/25 (64)	11/11 (100)	6/46 (13)
Imatinib intolerant	16/25 (64)	8/11 (73)	17/46 (37)
Imatinib resistant and/or intolerant	22/25 (88)	11/11 (100)	20/46 (44)
Imatinib resistant and intolerant	10/25 (40)	8/11 (73)	3/46 (7)
Discontinued imatinib treatment	15/25 (60)	10/11 (91)	17/46 (37)
Imatinib dose modification	20/25 (83)	9/11 (90)	26/46 (59)
Disease monitoring			
≥1 cytogenetic analysis per patient in the last 12 months	15/22 (68)	10/10 (100)	29/42 (69)
≥1 PCR analysis in the last 12 months	16/25 (64)	11/11 (100)	46/48 (96)
≥1 mutational analyses since diagnosis in imatinib-resistant patients	6/12 (50)	3/8 (38)	3/6 (50)

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DASATINIB EFFICACY IN PATIENTS WITH IMATINIB-RESISTANT/ -INTOLERANT CHRONIC MYELOID LEUKEMIA IN ACCELERATED PHASE: 24-MONTH DATA FROM START-A

D. Rea,[1] H. Dombret,[1] D.W. Kim,[2] G. Rosti,[3] L. Roy,[4] F.T. Garzon,[5] X. Yuan,[5] J. Cortes,[6] F. Guilhot[4]

[1]Hôpital Saint-Louis, PARIS, France; [2]St Mary's Hospital, The Catholic University of Korea, SEOUL, South-Korea; [3]Institute Seràgnoli - Bologna University Hospital, BOLOGNA, Italy; [4]CHU de Poitiers, POITIERS, France; [5]Bristol-Myers Squibb, WALLINGFORD, USA; [6]MD Anderson Cancer Center, HOUSTON, USA

Background. Imatinib resistance and intolerance are important issues in advanced chronic myelogenous leukemia (CML) and 40-50% of patients with accelerated phase (AP) CML develop imatinib resistance. Dasatinib is the most potent inhibitor of BCR-ABL and is 325-fold more potent than imatinib and 16-fold more potent than nilotinib *in vitro*. During the START program, dasatinib was demonstrated to be an effective treatment for patients with CML in any phase following imatinib failure. *Aims.* To provide a more complete assessment of the efficacy and safety of dasatinib 70 mg BID in patients with CML-AP, results from the START-A trial are reported with a minimum follow-up of 24 months. *Methods.* Patients with imatinib-resistant or -intolerant CML-AP were enrolled to START-A (an open-label, international study) between December 2004 and July 2005. Dose escalation (to 100 mg BID), reduction (50 or 40 mg BID), or interruption were permitted for lack of response or toxicity. Trial objectives included: evaluation of rates and duration of complete hematologic response (CHR), major cytogenetic response (MCyR), and complete cytogenetic response (CCyR); progression-free or overall survival; and safety of the study treatment. *Results*. Overall, 174 patients with imatinib-resistant (n=161) or -intolerant (n=13) CML-AP were recruited. Median time from original CML diagnosis was 82 months (range 4-359). Prior therapy included interferon-alpha in 72% of patients and stem-cell transplantation in 13%. In addition, 52% had received a prior imatinib dose of 800 mg/d and 59% had been treated with imatinib for >3 years. Best response to prior imatinib therapy was CHR in 79% and MCyR in 33%. Prior to dasatinib, BCR-ABL mutations had been detected in 56% of patients assessed (n=162). After a median of 13.5 months (range 0.1-29.4) of dasatinib treatment, CHRs were attained by 50% of patients, while MCyRs and CCyRs were attained by 40% and 33%, respectively. Responses were achieved irrespective of imatinib status (MCyR rates: resistant 40%, intolerant 38%) and were demonstrated in patients with all but one (T315I) of 29 baseline mutations. At 24 months, MCyRs had been maintained in 61% of responding patients (median duration not reached). The 24-month progression-free survival rate was 46% (median 19.5 months) and overall survival was 72% (median not reached). Grade 3/4 neutropenia and thrombocytopenia were reported in 76% and 82% of patients, respectively. Non-hematologic toxicity was generally mild to moderate; the most frequently reported grade 3/4 events associated with treatment were gastrointestinal bleeding (11%), diarrhea (7%), dyspnea (6%), fatigue (5%), pleural effusion (5%), and fever (5%). Changes from 12-month toxicity data were minimal. *Summary and Conclusions.* Dasatinib 70 mg BID is associated with durable efficacy in patients with CML-AP following imatinib treatment failure, with median durations of MCyR and progression-free survival not reached after 24 months. The overall benefit-risk evaluation is favorable in this poor-prognosis population.

0983

LENALIDOMIDE LEADS TO A PRO-INFLAMMATORY CYTOKINE RELEASE SYNDROME IN CLL WHICH MAY HAVE IMPLICATIONS ON THE MANAGEMENT

G. Aue, N. Njuguna, B. Vire, S. Soto, C. Boss, S. Pittaluga, A.W. Wiestner

National Institutes of Health, BETHESDA, USA

Background. Lenalidomide induces clinical responses in 35-45% of patients with relapsed CLL. Proposed mechanisms of action include immune modulation, effects on tumor-stroma interactions and direct effects on signaling pathways or mRNA stability. *Aims.* To determine the role of immune modulatory effects of lenalidomide in CLL. Here, we described the clinical syndrome and biologic mechanisms of an inflammatory reaction to lenalidomide treatment in CLL. *Methods.* Adverse events were recorded, and blood, serum and lymph node biopsies were collected on 10 patients (ages 45-79) with CLL (7 Rai III and IV) partici-

pating in an IRB approved phase II single agent study using lenalidomide cycled for 3 weeks on, 3 weeks off for up to 8 cycles. Cytokines were measured in the serum using Luminex technology, lymph node biopsies were analyzed by standard immunohistochemistry, PBMCs from CLL patients were cultured in-vitro using AIM media and analyzed by flow cytometry. *Results*. Most patients (90%) had a rapid reduction (33%, 7-54%) in absolute lymphocyte count within 4 days. Tumor flare reactions (TFR) were observed in the 1st, 2nd, and 3rd cycle in 9 (90%), 4 (40%), and 2 (20%) of the patients, respectively. The TFR in cycle 1 was characterized by fever >38°C (78%), pain (78%), fatigue (78%), increase in lymph node size/ lymphocyte count (56%), sweating (56%), decreased appetite (56%), chills (44%), dehydration (33%), hypotension (33%), and tender lymphadenopathy (22%). New onset of pre-renal azotemia (GFR <60 mL/min) occurred in 3 patients between days 3-8, in the setting of a TFR. Pro-inflammatory cytokines and chemokines (IL1ra, IL6, IL10, TNFa CCL2, CCL3, CCL4, and CXCL8) were rapidly upregulated. In 5 patients, TNFa serum levels on day 8 had increased to >3 times baseline. Four of these patients experienced a prominent TFR with severe bone or abdominal pain and debilitating fatigue. *In vitro* lenalidomide is not cytotoxic for leukemic cells but in-vivo it triggers an inflammatory reaction that is more pronounced in CLL than in other hematologic diseases. We therefore tested whether lenalidomide affects cell surface expression of immune regulatory molecules on CLL cells and T or NK-cells *in vitro*. Indeed, after exposure to 2 uM lenalidomide CLL cells expressed costimulatory molecules (CD80, CD86) and CD40 on average 2-fold higher compared to untreated controls. T and NK cells showed similar, albeit weaker changes. Lenalidomide had minimal on no effect on cell surface expression of these molecules on B or T cells from a normal donor. In three patients we could analyze matched lymph node biopsies (pre-treatment and day 8). In one patient who experienced tender lymph node enlargement we found a 2-fold increase in T-cells to 740 $CD3^+$ cells/hpf, while two other patients had no apparent change in lymph node resident T-cells. *Conclusions.* Lenalidomide leads to increased expression of immune stimulatory molecules on CLL cells that can trigger an immune reaction consisting of a prominent inflammatory cytokine release syndrome, T/NK cell activation and, at least in select patients, an increase in tumor infiltrating lymphocytes.

0984

CLINICAL AND BIOLOGICAL PROFILING OF THE IGG-SWITCHED VARIANT OF CHRONIC LYMPHOCYTIC LEUKEMIA: COMPARISON TO THE COMMON IGM/MD VARIANT AND ONTOGENETIC IMPLICATIONS

N. Stavroyianni,[1] V. Tachynopoulou,[1] A. Hadzidimitriou,[1] I. Athanasiadou,[1] K. Valianatou,[2] A. Athanasiadou,[1] G. Paterakis,[3] T. Papadaki,[4] C. Georgopoulos,[5] G. Kokkini,[6] A. Papadopoulos,[7] M. Papaioannou,[7] A. Tsompanakou,[1] C. Belessi,[2] N. Laoutaris,[2] K. Stamatopoulos,[1] A. Anagnostopoulos,[1] A. Fassas[1]

[1]G. Papanicolaou Hospital, THESSALONIKI; [2]Nikea General Hospital, PIRAEUS; [3]G. Gennimatas Hospital, ATHENS; [4]Evangelismos Hospital, ATHENS; [5]424 Veterans Hospital, THESSALONIKI; [6]Sismanogleion Hospital, ATHENS; [7]Ahepa Hospital, THESSALONIKI, Greece

IgG-positive CLL is a relatively rare variant of CLL (frequency 6-15%), whose origin and ontogenetic relationship to the common IgM/IgD variant remain unknown. In the present study, we compared IgG^+ vs. IgM/D^+ CLL cases with regard to clinical presentation, immunophenotypical features, IG repertoire and outcome. In a cohort of 372 patients with typical CLL, 64 (17%) were found to express sIgG, while the remainder (308/372; 83%) expressed sIgM/D. The two subgroups ($sIgG^+$ vs. sIgM/D+) did not differ with regard to: gender; bone marrow histopathology (cytology and patterns of neoplastic infiltration of the bone marrow); CD20, CD19, CD5 and CD23 expression; CD38 expression; and light chain isotype (however a tendency was noted for association of the sIgG variant with kappa light chain expression, p=0.07). In contrast, statistically significant differences were observed in terms of the following parameters: i) median age at diagnosis: $sIgG^+$, 62 years/ $sIgM/D^+$, 66 years (p=0.03), ii) advanced clinical stage at presentation: $sIgG^+$, 9/64 Binet-B/C cases (14%)/ $sIgM/D^+$, 79/308 Binet-B/C cases (25.6%, p=0.05), iii) ZAP-70 expression: $sIgG^+$, 4/18 analyzed cases (22.2%)/ $sIgM/D^+$, 47/93 analyzed cases (50.5%, p=0.03), iv) unmutated IGHV genes: $sIgG^+$, 16/64 cases (25%)/$sIgM/MD^+$, 137/308 cases (44.5%, p=0.004), v) IGHV gene repertoire: the $sIgG^+$ subgroup was characterized by under-representation of IGHV1 genes (p=0.03) and, in contrast, over-representation of IGHV4 genes (p=0.0004), in particular the IGHV4-34 gene (20/64 $sIgG^+$ cases vs. 27/308 $sIgM/D^+$ cases, p<0.0001), vi) progression-free survival (PFS): $sIgG^+$, 34.8 months/ $sIgM/MD^+$, 14.7 months (p=0.05), vii) B cell receptor (BCR) stereotypy: $sIgG^+$, 16/64 cases (25%)/ $sIgM/D^+$, 40/308 cases (12.9%, p=0.03). In detail, two major subsets of cases with stereotyped BCRs were identified in the $sIgG^+$ subgroup. 1) The first subset included 9 cases expressing mutated IGHV4-34/IGKV2-30 BCRs with a notably young median age at diagnosis (51 years); all cases were negative for $CD38^-$; 5/9 cases carried del(13)(q12q14) as the sole chromosomal abnormality; only two of nine cases experienced progressive disease requiring treatment. 2) The second subset included 5 cases expressing unmutated IGHV4-39/IGKV1-39(1D-39) BCRs; four of five cases expressed CD38; three of four analyzed cases carried trisomy 12 as the sole chromosomal abnormality; three of five cases experienced progressive disease requiring treatment. In conclusion, the $sIgG^+$ variant of CLL is a distinctive subgroup with significant clinical and biological differences from the common $sIgM/D^+$ variant of the disease. Genetic and clinical stereotypy in the context of IgG isotype switch may be considered as further strong evidence for the important role of antigen in the development and evolution of the leukemic clone.

0985

ANALYSIS OF CD4+CD25HI REGULATORY T CELLS IN THE PERIPHERAL BLOOD OF PATIENTS WITH B CELL CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

V. Pappa,[1] V. Giannopoulou,[2] K. Spyridaki,[3] M. Chatzouli,[4] V. Karayanni,[3] E. Liakata,[2] F. Kontsioti,[2] K. Girkas,[2] A. Vassilatou,[2] S. Papageorgiou,[2] E. Giamarellos,[3] N. Laoutaris,[4] J. Dervenoulas,[2] T. Economopoulos[2]

[1]Attikon University General Hospital, ATHENS; [2]Second Department of Internal Medicine,Attikon University General Hospital, ATHENS; [3]Fourth Department of Internal Medicine, Attikon University General Hospital, ATHENS; [4]Hematology Department, General Hospital of Nikea, ATHENS, Greece

Background. Naturally occurring regulatory T cells (T reg) prevent autoimmune and inflammatory diseases, represent one important factor contributing to the inhibition of antitumor immune response in cancer and therefore constitute a potential target for immunotherapy. CLL is a disease characterized both by immunodeficiency and sometimes by autoimmunity. *Aims.* We analyzed the frequency of Treg population in the peripheral blood of patients with B-CLL and correlated them with clinical and laboratory characteristics. *Methods.* We analyzed prospectively 49 patients with B-CLL (29 males, 20 females) with median age of 63 and 24 normal healthy volunteers. Freshly isolated peripheral blood mononuclear cells were analysed by flow cytometry using the EPICS XL/MSL cytometer (Beckman Coulter). We determined the level of apoptosis by the method of annexin-V and the frequency of Treg as cells positive for CD4, CD25hi and intracellular staining of Foxp3 using the PE anti-Human Foxp3 staining set protocol. Mutation status of IGHV genes was determined by RT-PCR and direct sequencing. We recorded clinical information regarding Rai and Binet staging, hematological and biochemical parameters and the presence of autoimmune hemolytic anemia. *Results.* The level of apoptosis as determined by the annexin V method was significantly lower on $CD19^+$ cells of patients compared to normal controls (4,7 *vs* 11,34, p=0.02). Moreover patients under treatment had significantly lower apoptosis level *vs* untreated (4 *vs* 6,4, p=0,023). Δhe mean and median values of Treg cells in patients with CLL were higher but not significantly compared to controls. However the log10 values of the Treg frequencies were significantly higher in the group of CLL (0,6287 *vs* 0,1021, p=0,03). There was not any statistically significant association of Tregs with age, Rai and Binet stage, LDH values and the level of apoptosis. Cases with CD38 expression >7% had significantly lower median values of Treg (11,83 *vs* 1,28, p=0,01). This difference was not observed when the cutoff level of CD38 expression used was at 30%. Unmutated cases had significantly higher median Treg values compared to mutated (14,2 *vs* 0,3, p=0,02). Furthermore, unmutated cases presented higher median CD38 expression and lower apoptosis level, even if these differences were not statistically significant. *Summary and Conclusions.* The log10 values of Treg frequencies in patients with B-CLL were significantly higher compared to normal controls in accordance with previously published data. We did not observe any significant association with any laboratory or clinical characteristics or the level of apoptosis. Importantly unmulated CLL cases were associated with significantly higher Treg values providing one more explanation for a defective antitumor immune response and an aggressive behavior. Functional studies of these cells are under way to shed more light on their exact role in the pathogenesis of the disease.

0986

IMMUNE THROMBOCYTOPENIA IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS TREATED WITH CHLORAMBUCIL OR CLADRIBINE

J.Z. Blonski,[1] J. Trelinski,[1] K. Chojnowski,[1] L. Konopka,[2] B. Ceglarek,[2] M. Calbecka,[2] A. Kostyra,[2] B. Stella-Holowiecka,[2] J. Kloczko,[2] K. Warzocha,[2] I. Seferynska,[2] A. Dmoszynska,[2] M. Kowal,[2] K. Lewandowski,[2] J. Dwilewicz-Trojaczek,[2] E. Wiater,[2] K. Kuliczkowski,[2] S. Potoczek,[2] A. Hellmann,[2] A. Mital,[2] A. Skotnicki,[2] M. Piotrowska,[2] K. Sulek,[2] A. Zdunczyk,[2] J. Dybowicz,[2] K. Zawilska,[2] T. Robak[1]

[1]Med Univ of Lodz, LODZ; [2]PALG-CLL[1]&,[2] LODZ, Poland

Introduction. Immune thrombocytopenia (IT) is second frequent autoimmune condition associated with chronic lymphocytic leukemia (CLL). As reported recently IT can be also triggered by fludarabine. The aim of this study was to compare the frequency of IT in CLL patients treated with chlorambucil or cladribine. *Patients and Methods.* Study population consisted of 777 patients treated in 1999-2004 years according to two randomized clinical trial protocols coordinated by Polish Adult Leukemia Group (PALG) CLL1&2. The details of the treatment schedules were published previously (Blood, 2000; 96(8):2723, Blood 2006;108(2):473). In 104 patients chlorambucil plus prednisone and in 673 cladribine alone or in combinations was applied. In the cladirbine group 187 were given monotherapy, in 128 combination therapy with prednisone (CP), in 170 with cyclophosphamide (CC) and in 188 with cyclophosphamide and mitoxantrone (CMC) were used. IT patients had to fulfill the following diagnostic criteria: rapid and severe fall of the platelet count, normal or augmented number of megakariocytes in bone marrow, no reaction to platelet transfusions, no palpable splenomegaly and no chemotherapy in the last 30 days. No patient had IT before enrolment to any cytotoxic treatment. *Results.* In chlorambucil group IT was diagnosed in 5/104 patients (4.8%) what was less frequent than in cladribine group 50/673 (7.43%), but without statistical significance. The differences in IT prevalence between patients treated with cladribine in monotherapy - 6.9%, or in combination: CP - 9.4%, CC - 8.8% or CMC - 6.9%, were also not significant. Overall survival (OS) was shorter in patients with IT both in chlorambucil and cladribine groups but with no significance. Markedly lower remission rate (RR) in IT patients treated with cladribine (63% *vs* 82%, p=0.004) in contrary to patients with IT in chlorambucil group (40% *vs* 58%, p=0.36) was observed. *Conclusions.* The application of cladribine either in monotherapy or in combination did not significantly increase the frequency of IT in the studied population. Although the remission rates in IT patients treated with cladribine was lower than in IT patients treated with chlorambucil the occurrence of this autoimmune complication had no significant influence on overall survival.

0987

CODOX-M/IVAC CHEMOTHERAPY IS AN EFFECTIVE THERAPY FOR HIV-ASSOCIATED BURKITT'S LYMPHOMA (BL)

S. Montoto,[1] J. Wilson,[2] K. Shaw,[3] M. Heath,[1] A. Wilson,[1] C. Orkin,[4] C. McNamara,[2] M. Bower,[3] M. Johnson,[5] K. Cwynarski[2]

[1]Barts and the London School of Medicine and Dentistry, LONDON; [2]Department of Haematology, Royal Free Hospital, LONDON; [3]Department of Medical Oncology, Chelsea & Westminster Hospital, LONDON; [4]Infection and Immunity Department, Barts and the London, LONDON; [5]Department of HIV Medicine, Royal Free Hospital, LONDON, UK

Background. The outcome of patients with HIV-associated Burkitt's lymphoma (HIV-BL) treated with infusional regimens remains poor, even in the HAART era. In contrast, promising results have been published in HIV-BL with the same intensive chemotherapies used in the non-HIV population. *Aims.* The objective of this multicentre study was to analyse the outcome of patients with HIV-BL, treated with the intensive chemotherapy regimen CODOX-M/IVAC and HAART. *Patients and Methods.* 25 patients (20 male; median age: 38) from 3 UK centres consecutively treated with CODOX-M-IVAC from 2003 to 2007 were included in the study. CODOX-M/IVAC consisted of 2 alternating cycles of CODOX-M (cyclophosphamide 800 mg/sqm day 1 and 200 mg/sqm days 2-5, doxorubicin 40 mg/sqm day 1, vincristine 1.5 mg/sqm days 1 and 8, methotrexate 3 g/sqm day 10) and IVAC (etoposide 60 mg/sqm days 1-5, ifosfamide 1 g/sqm days 1-5, cytarabine 1g/sqm bd days 1 and 2) for patients with high-risk disease and 3 cycles of CODOX-M for patients with low-risk disease. High-risk disease was defined by the presence of at least 2 of the following: stage III-IV, ECOG>2, extranodal sites>2 or high serum LDH. All patients received concomitant treatment with HAART (NRTI + NNRTI: 18; NRTI + PI: 7) and prophylactic antimicrobials as well as intrathecal prophylaxis and G-CSF as per protocol. Four patients received rituximab in combination with the chemotherapy. *Results.* HIV was diagnosed concomitantly with BL in 11 patients. The median CD4 at BL diagnosis was 159 (range: 4-848) and HIV viral load was undetectable in 2 (8%) patients. Six (26%) of the patients previously known to have HIV infection were on HAART before the diagnosis of BL. The majority of the patients (72%) had high-risk disease. The IPI was high (3-5) in 12 (48%) patients. BM involvement was detected in 4 (16%) patients, CNS infiltration in 4 (16%), and 16 (64%) presented with stage III-IV. Grade 3-4 non haematological toxicity was as follows: infection, 73% of the cycles; mucositis, 8%; and diarrhoea, 8%. Eight patients died during treatment, due to disease progression in 3 cases, toxicity in 4 (3 infections, 1 GI bleeding) and 1 patient died with a CNS lesion that was not biopsed. Response at the end of treatment was as follows: complete response (CR)/CR uncertain (CRu): 13 patients (52%); partial response (PR): 4 (16%); treatment failure: 8 patients (32%). One patient in CR died of HIV-related causes HAART non-compliance. After a median follow-up of 14 months (range: 5-60), 14 patients remain alive without disease progression, whilst 2 patients (1 CR, 1 PR) have relapsed at 2 and 1 months after finishing treatment and died. Three patients in PR at end of treatment survive without evidence of disease progression at 20, 27 and 48 months. Overall survival (OS) and disease-free survival (DFS) at 2 years were 52% and 85%, respectively. *Summary and Conclusions.* This retrospective analysis demonstrates that the intensive regimen CODOX-M/IVAC is feasible and effective in HIV positive patients on HAART with BL. Whether concomitant treatment with rituximab will improve outcome warrants further study.

0988

EFFICACY AND TOXICITY OF A NEW SHORT-TERM HIGH INTENSIVE PROTOCOL BL-M-04 FOR ADULT PATIENTS WITH BURKITT LYMPHOMA: INTERMEDIATE RESULTS

E.A. Baryakh, S.K. Kravchenco, A.M Kremenetskaja, E.E Zvonkov, A.I Vorobjov

Russian Hematological Scientific Center, MOSCOW, Russian Federation

Background. Burkitt lymphoma (BL) is the most aggressive B-cell lymphoid neoplasm, whose growth fraction approximates 100%. BL has specific chromosomal abnormalities (t(8;14)(q24;q32), rarely - t(2;8)(p12;q32), t(8;22)(q24;q11)). Despite rapid proliferative rates BL is one of the most chemosensitive lymphoid neoplasms. *Aims.* to evaluate the efficacy and toxicity of therapy protocol BL-M-04 for adult patients with Burkitt lymphoma (BL). *Methods.* 30 patients: twenty seven previously untreated and three pretreated patients with BL (they had specific for BL cytogenetic aberration t(8;14)(q24;q32)) were eligible for our study. All the patients (19 males and 11 females, mean age 26 years (from 15 to 56 years)) participated in the study performed in the Russian Hematological Research Center between August 2003 and November 2007. The treatment was based on experimental high intensive protocol BL-M-04. BL staging criteria developed by S. B. Murphy were used to stage the patients. Stage I, II, III, IV was diagnosed in 1, 2, 15 (50%), 3 patients respectively. B-acute lymphoblastic leukemia (L3) was diagnosed in 9 (30%) patients. B-symptoms (night sweets, fever and weight loss) were revealed in 25 (83%) patients. Serum lactate dehydrogenase level (LDH) was increased in 25 (83%) patients. Acute renal failure was found in 10 patients: specific renal involvement in 6 patients, ureter compression with urine flow block and postrenal anuria by abdominal, retroabdominal and/or pelvis tumor in 2 patients, tumor lysis syndrome with uric acid nephropaty in 2 patients. Hemodilysis was used in 4 patients, urgent nephrostomy - in 1 patient. Acute renal failure regressed due to the institution of chemotherapy and intensive care in 5 patients. The main aim of a new treatment regimen was an intensification and treatment duration reduction in patients with BL. The new treatment protocol is based on modified NHL-BFM-90 protocol for a group of high risk patients (methotrexate dose 1500 mg/m^2). BL being a chemosensitive tumor which regresses after 1-2 courses of chemotherapy we decided to treat BL in 4 courses (2 inductional and 2 consolidational) irrespective of the initial tumor mass. As BL is the most sensitive to high dose methotrexate and cytarabine we used these drugs in the induction phase to achieve the maximum cytoreductive effect. Courses A and C were used to achieve remission. Doxorubicine was added to course A, methotrexate - to course C. Consolidation courses were similar to induction courses. Hence, we used A and C courses (without course B), intensified with course B drugs (doxorubicin and methotrexate), the interval between the courses being 21 days. *Results*. twenty seven patients (90%) achieved

a complete remission (CR) after 1-2 courses (12 patients - after the 1st course, 15 - after the 2d). Twenty six are alive in the first CR during 25 months (median 2-48 months). Four patients died. The cause of death was chemotherapy complications: in three cases and early relapse in one. A 3-year disease-free survival - 97%, overall survival - 87%. BL-M-04 therapy resulted in higher CR rates and longer disease-free survival in adult patients with BL and confirmed high efficacy of short-term intensive therapy. Treatment duration was 3-3,5 months. Grade III-IV neutropenia toxicity was universal. Most infectious and hemorrhagic complications were registered during the first course A, which can be explained by the initial poor performance status of the patients. Unfavorable prognostic factors, which increase the number of chemotherapy complications, are: stage IV/B-ALL, acute renal failure, inadequate previous treatment (surgery and chemotherapy). *Summary*. BL-M-04 is a highly effective protocol: 3-year disease-free survival -97%, 3-year overall survival -87%. The use of this protocol can achieve rapid tumor mass regression and treatment duration reduction, due to chemotherapy intensification and acceptable toxicity. As the majority of relapses occur after 8-12 months of treatment, we can speak of full recovery in most patients.

0989

DOSE-DENSE THERAPY (R-CHOP14) SEEMS TO OVERCOME THE NEGATIVE PROGNOSTIC SIGNIFICANCE OF B CELL ORIGIN IN DLBCL LYMPHOMA PATIENTS

L. Rigacci,[1] M. Martelli,[2] B. Puccini,[3] S. Di Lollo,[4] E. Finolezzi,[2] L. Nassi,[3] M. Doria,[4] R. Alterini,[3] V. Carrai,[3] A. Bosi[3]

[1]Azienda Ospedaliero Universitaria Careggi, FLORENCE; [2]Hematology Institute University La Sapienza, ROMA; [3]Department of Hematology Azienda Ospedaliero Universitaria Careggi, FLORENCE; 4Department of Pathology Azienda Ospedaliero Universitaria Careggi, FLORENCE, Italy

Background. Diffuse large B cell lymphoma (DLBCL) is one of the most common types of non-Hodgkin's lympoma. Approximately half of all patients (pts) will be cured of their disease by primary therapy the remaining die of the disease. Gene-expression profiling in DLCBL has brought an insight into the biological heterogeneity of the disease. Two major subgroups were identified: germinal centre B (GCB) cell or non-germinal centre (non-GCB). In some recent papers the GCB group showes a significantly better survival than the non-GCB group. Immunohistochemistry has been evaluated as a surrogate for this molecular classification. *Aims.* The aim of this study was to define retrospectively the B-cell origin of 40 pts treated with R-CHOP14 and to evaluate if the dose-dense immuno-chemotherapy could improve their clinical outcome. *Methods.* We performed a centralized immunohistochemical stains on formalin-fixed paraffin-embedded tissues from diagnostic biopsies with the following antibodies: CD10, bcl-6, bcl-2, MUM1 and Mib1. Based on the algorithm published by Hans *et al.* we subdivided the patients in GCB origin and non-GCB origin. We evaluated also the prognostic value of single protein expression. *Results.* Twenty-seven pts were male and 13 female, 18 were stage I-II and 22 stage III-IV, 17 presented symptoms at diagnosis and 17 showed bulky disease. Twenty-two pts showed abnormal LDH value, the IPI was intermediate-high risk or high risk in 13 pts. According to immunohistochemistry analysis 16 pts derived from germinal centre and 24 from non-germinal centre, 12 pts presented a positive CD10, 30 a positivity for bcl6, 19 a positive bcl2 and 27 a positive MUM1. Twenty-nine pts (73%) obtained a complete remission (CR), 8 a partial response (PR) and 3 were non responders (NR). Four out 29 CR pts experienced relapse, three (75%) derived from non-germinal centre. Eight pts died, 4 derived from GCB and 4 from non-GCB. After a median period of observation of 16 months (range 3-70 months) the overall survival (OS) was 75% and the failure free survival (FFS) was 57%. The statistical analysis was performed comparing the B cell origin and clinical characteristics, moreover was also evaluated the expression of bcl2 either in GCB or in non-GCB lymphomas. In univariate analysis normal β2 microglobulin and low-intermediate risk IPI were significantly associated with longer overall survival. In univariate and multivariate analysis FFS was significantly higher in low and low-intermediate IPI risk patients it was the only factor that influenced the FFS. No differences were reported in OS and FFS evaluating the B cell origin. *Conclusions*. In conclusion even if few patients were evaluated we can point out that the intensification could enhance the efficacy of R-CHOP regimen improving the overall survival and FFS in patients with non-GCB lymphoma. In this analysis the only significance was the IPI index that affected either OS or FFS. Further analysis with larger sample sizes of DLBCL patients are needed to verify this preliminary observations.

0990

PROGNOSIS VALUE OF POSITRON EMISSION TOMOGRAPHY USING FLUORINE 18-FLUORODEOXYGLUCOSE BEFORE AND AFTER AUTOLOGOUS TRANSPLANTATION IN HIGH GRADE LYMPHOMAS

V. Roland, C. Bodet-Milin, T. Gastinne, P. Moreau, F. Kraeber-Bodéré, J.L. Harousseau, S. Le Gouill

Nantes Medical University, NANTES, France

Background. Positron emission tomography using fluorine 18-fluorodeoxyglucose ([18F]FDG-PET) is required to evaluate response in high grade non-Hodgkin lymphoma (HG-NHL) and Hodgkin's disease. However, only limited studies assessed the interest of [18F]FDG-PET and its prognostic value for high-grade NHL patients before and after autologous stem cells transplantation (ASCT). *Aims.* The aim of this single-institution study was to evaluate the prognosis value of [18F]FDG-PET. We retrospectively analysed a cohort of 42 HG-NHL patients treated between 2002 and 2005 in Nantes Medical University. *Methods.* Patients characteristics were as followed: male gender in 23 cases; median age at diagnosis was 50 years [16-65], all patients had a HG-NHL (DLBCL in 34 cases and Richter's syndrome in 8 cases), a stage IV disease in 21 cases, bulky disease in 21 cases (7 cm), IPI score >1 in 25 cases. ASCT was performed as part of the first-line therapy in 32 cases (76%) followed CHOP regimen as initial chemotherapy (with Rituximab in 5 cases) or at the time of relapse followed R-DHAP regimen (n=10). Conditioning regimen consisted of BEAM in all cases (except one patient who received melphalan alone). Eighteen patients received radiotherapy after ASCT: they either had a positive [18F]FDG-PET before ASCT or an initial bulky disease (n=6). [18F]FDG-PET and computed tomography (CT) were systematically performed at two time points: before and 3 months after ASCT. [18F]FDG-PET images was analyzed by local nuclear medicine physician in a retrospective blind control. *Results.* The median follow-up (from the time of ASCT) for alive patients was 30 months [8-49 months]. 2 years EFS and OS rates for all patients were: 85,7% and 90,5% , respectively. According [18F]FDG-PET images before and after ASCT, patients were classified into 4 groups: NEG/NEG (n=25), NEG/POS (n=0), POS/POS (n=8) and POS/NEG (n=9). Among NEG/NEG patients, fourteen were in PR according to CT staging. In this group, three patients experienced relapse (two patients died because of disease progression). Interestingly, sites of relapse were bone and spinal fluid which are not well expertized by [18F]FDG-PET. One patient had a follicular lymphoma at relapse. In the POS/NEG group, seven patients were in PR according to CT staging and no patients relapsed. In comparison, three patients out of 8 relapsed in the POS/POS group. In this last group, six patients reached PR according to CT. The 2 years EFS and OS rates for NEG [18F]FDG-PET and POS [18F]FDG-PET after ASCT patients were: 91,2% versus 62,5% and 94,2% versus 75%, respectively. Thus, 92% of the patients with a NEG [18F]FDG-PET after ASCT (n=34) remained disease free versus only 63% (n=8) for POS [18F]FDG-PET patients. *Conclusions.* Our study demonstrate that only [18F]FDG-PET after ASCT is correlated with a better EFS. As expected patients with a negative [18F]FDG-PET after ASCT have a better EFS as compared to patients with a positive [18F]FDG-PET. In our series, [18F]FDG-PET before ASCT has no prognosis value on both EFS and OS (EFS at 2 years: negative before ASCT 88% versus 83% for [18F]FDG-PET positive patients) suggesting that ASCT should not be reported because of a [18F]FDG-PET remaining positif. This results will have to be confirmed in a larger prospective study.

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R-CHOP + THALIDOMIDE FOR PREVIOUSLY UNTREATED MANTLE CELL LYMPHOMA

W. Drach,[1] G. Hopfinger,[2] M. Fridrik,[3] R. Greil,[4] J. Thaler,[5] F. Keil,[6] A. Lang,[7] U. Jaeger,[8] M. Raderer[8]

[1]Medical University of Vienna, VIENNA; [2]Hanusch KH, [3]rd Dept. of Medicine with Hematology, VIENNA; [3]AKH Linz, Dept. of Oncology, LINZ; [4]Paracelsus University, Dept. of Oncology, Hematology, Immunology & Rheumatology, SALZBURG; [5]KH Wels, Dept. of Medicine, WELS; [6]KH Leoben, Dept. of Hematology and Oncology, LEOBEN; [7]LKH Feldkirch, Dept. of Medicine, FELDKIRCH; [8]Medical University of Vienna, Dept. of Medicine I, VIENNA, Austria

Background. Despite favorable response rates of R-CHOP in mantle cell lymphoma (MCL), R-CHOP has only a moderate effect on progression-free survival (PFS), which highlights the need for additional therapeutic strategies in MCL. We have previously shown that rituximab plus

thalidomide (Thal) has marked activity in patients (pts) with relapsed/refractory MCL (Blood 2004;104:2269). To further explore the role of Thal in MCL, we have performed a phase II trial of rituximab plus Thal combined with standard CHOP in pts with previously untreated MCL (NHL-11 trial of the Austrian AGMT - Working Group Medical Oncology). *Methods.* Treatment consists of standard R-CHOP being administered every 3 weeks for a total of 6 cycles. In addition, Thal is administered concomitantly with R-CHOP at a daily dose of 100 mg during the first cycle, and increased to 200 mg at cycle 2 and beyond (continued at this dose level throughout R-CHOP and as maintenance until progression or relapse). Dalteparin (5000 I.E. sc daily) is included as prophylaxis for venous thromboembolism. Pts with CD20 positive, newly diagnosed MCL up to the age of 75 years are eligible. The primary endpoint is the activity of R-CHOP-Thal on PFS, secondary endpoints are tolerability, overall response, CR-rate and survival. *Results*. To date, we have enrolled 33 pts (median age, 65 years; range, 51 to 75 years). A planned safety interim analysis after the first 10 pts revealed grade III peripheral neuropathy in 1 pat (concomitant diabetes mellitus) and pulmonary embolism in 1 pat, who received full oral anticoagulation due to atrial fibrillation. The low rate of venous thromboembolism is further documented by only one deep venous thrombosis among 30 pts receiving R-CHOP-Thal and dalteparin prophylaxis. No unexpected adverse events of R-CHOP were noted by the addition of Thal. All 10 pts experienced a response to R-CHOP-Thal (with 8 patients in CR; 4 pts were also negative by FDG-PET). Results are currently updated and evaluated in comparison with a reference population of MCL patients treated with CHOP/R-CHOP without Thal, and data will be presented at the meeting.

0992

Y90-IBRITUMOMAB TIUTEXAN RADIOIMMUNOTHERAPY FOR FOLLICULAR NON HODGKIN'S LYMPHOMA IN RELAPSE AFTER AUTOLOGOUS TRANSPLANT OR REFRACTORY TO CHEMOTHERAPY

M. Capponi,[1] F. Falcinelli,[1] L. Flenghi,[1] P. Minga,[1] F. Falzetti,[1] I. Sabalich,[2] B. Palumbo,[3] A. Tabilio[4]

[1]Hematology and Clinical Immunology, PERUGIA; [2]Nuclear Medicine, Ospedale S. Maria della Misericordia, PERUGIA; [3]Nuclear Medicine, University of Perugia, PERUGIA; [4]Hematology, University of L'Aquila, L'AQUILA, Italy

Background. The relapse rate after autologous stem cell transplant (ASCT) ranges from 30 to 35% in patients with follicular lymphoma (FL). As they have already been heavily treated, administration of standard chemo- and immuno- therapies is often not feasible. Radioimmunotherapy offers tumour eradication by a monoclonal antibody bound to a beta-emitting radioisotope and has provided encouraging results in patients in relapse after high dose therapy and ASCT (Jacobs Cli Cancer Res 2005; 11(19 suppl):7146s, Vose JM Leuk Lymphoma 2007; 48(4):683). Y90 Ibritumomab tiutexan, which has been approved by the FDA and the EMEA is available only for adults with FL who are in relapse or are refractory to Rituximab treatment. *Aims.* To assess safety of, and response to, (as determined by progression- and event- free survival) radioimmunotherapy with standard dose Y90 Ibritumomab tiutexan for FL in relapse after ASCT. *Methods.* We recruited 11 adult patients with FL: 4 who were in relapse after ASCT (i.e. supralethal chemotherapy±radiotherapy followed by autologous haematopoietic stem cell rescue) performed at least 6 months previously and 7 who were partially refractory to standard chemotherapy. Inclusion criteria were: satisfying FDA recommendations for treatment with Ibritumomab i.e. previous treatment with the anti-CD20 monoclonal antibody, under 25% bone marrow infiltration, platelet count ≥100000/mmc and neutrophil count ≥1500/mmc. Patients in relapse after ASCT had autologous haematopoietic stem cells ($CD34^+$ ≥3×10^6/kg) to be used for rescue only when grade 4 thrombocytopenia persisted for more than 4 weeks, severe neutropenia did not respond to lenograstim or was associated with severe infections or the patient was dependent on blood transfusions for more than 12 weeks. In all patients pre-treatment with rituximab was followed by administration of standard doses of Y90 Ibritumomab tiutexan (0,4 mCu/kg). *Results.* To date we have treated 4 patients in relapse after ASCT and 7 who were refractory to standard chemotherapy. One relapsed patient had received total body irradiation (TBI). Three of the patients in relapse after ASCT are evaluable. After radioimmunotherapy there was platelet loss (grade 2-3 WHO) and white blood cell loss (grade 1-2), but no major anaemia. All parameters recovered by week 12 post-therapy. Two patients required G-CSF support. Autologous rescue and transfusions were not needed. No patient presented major extra-hematological toxicity, not even one with chronic HBV hepatitis who was under therapy with lamivudine. No changes were observed in liver function indices and HBV-DNA negative status was maintained. At a median follow-up of 246 days, all 3 patients have an excellent quality of life and maintain complete response. The 7 refractory patients have a median follow-up of 398 days: 5 maintain a complete response and excellent quality of life; the two non-responders underwent salvage chemotherapy. Hematological toxicity did not differ from relapsed patients; no patient presented extra - hematological toxicity. *Conclusions.* Radioimmunotherapy with Ibritumomab tiutexan seems safe even in patients with FL who have already received supra-lethal chemo- and/or radio- therapy.

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SUCCESSFUL RESPONSE TO RITUXIMAB IN TWO CASES OF ACQUIRED HAEMOPHILIA REFRACTORY TO STANDARD-THERAPY

P. Machado, J.M. Raya, T. Martin, L. Morabito, M.J. Rodriguez-Salazar, L.M. Perez-Hernandez, M.L. Brito, J.M. Rodriguez-Martin

Hospital Universitario de Canarias, LA LAGUNA, Spain

Background. Acquired autoantibodies against coagulation factors (acquired haemophilia) constitute a life-threatening bleeding situation requiring a prompt therapeutic intervention, including control of bleeding and eradication of the inhibitor. The combination of oral corticosteroids and cyplophosphamide seems to be effective to eradicate the autoantibody, but some patients may be resistant. Another therapeutic approach, recently described, observes treatment with the chimeric anti-CD20 monoclonal antibody rituximab. *Aims.* We report two consecutively treated patients whose acquired FVIII inhibitors did not respond to standard immunosuppressive regimens. Complete response and prolonged remission were obtained when rituximab was added to therapy. *CASE #1.* A 59-year old male with antecedents of bronchial asthma presented with a great hematoma in the back. Haemoglobin was 68 g/L and MCV 91 fL: aPTT 61 seconds (control 24), which did not correct when normal plasma was added. Factor VIII level was 1.7% (normal 70-140). An inhibitor with anti-factor VIII specificity was detected (titre of 85 Bethesda units, BU). Initial treatment included packed red blood cell transfusion, recombinant FVIIa (a bolus of 90 μg/Kg), prednisone (1 mg/Kg orally daily) and intravenous immunoglobulins (1 g/Kg/day, x2). After several days, intravenous cyclophosphamide (150 mg daily) was added because of persistence of bleeding. After one week, aPTT followed between 2-2,5 times over the control, reason why cyclophosphamide dosage was increased to 200 mg, and rituximab was initiated (375 mg/m^2 every week). Globally, twelve dose of rituximab were necessary to aPTT and FVIII normalization. Twenty months after the end of the treatment, the patient follows in remission. *CASE #2.* A 40-year old female with a history of eutocic delivery eight months before, attended our clinic referring the sudden, spontaneous appearance of a hematoma in the left arm. Haemoglobin was 118 g/L, MCV 86 fL, aPTT 46/27 seconds (which did not correct when mixing with normal plasma) and FVIII level 13.5%. An anti-FVIII inhibitor was identified (titre of 1.7 BU). Ambulatory treatment with prednisone (1 mg/Kg/day) was started; lack of analytical response induced the addition of cyclophosphamide (100 mg/day) two weeks later. After two weeks, the appearance of new multiple hematomas in legs and arms and aPTT 69/25 s, determined the administration of intravenous immunoglobulin (1 g/kg/day, x2) and azathioprine (50 mg/day), and cyclophosphamide dose was increased to 150 mg. Four weeks later aPTT was 65/25 s, and rituximab (375 mg/m^2 every week) was introduced. The patient received four doses of rituximab, new hemorrhagic episodes did not occur, and a progressive normalization of aPTT and factor VIII activity was observed. After two years from the end of treatment, the patient remains in remission, asymptomatic, with normal aPTT and Factor VIII level. *Conclusions.* Some questions about the use of rituximab in these situations (exact mechanism of action, optimum therapy schedule, differential utilisation depending on inhibitor titres, long-term side effects) remain without a precise answer, but its valuable effect makes this agent a therapeutic option for patients with acquired haemophilia. Although the rarity of acquired haemophilia makes difficult large, prospective, randomized trials, efforts must be done to make such a study.

0994

OFF-LABEL USE OF ACTIVATED RECOMBINANT FACTOR VII. A SPANISH THREE LEVEL HOSPITAL'S EXPERIENCE

R. Perez-Montes, B. Gonzalez-Mesones, M. Ruiz, A. Uresandi, J. Nuñez, C. Sedano, A. Iriondo

HUMV, SANTANDER, Spain

Introduction. Although numerous studies about the use of activated recombinant Factor VII (rFVIIa) in hemophilic patients with inhibitors, congenital FVII deficiencies, and Glanzman´s trombasthenia have been published, the role of this hemostatic agent in other not approved indications is yet not well defined. *Objetives.* To evaluate efficacy and safety of rFVIIa in a wide variety of pathologies with critical bleeding refractory to standard treatment. *Patients and Methods.* From January 2003 to March 2008, 41 patients (27 males /14 females) with massive hemorrhage and no response to standard hemostatic support received rFVIIa under off-label use in our centre. Median age was 54 years (range 15-87 years). The patients received a median dose of 90 mg/Kg (single dose). *Results.* We observed efficacy without adverse events in 30/41 patients (73%), 24 of them (58%) were alive 30 days after treatment and the other six patients died due to infectious causes. Indications and efficacy in these clinical situations are described: Critical bleeding in major trauma (11, efficacy 8/11, 72%), cardiovascular surgery (12, efficacy 7/12, 58%), thrombopathy refractory to platelets transfusion (4, efficacy 4/4, 100%), massive perioperative hemorrhage, post-cesarean or delivery (6, efficacy 5/6, 83%), pulmonary hemorrhage in hemopathies (3, efficacy 1/3, 33%), bleeding in cirrhotic patients (3, efficacy 0/3, 0%), and massive abdominal hemorrhage in amyloidosis and after liver biopsy (2, efficacy 2/2, 100%). Normalization of coagulation tests, specially the protrombine time was observed in 85% of patients (35/41), as well as a noted reduction in blood requirements, red cell units (p=0.008), fresh frozen plasma (p=0.006) and platelets (p=0.045). We observed an impressive response in 4 patients with life-threatening hermorrhage and coagulation tests in normal range. *Conclusions.* The great variety of clinical situations complicated with critical hemorrhage, makes it extremely difficult to get treatment guidelines supported by controlled trials. In our experience, rFVIIa showed efficacy in 73% of critical patients, when other hemostatic measures had failed, and without thrombotic events. We observed less efficacy in cirrhotic and haematological patients, as well as a impresive response in a group of patients with life-threatening bleeding and coagulation tests in normal range. Furthermore, the reduction of blood requirements could avoid or diminish complications related to a massive transfusion

0995

COMPARATIVE MEASUREMENTS OF FVIII INHIBITORS IN HEMOPHILIA A PATIENTS BY ELISA AND BETHESDA ASSAY

S.Y. Kim, W.I. Lee, S.Y. Kang

East-West Neo Medical Center, Kyung Hee University College of Medicine, SEOUL, South-Korea

Background. Factor VIII (FVIII) inhibitors of hemophilia A patients have usually polyclonal IgGs antibodies that neutralize or inhibit coagulation, after or during therapy of factor VIII. The responses of factor antibodies include noninhibitory antibodies against nonfunctional epitopes of FVIII as well as inhibitory antibodies. *Aims.* The most commonly used method for detection of antibody response to FVIII in hemophilia patients is Bethesda assay. However, there are some difficulties to quantitate the low and high titers inhibitors as well as detect non inhibitory antibodies. ELISA assay could be suggested to detect nonhibitory antibodies with high sensitivity and specificity and with other advantages. *Methods.* In this study, 75 samples with hemophilia A were de-identified by Bethesda assay and ELISA assay. *Results.* Antibody incidence was 16.6%, of which concordance rate between ELISA and Bethesda assay was 65.7% (23/35) and discrepancy rate was 34.3%(12/35) (Table 1).

Table 1. Results of ELISA and Bethesda assay.

		Bethesda assay		Total
		+	–	
ELISA	+	4	12	16
	–	0	19	19
Total		4	31	35

The discrepant cases with negative by Bethesda and positive by ELISA were younger age and their initial titers of Bethesda unit were lower than patients with concordance (Table 2). *Conclusions.* These findings indicate that we should review of Bethesda assay to detect FVIII antibody according to clinical correlation and standardize all the procedures. Moreover, ELISA method to detect FVIII antibody should be considered supplementary method or replacement for rapid screening of factor VIII inhibitors.

Table 2. Sample data of ELISA positive and Bethesda negative results.

Samlpe	Age	Initial VIII	Initial Bethesda assay(BU/ml)	ELISA	Secondary* Bethesda assay(BU/ml)
1	18	2	1	597	-
2	36	4	0.5	901	-
3	17	3	1.3	859	-
4	36	30	0.3	829	-
5	24	0	0.3	681	-
6	36	30	0.3	651	-
7	36	30	0.3	561	-
8	36	30	0.3	788	-
9	17	3	1.3	1034	-
10	17	3	1.3	531	-
11	14	1	0.3	528	-
12	14	1	0.3	542	-

*secondary Bethesda assay was performed after detection by initial Betehesda assay

0996

DIAGNOSIS OF A PREVIOUSLY UNNOTICED FACTOR XIII DEFICIENCY AFTER OVARIAN HEMORRHAGE IN A 13-YEAR-OLD GIRL WITH EXTRADURAL HAEMATOMA AND DEFECTIVE WOUNDHEALING IN HER MEDICAL HISTORY

S. Schouwers,[1] E. Vandecruys,[2] A. Vantilborgh,[3] K. Devreese[4]

[1]University Hospital Ghent, GHENT; [2]Pediatric Hemato-Oncology, Ghent University Hospital, GHENT; [3]Department of Clinical Haematology, University Hospital Ghent, GHENT; [4]Coagulation Laboratory Ghent University Hospital, GHENT, Belgium

We report on a girl newly diagnosed with congenital factor XIII deficiency at the age of thirteen. She was born after 39 weeks of pregnancy. There were no reports of umbilical cord bleeding, the first and most characteristic symptom of FXIII deficiency. At the age of 3 she was admitted to the hospital with an extradural haematoma after minor trauma capitis. Trepanation was performed. Laboratory analysis showed no abnormalities in screening coagulation test results (PT, APTT and platelet count). Levels of Von Willebrand (VW) factor antigen and activity were normal as well as platelet function tests. Later on there were several reports of extensive, but superficial bruising over the whole body, which even lead to the suspicion of child abuse. When she was 12 years old a botriomycoma was removed from her right flank with seriously impaired wound healing. Since no abnormalities in routine coagulation were detected, self mutilation was considered as a possible cause. Presently, while still in wound care follow-up she presented at the emergency room with nausea and abdominal pain. Ultrasound and MRI showed an abdominal mass near the left ovary. An explorative laparatomy was performed and revealed a massive bleeding (most probably due to rupture of the follicle during first ovulation) and 1150 mL of blood was removed. Eventually a clot solubility test (CST) was performed because routine coagulation, platelet function tests, screening for VW disease and factor VIII and IX were normal. The qualitative CST turned out positive, indicating severe factor XIII deficiency. Therefore, we evaluated an automated quantitative assay for FXIII measurement to replace the qualitative CST. The Hexamate™ (MBL) is a latex immuno-assay (LIA) which can be implemented on a routine coagulation analyser. The polyclonal antibodies used, are directed against the subunit A of factor XIII. Intra- and between run imprecision was evaluated using 2 levels of commercial control material; inaccuracy was calculated based on a dilution study in factor XIII deficient plasma and the limit of detection (LOD) was determined using diluent. The reference values proposed by the manufacturer (60-125%) were checked using plasma of 20 normal individuals. The evaluation showed an intra-run imprecision <2,75% and an

between-run imprecision <6,33% for both control levels; there is a mean inaccuracy of 8,23%. The LOD is 4% and factor XIII levels of all normal individuals were above 60%. The FXIII levels detected in the patient's plasma was below 4%. After administration of fresh frozen plasma postoperatively, factor XIII was 22%. To stop bleeding 30 U/kg FXIII-concentrate was administrated IV. 14 days after treatment her FXIII level was 12%. Today, she recieves prophylactic administration of 10 U/kg F XIII every two to four weeks. Her wound is finally healing and she no longer presents easy bruising.

0997

EFFECTIVE TREATMENT OF GIANT HEMANGIOENDOTHELIOMA ASSOCIATED WITH KASABACH-MERRITT SYNDROME IN A 14 YEARS OLD BOY

T. Ociepa, E. Maloney, T. Urasinski, E. Kamienska

Pomeranian Medical University, SZCZECIN, Poland

Kasabach-Merritt syndrome (KMS) is a disorder characterised by consumption coagulopathy occurring due to cavernous hemangioma. A growing tumor and coagulopathy can lead to a life-threatening haemorrhages resulting from excessive consumption of platelets and clotting factors in hemangioma. Up to now over 300 hundred cases were reported. There is no guidelines regarding the effective therapy in this disorder. Management of KMS includes: steroids, interferon-α, radiation therapy, surgery and chemotherapy. We report on a case of 14 years-old boy diagnosed at the age of 2 with giant, cavernous hemangioma of pelvis, right hip and thigh complicated by KMS. Despite prolonged (over ten years?!) prednisone and interferon-α administration as well as radiotherapy, remission wasn't achieved. For all that period many complications were presented such as chronic bleeding from gastro-intestinal tract, osteoporosis, cachexia resulting in marked motor deficits. Giant hemangioma lead to acquired dislocation of the right hip. After ten years of unsuccessful treatment the patient was transferred to our unit (Figure 1). Based upon MRI the giant hemangioma of pelvis, hip and thigh including surrounding skin was confirmed. Laboratory tests showed profound thrombocytopenia, hipofibrynogenemia, prolonged PTT and microcytic anemia. Vincristin in a single dose 1,5 mg/m^2 given once a week for four weeks was introduced followed by the protocol suggested by the Hauer *et al.* (Pediatr Blood Cancer, 2007;49: 852-4). He received 8 courses of Vincristine (1,5 mg/m^2), Actinomycin-D (1,5 mg/m^2) and Cyclophosphamide (500 mg/m^2) with a four-week interval. This resulted in substantial regression of the tumor volume (reduction of the thigh circumference by 75%, total regression of the pelvic hemangioma) as well as normalization of platelets, PTT, fibrinogen and hemoglobin levels. General condition of the patient improved, his weight has increased 25%, and after several courses of physiotherapy he is able to walk with some support. Intensive chemotherapy comprising Vincristine, Actinomycin-D and Cyclophosphamide is beneficial in children with giant hemangioma complicated by KMS and seems to be more effective than other forms of treatment.

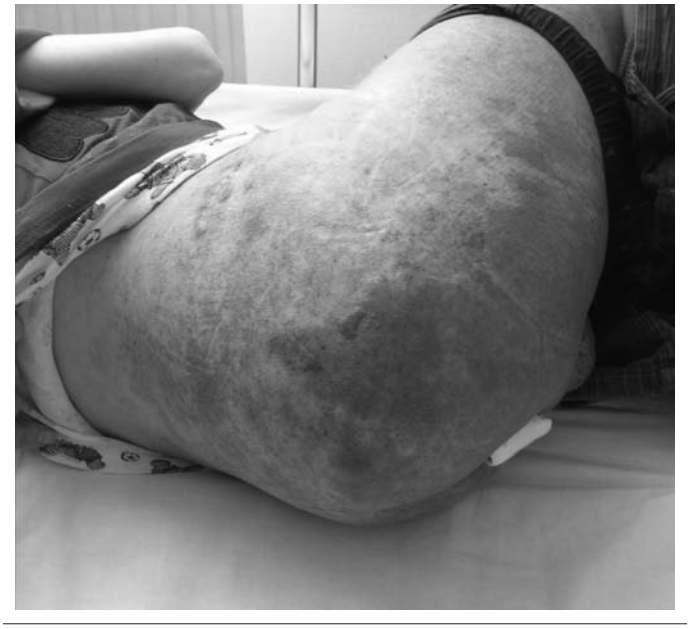

Figure 1.

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INCIDENCE AND RISK FACTORS FOR SEVERE ACUTE RENAL FAILURE FOLLOWING HIGH-DOSE METHOTREXATE THERAPY IN ADULTS WITH HAEMATOLOGICAL MALIGNANCIES: A RETROSPECTIVE REVIEW

D. de Miguel, J. García-Suárez, H. Bañas, Y. Martin, J.J. Gil-Fernández, P. Massó, C. Burgaleta

Hospital Principe de Asturias, ALCALA DE HENARES-MADRID, Spain

Background. Multiple factors may influence the development of severe ARF following HDMTX therapy. Our data suggest that a significant number of HDMTX-associated nephropathy might result from potential drug-drug interaction between HDMTX and several agents. *Aims.* The goal of this study was to define the incidence and risk factors for severe acute renal failure (ARF) following high-dose methotrexate (HDMTX) therapy in adults with haematological malignancies treated at our institution. *Methods.* All consecutive patients above 18 years of age developing HDMTX-induced severe acute renal failure (ARF), were retrospectively analysed. The diagnosis was based on markedly elevated serum MTX levels (>10 µmol/L at 48h after HDMTX) and severe ARF (grade 3-4 increases in serum creatinine, according to WHO criteria). Analysis of laboratory data included serial evaluation of serum creatinine levels, MTX levels, and haematological parameters. Plasma for MTX determination was obtained 24h post MTX-infusion, and then once daily until recovery of kidney function and decrease of MTX serum levels to less than 0.1 µmol/L. In addition, samples for MTX determination were obtained immediately before and 30 min following the dose of carboxypeptidase G2 (CPDG2). MTX was quantified using a fluorescence polarisation immunoassay (Widemann & Adamson, 2006). *Analysis of risk factors* . The following predisposing factors were analysed: pre-existing renal impairment; urinary pH <7 prior, during and after the administration of HDMTX; hypovolemia; exposition to nephrotoxic agents, existence of a third compartment (eg. pleural effusion); presence of the methylentetrahydrofolate reductase (MTHFR) C677T polymorphism; and use of drugs known to alter the clearance oh MTX. Concomitant therapy was also reviewed. The Naranjo criteria were used for determination of causality for suspected adverse drug reactions (Naranjo *et al.,* 1981). *Treatment strategies.* During the 5-year study period, we have used pre-treatment alkaline hyperhydration and postinfusion guided leucovorin (LV) rescue initiated at 24-36h after beginning of HDMTX (first administration 60 mg i.v. then 30 mg q6h until the serum MTX level was below 0.1 µmol/L). *Results.* Case reports. A total of 158 courses of HDMTX (in 31 patients) were administered in our institution. During the study period, 2 cases (6.4%) of HDMTX-induced severe ARF occurred. *Patient 1.* A 58-year-old male with primary cerebral lymphoma received chemotherapy with HDMTX cycles (2.5 g/m^2 over 3h) according to the DeAngelis protocol. The first course was delivered without problems and the patient had eliminated MTX without delay. During the 3 h following the second course of HDMTX the patient developed fever (38.5°C) and piperacillin-tazobactam (4 g/500 mg q6h) was initiated as empirical therapy. The next day (day 2) unexpectedly the serum creatinine level had increased from 60 to 210 µmol/L. At this point, the plasma MTX level was 93 µmol/L. ARF was diagnosed and treated according our treatment protocol for this condition. On day 3 the serum MTX level was very high at 40 µmol/L and CPDG2 was promptly administered. The response was excellent, the MTX level falling to 4.75 µmol/L within 1h. Concomitantly, a charcoal haemoperfusion (ChH) program seemed also effective. However, cytotoxic MTX concentrations were sustained for 26 days and were only abated by the improvement of renal function. The patient developed grade 4 myelosuppression (nadir day 8 after HDMTX), and grade 3 mucositis. He died 30 days after HDMTX due to septic complications. Concurrent medications, aside from piperacillin-tazobactam, did no differ between the two cycles of HDMTX. *Patient 2.* A 39-year-old man with precursor B-lineage acute lymphoblastic leukaemia received chemotherapy according to PETHEMA ALL-AR-03 trial. He was taking gemfibrozil, a cholesterol lowering agent, for more than 1 year. Following the achievement of complete remission the patient received the first cycle of early intensification chemotherapy that included HDMTX 3 g/mV. Twenty four hours post MTX-infusion, unexpectedly creatinine and MTX levels were 166 µmol/L and 24 µmol/L, respectively. Creatine kinase level and renal ultrasound were normal. With the diagnoses of ARF and impaired clearance of MTX, our treatment approach for this complication was initiated. CPDG2 was administered at 152 h after HDMTX and the plasma MTX concentration decrease from10.8 µmol/L to 3.7 µmol/L within 1h. Serum MTX and creatinine levels were decreased to <0.02 µmol/L and 80 µmol/L, respectively, 16 days after HDMTX. The patient developed grade 2 mucositis. Evidence of other MTX toxicity, such as myelosup-

pression or elevation of transaminase concentrations, was not observed. The patient fully recovered and no further complications occurred. *Risk factors.* Initially, our two patients showed markedly increased MTX concentrations without apparent risk factors. However, when both cases were reviewed in retrospect, a potential drug interaction between HDMTX and either piperacillin-tazobactam (patient 1) or gemfibrozil (patient 2) were found. Use of the Naranjo probability scale registered causality as probable in both patients. *Conclusions.* the incidence of HDMTX-induced severe ARF in adults with haematological malignancies may be considerably higher to that observed in other cancer patients. Our data and previously published case reports suggest that a significant number of HDMTX-associated nephropathy might result from potential drug-drug interaction between HDMTX and several agents. Clinicians should be aware of the potential pharmacokinetic interaction between HDMTX and either piperacillin-tazobactam or gemfibrozil. CPDG2 appears to be the treatment of choice when rapid elimination of MTX is indicated.

0999

VALIDATION OF RESPOND, A WEB-BASED CLINICAL GUIDANCE SYSTEM BASED ON THE EORTC GUIDELINES FOR THE USE OF ERYTHROPOIETIC PROTEINS IN CANCER PATIENTS WITH ANEMIA

L. Abraham,[1] J. Van Erps,[2] M. Aapro,[3] K. MacDonald,[1] P. Soubeyran,[4] M. Muenzberg,[5] T. Albrecht,[6] H. Warrinnier,[7] I. Abraham[8]

[1]Matrix45, EARLYSVILLE, USA; [2]Algemeen Stedelijk Ziekenhuis, AALST, Belgium; [3]Clinique de Genolier, GENOLIER, Switzerland; [4]Institut Bergonie & Universite Victor Segalen, BORDEAUX, France; [5]F. Hoffmann-La Roche, BASEL, Switzerland; [6]Matrix45 & University of Virginia, EARLYSVILLE, VA, USA; [7]Roche, BRUSSEL, Belgium; [8]Matrix45 & University of Arizona & University of Pennsylvania, EARLYSVILLE, VA, USA

Background. The EORTC has published evidence-based guidelines for the management of anemia using erythropoiesis-stimulating agents (ESA), however the challenge of bringing evidence-based guidelines to the point-of-care remains. Information technology may assist in this process, yet most systems developed for clinical support tend to be poorly if at all validated. We previously described the background to this technology the scientific methodology for its validation (Foubert *et al.*, EHA 2007, Haematologica/The Hematology Journal, 92(Suppl.1), 441-442) for the RESPOND system. *Aims.* To determine the content, concurrent, discriminant, and predictive validity of the RESPOND system. *Methods.* Sample of 34 patients matched by gender, cancer type, and treatment, and managed prior to (pre-cohort; without RESPOND support) and after introduction of the EORTC guidelines (post-cohort; with RESPOND cohort). Endpoints: 1) interclass correlation coefficient (ICC) as index of content validity, i.e., the extent to which experts agreed that a given algorithmic operationalization was an accurate representation of a given guideline; and 2) congruence score (CS; range 0-10) with EORTC guidelines as parameter for calculating concurrent, discriminant, and predictive validity. *Results.* Following an iterative process, all algorithmic operationalizations had an ICC=1.00.

Table 1. Congruence Scores (0-10) for Pre- and Post-Cohorts.

Guideline	Score	Pre-cahant (no RESPOND)	Post-Cahant (RESPOND)	p
Rule out or treat other cause of anemia: iron deficiency.	0.25	0.0%	100.0%	<0.0001
Rule out or treat other causes of anemia: bleeding.	0.25	0.0%	85.3%	<0.0001
Rule out or treat other causes of anemia: nutritional deficits.	0.25	0.0%	85.3%	<0.0001
Rule out or treat other causes of anemia: hemolysis.	0.25	0.0%	85.3%	<0.0001
ESA treatment initiated at Hb levels between 9 and 11 g/dL	1.00	5.9%	100.0%	<0.0001
IfHb<9g/dL: patients was considered for blood transfusion, or no blood transfusion needed.	1.00	79.5%	88.2%	n.s.
IfHB between 9 and 11 g/dL: patient individually considered for ESA treatment; or: not applicable	1.00	100.0%	100.0%	n.s
Target HB range set at 12-13 g/dL	1.00	0.0%	100.0%	<0.0001
Initial ESA dose: 40,000 IV epoetin alfa Q1W; 30,000 IV epoetin beta Q1W; darbepoetin Q1W or Q3W.	1.00	41.2%	100.0%	<0.0001
Initial ESA dose was fixed dose.	1.00	2.9%	100.0%	<0.0001
Target Hb level achieved between 4 and 8 weeks: if not, individualized dose escalation.	1.00	20.6%	52.9%	<0.0006
Hb maintained at 12-13 g/dL for up to 8 weeks	0.125 per week	20.6%	38.2%	n.s
ESA discontinued if Hb at or exceeding 13 g/dL (or Hb bewteen 12 and 13 g/dL at end of study)	1	29.4%	50.0%	n.s
M±SD		3.00±1.48	8.18±1.38	
95%CI		2.48±3.52	7.70±8.66	

ESA: erythropoiesis-stimulating agent; Hb: hemoglobin; Q1W: once weekly; Q3W: once every three weeks

See Table 1 for tabulated results on the congruence scores. The mean congruence score was 3.0 (SD=1.5; 95%CI 2.5-3.5) for the pre-cohort and 8.2 (SD=1.4, 95%CI 7.7-8.7) for the post-cohort. Concurrent validity was inferred from the high mean congruence score observed for the post-cohort. Discriminant validity was inferred from the statistically significant difference between the mean congruence scores of both cohorts (p<.00001) and from the higher likelihood of the post-cohort to achieve hemoglobin levels at or exceeding 11 g/dL (OR=3.6, 95%CI 1.1-11.8) and Hb levels at or exceeding 12 g/dL (OR=2.9, 95%CI 1.1-8.1). Predictive validity was inferred from the following: 1) statistically significant cross-sectional differences in mean hemoglobin levels at visits 2 (p=0.026), 3 (p=0.019), and 4 (p=0.007) between both cohorts; 2) statistically significant difference in the change in hemoglobin (HbΔ) from visit 1 to 4 (p=0.006); 3) the interaction effect for time and cohort (p=0.006); 4) the regression slope coefficient of 0.3 g/dL HbΔ increase for every 1 point increase in the congruence score; and 5) the congruence scores' ability to predict the likelihood of achieving hemoglobin levels of at least 11g/dL (OR=1.5, 95%CI 1.2-2.0) and at least 12 g/dL (OR=1.5, 95%CI 1.2-1.8). *Summary and Conclusions.* RESPOND has now been shown to have content, concurrent, discriminant, and predictive validity. Thus the RESPOND system can be implemented safely and accurately in hematology and oncology practice.

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EARLY LENOGRASTIM SUPPORT TO ENHANCE ENGRAFTMENT IN A TANDEM AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (APBSCT) PROGRAM FOR ADULT HIGH RISK MULTIPLE MYELOMA (MM) PATIENTS: A SINGLE CENTRE EXPERIENCE

A. Chierichini,[1] B. Anaclerico,[1] O. Olimpieri,[2] V. Bongarzoni,[1] P. Anticoli Borza,[1] S. Fenu,[1] M. Cedrone,[1] P. Iacovino,[1] B. Ronci,[1] L. Annino[1]

[1]Azienda Ospedaliera S.Giovanni Addolorata, ROMA; [2]Università Campus Biomedico, ROMA, Italy

Background. It is still debated if the dosage and timing of G-CSF support may have a significant impact on haematological recovery following APBSCT. Recently some changes occurred in recommendations to optimize G-CSF schedule and to improve the clinical outcome (Nolan, 2007;Jang, 2008). Since tandem APBSCT program is the gold standard to obtain prolonged response in adult MM patients (Harousseau 2005; Moreau 2006; Cavo 2007), early engraftment allow to complete the whole program. *Aim.* We retrospectively reviewed: a) the impact of an early administration (day +1) of lenograstim to support fast engraftment (ANC > 1.0×10^9/L within day +10); b) the incidence of non haematological toxicities (WHO>2) during all the therapeutic program; c) the overall time to complete the program in selected high risk MM patients aged <65 yrs who underwent tandem APBSCT as frontline treatment. *Methods.*From April 2002 to October 2007, 10 among 43 MM pts <65 yrs - 5 M, median age 58 yrs (range 39-63), high risk (7 DS stage III A, 2 DS stage III B and 1 adverse cytogenetics)- entered in an intensive program including induction chemotherapy (VAD, 2 median cycles) followed by PBSC harvest induced by cyclofosfamide 4 g/m^2 and a MEL200 conditioning regimen. Lenograstim was administered at a daily dosage of 5 µg/kg from day +1 until the third day after ANC>1.0×10^9/L. *Results.* Median CD34$^+$ harvest was 10.34×10^6 (range 6,44-28,85). First APBSCT was performed in 1 CR and 9 PR pts (according to EBMT criteria); median CD34 infused were 4.27×10^6 (range 3.08-13.44) and median days to ANC > 1.0×10^9/L were 9 (range 8-11). Lenograstim was administered for a median time of 12 days (range 11-14). The second APBSCT was performed in 5 CR and 5 PR pts; median CD34 infused were 5.2×10^6 (range 2.95-16); median days to ANC>1.0×10^9/L were 9 (range 8-11). Lenograstim was likewise administered for a median time of 12 days (range 11-14). During the treatment no patient developed a non-haematological toxicity >2 (WHO). The median time to complete the program was 10.1 m (range 5.5-27); 5,6 m (range 3.2-22) from induction to the first APBSCT, 4.5 m (range 1.9-5.8) from first to second APBSCT. At the end of program, 7 pts achieved CR and 3 PR. As of January 2008, 7 pts are alive, 4 in continuous CR (+4, +9, +15, +40 m) and 3 in PR (+10, +10, +16 m). 2 patients died for disease progression at month 3 and 14, 1 died for hepatitis B reactivation at month 1 from CR achievement, after the end of the tandem transplantation program. Median length of response (CR+PR) is 10 m (range 1-40). Median overall survival is 16 m (range 8-68 m) after a median follow up of 17.9 m (range 16-69 m). *Comments.* The early administration of lenograstim allowed a fast recovery of ANC in the first as well as in the second APBSCT, without significant differences regarding time to engraftment; during both procedures no relevant toxicities were experi-

enced and the overall time to complete the program (median time 10. 1 m) was consistent with a dose intensity purpose. In our experience double APBSC supported with early lenograstim is a feasible and effective procedure with acceptable toxicities in high risk young MM patients.

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HBV REACTIVATION IN HBSAG NEGATIVE PATIENTS WITH LYMPHOPROLIFERATIVE DISEASES POST CHEMOTHERAPY: CASE REPORTS

F. Graziani, D. Esposito, G. De Falco, G. Caparrotti, E. Iovane, D. Pagnini

ASL CE,[1] SAN FELICE A CANCELLO (CE), Italy

Background. HBV reactivation in HbsAg negative patient under immunosuppressive therapy is a phenomenon that usually leads to exitus in the patients because of the lack of a prophylaxis with lamivudine. *Methods.* Here we report seven cases of HBV reactivation in HbsAg negative patients but with HbcAb and sometimes HbsAb. Six patients were affected by B-CLL, while one by splenic MZL; at baseline HbcAb were present in 7, HbsAb in 3 and HbeAb in 2 patients. HbsAg, HBVDNA, Anti HCV were negative in all patients. These results were unchanged until the last therapeutic line preceding the reactivation. First line included: FC x4, FC x6, CEOP x3, VCP x6, fludarabine x6, chlorambucil at standard dose, chlorambucil at intermediate dose x4 cycles. After first line therapy maintenance was administered in 2 patients, respectively with rituximab for twelve and interferon for eight months. Second line was given in 4 patients; it included FCR x6, fludarabine x6 in 2, and chlorambucil x7. Two patients needed ≥3 therapeutic lines. In order to therapeutic lines HBV reactivations realized: 1) during first line therapy with chlorambucil at intermediate doses; 2) after 1 month from FC as first line; 3) after 35 months from twelve monthly doses of rituximab as maintenance following first line; 4) during second line at fourth dose of fludarabine x os; 5) after 11 months from FCR as second line; 6) during third line at fifth dose of FCR; 7) during rituximab monthly maintenance following five therapeutic lines. At reactivation HbsAg were always present, HbcAb in 6/7, HbeAg in 3/7, while HbeAb appeared in 2/7 patients *de novo*. Liver toxicity for transaminase were: grade 0 in 1/7, grade 2 in 1/7, grade 3 in 4/7, grade 4 in 1/7. HBVDNA was positive in all patients. At reactivation lamivudine started in six patients. *Results.* Four patients resolved their reactivation with HBV DNA disappearance in a median time of 3.5 months (range 2-4 months). A patient died after 15 days from reactivation and 45 days from the last dose of rituximab maintenance following five therapeutic lines. Another patient died for acute renale failure, despite his HBV DNA improved under lamivudine therapy. At the moment a patient is lost at follow up. *Discussion.* Here we have shown seven cases of HBV reactivation in HbsAg negative patients: all patients but one were affected by CLL; in 5/7 patients reactivation realized after chemotherapy lines >1; in 6/7 cases reactivation realized after powerful immunosuppressive drugs as fludarabine or rituximab, but in one case it happened during first line of chlorambucil at intermediate doses; in 5/7 patients reactivation occurred during or just after chemotherapy, but in 2/7 cases it realized after 11 and 35 months from the chemotherapy stop. Despite the lack of lamivudine prophylaxis, four patients cured their reactivation, while a patient died because heavily pretreated and another died for causes not apparently related to reactivation. Actually HbcAb positive patients are considered occult infection carriers and they should be regularly treated with lamivudine prophylaxis during immunochemotherapy and for several months thereafter.

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READING AGE HAS TO BE ACCOUNTED FOR IN PATIENT SUPPORT MATERIAL

C.X.L. Tai,[1] R. Soutar,[1] E. Low[2]

[1]Western Infirmary Glasgow, GLASGOW; [2]Myeloma UK, EDINBURGH, UK

Background. Studies have shown that written material is an inexpensive, portable method of helping patients retain information and expands understanding of their condition. It builds on the clinic consultation and increases rates of patient satisfaction. 1 in 5 people in the UK have been found to be functionally illiterate, with similar rates elsewhere in the developed world. The appropriate reading age for written material should be 13 years old or less. Highest level of education attained has not been found to correlate well with reading age as the reading age is often 3 to 5 years lower than years spent in education. *Aims.* The objective of this paper was to evaluate the readability of patient information/education materials supplied by Myeloma UK, a support charity for patients with multiple myeloma, using readability formulae that were readily available, e.g. in a word processing software such as Microsoft Word®. *Methods.* In conjunction with Myeloma UK, their information sheets were located on the Myeloma UK website (http:\\www.myelomaonline.org.uk) and were downloaded in PDF form. Using Adobe Acrobat 6.0 Professional®, the PDF files were converted into Word® format. Each file was then evaluated for its readability statistics using the Flesch Reading Ease and Flesch-Kincaid Grade Level formulae embedded in the Word® software. *Results.* 34 brochures were analysed of which 5.9% (n=2) were classified as *Infopacks*, 26.5% (n=9) were *Infoguides* and 67.6% (n=23) were *Infosheets*. The average Flesch Reading Ease was 40.84 (range 30.3 to 51.1), and the average Flesch-Kincaid Grade Level was 11.61 (range 10.0 to 12.0), making the average reading age 16.61 years (range 15.0 to 17.0). *Summary and Conclusions.* The reading ages of patient information materials produced by Myeloma UK were above the recommended reading level of grade 8 or age 13 years. Although patients were receiving reading material, they might not necessarily understand what they were reading. Authors, usually with medical and/or nursing backgrounds, of the patient information materials might not be familiar with the background of these readability formulae. There are various methods that can be used to improve readability. In the UK, the Royal National Institute for the Blind (http:\\www.rnib.org.uk/xpedio/groups/public/documents/publicwebsite/public_printdesign.hcsp), and the Department of Health (http:\\www.nhsidentity.nhs.uk/patientinformationtoolkit/patientinfotoolkit.pdf) have all released guidelines to improve readability of written material. In light of these findings, Myeloma UK is now revising its written information. Other support groups and charities might also find this worthwhile to aid patient understanding and satisfaction. These findings highlight the importance of a contact person / help line to clarify lack of understanding / confusion that might arise from written material alone. Given known literacy levels, it would be prudent to run final versions of written patient support materials through Word® to obtain readability statistics before publication.

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EFFICACY OF PERCUTANEOUS VERTEBROPLASTY IN PATIENTS WITH MULTIPLE MYELOMA PRESENTING WITH SYMPTOMATIC VERTEBRAL FRACTURES; A SINGLE CENTRE EXPERIENCE

J.S. Travers,[1] B. Sriharsha,[2] R.L. Soutar,[1] R. Edwards[2]

[1]Western Infirmary, GLASGOW; [2]Gartnavel General Hospital, GLASGOW, UK

Introduction. Vertebral compression fractures are a significant cause of morbidity in multiple myeloma (MM), associated with functional and quality of life impairment, high analgesia requirements, multiple radiotherapy treatments and high hospitalisation costs. Percutaneous vertebroplasty first described in 1987 has been reported to be successful[1] in multiple myeloma to stabilise vertebral fractures, provide pain relief and improve function with a low complication rate. *Aims.* We performed a prospective analysis at a single institution assessing the outcome of 23 patients with MM treated with percutaneous vertebroplasty. *Methods.* Patients were referred to a tertiary centre because of back pain refractory to analgesia and radiotherapy. Their vertebral fractures were assessed for suitability of vertebroplasty by plain x-ray, CT and MRI imaging of cervical, thoracic and lumbar spine. Procedures were carried out under local anaesthetic and light sedation and a percutaneous transpedicular approach was used. Bone cement was injected under radiological guidance to ensure appropriate distribution within the vertebral body. Patients' pain scores, analgesia requirements and quality of life measures were assessed pre vertebroplasty, at 1 month and again at 12 months. *Results.* Between 2001 and 2008 30 procedures were performed in 23 MM patients by a single interventional radiologist in a single centre. The median age of patients was 65 years (76-49 years). Of the 23 patients treated, only 1 patient (4%) experienced the complication of cement leakage into the vertebral vein however the patient was asymptomatic and did not experience any serious side effects. Subgroup analysis was possible for post vertebroplasty re fracture rates, radiotherapy treatments and quality of life. 100% of the subgroup (14 patients) required no further radiotherapy and the re fracture rate of the treated vertebral bodies was 0%. Quality of life data and pain reporting was of variable quality and is currently being reanalysed retrospectively via patient interview. *Conclusions.* Percutaneous vertebroplasty in multiple myeloma is well tolerated with very low complication rate when performed by an experienced operator. Objective efficacy was high with no re fracture and no further radiotherapy required to treated lesions. This study highlighted the difficulty in assessing benefit of any new procedure using subjective data such as pain scores and quality of life measures.

Reference

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ANESTHESIA UNDER SEDATION DURING BONE MARROW BIOPSY. RESULTS OF A SATISFACTION SURVEY

A. Lemes,[1] J. López,[2] T. Molero,[1] C. Partida,[1] C. Rodríguez,[1] P. Martín,[1] S. De la Iglesia[1]

[1]Hospital Universitario de Gran Canaria Dr Negrín, LAS PALMAS DE GRAN CANARIA; [2]Hospital Universitario de Gran Canaria Dr. Negrín, LAS PALMAS DE GRAN CANARIA, Spain

Background. Bone marrow biopsy is a necessary diagnostic method in several malignancies. Its main problem is the pain it causes. Fortunatelly, some years ago they were started to be made with anesthesia under sedation, which has avoided the suffering for the patients. *Aims.* To evaluate patients degree of satisfaction during the process of bone marrow biopsy. *Patients and Methods.* We made a survey to 77 random selected patients, all of whom answered them. Every survey had questions about different topics, such as the fact of using anesthesia under sedation with Midazolam and Propofol, or the pain they felt when biopsy was made. In the same way, patients were also questioned about the information they received before the biopsy: the explanation of the procedure or the necessitiy of signing a previous consent to make the biopsy. Another aspect analyzed was the opinion of the patients about the staff and the way they dealt with them; not only about the hematologist, but also about the anesthetist and nurses. Finally, the place where biopsies were made was also evaluated. *Results.* 96.1% of the patients answered that they were under sedation during the biopsy and the same percentage did not feel pain. 98.7% think that anesthesia under sedation should be used to make biopsies. When they were asked about the information received before biopsy a 54.5% answered to be quite satisfied. A 24.6% thought that it was good and a 9% were poorly informed. A 10% did not received any information. All of the patients (100%) signed a consent before making the biopsy. In relation to the staff, an 87% thought that the way the anesthetist dealt with them was excellent. An 80.5% thought the same about the hematologist and an 89% was also satisfied with the nurses. Finally a 92,2% of the patients was agree with the place where biopsies were made. *Conclusions*. Overall it could be said that patients are satisfied with nearly all the aspects related to bone marrow biopsies. However, some of them could be improved. I this way, and having into account our results, doctors should give more information about the procedure. Another important fact is the privacy. Sometimes patients do not have the intimacy they would like. It may be due to the place where biopsy is made or because of the people who are around them.In the same way it is also relevant not to make patients wait and start biopsies on time. Finally, all these results make us wonder two important questions: -Is it ethical to cause suffering to our patients when it could be eluded? -Is it a right of the patient and a duty of the doctor to avoid the pain if the economic resources allowed that?

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PER CASE PAYMENT IN IN-PATIENT ONCOLOGY AND HAEMATOONCOLOGY - FIVE YEARS EXPERIENCES WITH DIAGNOSIS RELATED GROUPS IN GERMANY

D. Franz

University Hospital Muenster, MUENSTER, Germany

Background. In-Patient reimbursement structures changed fundamentally in Germany in 2003. Per-day-payment was converted into per-case-payment based on Diagnosis Related Groups. The Australian AR-DRG-System provided a basis for the German G-DRG-System. Since implementation structures and valuations of the G-DRG-System have been advanced anually based on actual costs from by now app. 220 German hospitals. *Aims.*This analysis describes and comments the developments of the G-DRG-System since 2003 for non-surgical oncological and haematooncological treatments. *Methods.* Analysis of relevant diagnoses, procedures, G-DRGs and co-payments in the G-DRG-Versions 2003 - 2008 based on the published data from the German DRG-Institute (InEK) and the German Institute for medical documentation and information (DIMDI). *Results.* The number of G-DRGs for non-surgical oncological and haematooncological treatments and for radiotherapy tripled between 2003 and 2008 (2003: n=33, one for radio-therapy included, 2008: n=103, 39 for radiotherapy included). Parallel G-DRG-reimbursement was differenciated. The range of DRG-valuations of the 33 relevant G-DRGs in 2003 was 2,043, in comparison to 34,378 in 2008. As a result there have been reallocations of DRG-reimbursements to more complex cases that are usually treated in large tertiary care hospitals and university hospitals. 2003 DRG-Differenciation was based only according to the patients' age and comorbidity. In 2008 there are 20 different parameters (e. g. complexity of chemotherapy, complicating diagnoses or complicating procedures, length of stay, type of stem cell transplantation, use of intensive care therapy etc) in order to differenciate the expenditures by allocation to different G-DRGs. Very important for improving the quality of reimbursement was the implementation of co-payment structures for expensive pharmaceuti-cals, blood products, expensive diagnostic and therapeutic procedures (2003: n=0, 2008 n=115) and for innovations. Co-payment structures can be realized in addition to the DRG-reimbursement and are used frequently by large tertiary care hospitals and university hospitals. Furthermore the number of available codes for diagnoses and procedures has been increased to improve the identification of expensive case configurations. *Summary and Conslusions.* The G-DRG-System shall be used as a pricing system. Therefore sophisticated structures are necessary to properly identify and consider cases of different costs. The G-DRG-Version 2003 was not able to meet these requirements. In 2003 reim-bursement for complex cases was too low while less complex cases were overpaid. In 2008 based on the different improvements these criticism cannot held up for most cases. Persisting problems are the valuation of relevant comorbidities and the consideration of more than one relevant main diagnosis. Parallel to the improvement of G-DRG-differenciation the complex-ity of the system increased as well. This may lead to lower system-acceptance by clinicians. Regulary analysis and development is needed to assure proper allocation for in-patient oncological and haematooncological treatments in Germany in the future.

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β THALASSEMIA PREVENTION IN NORTHERN ISRAEL - COST BENEFIT ANALYSIS

A. Koren, C. Levin, L. Profeta, L. Zalman, O. Blondhaim

Ha'Emek Medical Center, AFULA, Israel

Introduction. β thalassemia major is a hemoglobinopathy characterized by chronic hemolytic anemia, ineffective erythropoiesis, progressive hemosiderosis and early death whitout adequate treatment. The management of β thalassemia major includes regular blood transfusions and iron chelation. This treatment and the regular follow up are expensive and carriers a significant burden to the health services. Population screening for detection of β thalassemia carriers seems to be significantly less expensive and cost effective. The purpose of this study is to analyze the cost benefit of the prevention of β thalassemia in northern Israel. *Materials and Methods.* The cost of treatment for β thalassemia major patients was calculated according to the actual management used and based in the fares used at the Clalit Health Insurance Services and the Iron chelator providers. The cost of the prevention program was calculated on the same basis. The data for the prevention program was obtained from the results of the prevention program in northern Israel during the year 2004. *Results.* The cost of the diagnostic workup of a new thalassemia patient is 325 U$S (1 U$S = 4.36 NIS), the yearly treatment cost during the first five years of life; whitout chelation therapy is estimated as 4,619 U$S. This cost increases to 24,670 U$S per year from the sixth year to the minimal estimated survival of 50 years. Another 463 U$S are needed for the regular yearly follow up. The total cost per patient for 50 years of life is 1.180.000 U$S or an average of 25.134 U$S per year. The cost of treating a patient with cardiac or endocrine deterioration is not included in the calculations, nor the splenectomy. In the screening program about 4000 blood tests were analyzed each year. 25 couples were found to be at risk of having an affected offspring and performed prenatal diagnosis and about six therapeutic abortions / year were performed. The annual cost of running the prevention program is 285,000 U$S and the calculated cost of each pathological fetus diagnosed is 45,340 U$S. *Discussion and Conclusions.* The burden of treating β thalassemia major patients is significant and the prevention program is relatively cheaper compared to other screening programs that needed molecular analysis for carrier detection. Each new thalassemia patient born induced a direct budget of about 1.180.000 U$S for the minimal life expectancy of 50 years. This budget can pay a prevention program for more than four years and prevent the born of 26 affected patients. These calculations do not include treatment of a deteriorating patient due to poor chelation compliance; blood related acquired infections, and the budget expended directly by the families and the National Insurance Found payment given to the patients. The indirect costs related to the affected poor quality of life are also not included in this analysis. With the introduction of the new oral iron chelator, Desferasirox, the annual cost of treatment increased by approximately 20%, but the improvement in quality of life and probably the compliance also, should be considered.

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A REVIEW OF THE COSTS OF TREATMENTS FOR FOLLICULAR LYMPHOMA PATIENTS WITHIN THE UK NATIONAL HEALTH SERVICE

R. Pettengell,[1] J. Ryan[2]

[1]St George's University of London, LONDON; [2]Bayer Schering Pharma, NEWBURY, UK

Background. Current standard regimens for the treatment of follicular non-Hodgkin's lymphoma (FL) are typically administered over a period of 3-6 months, generating substantial treatment, administration and indirect costs. Increasingly, first line treatments comprise a combination of CVP or CHOP with rituximab, although consolidation of induction therapy using Yttrium-90 (90Y)-ibritumomab tiuxetan, is also likely to be indicated for first line treatment during 2008. Non rituximab induction therapies, including CVP, CHOP, chlorambucil (CHL) and consolidation with transplantation are also available options. *Aims.* This analysis compared the costs of treating patients with FL at diagnosis or relapse within a UK NHS setting. *Methods*. All treatments indicated for the patient group, including induction, consolidation, maintenance and transplantation therapies were investigated using the latest list prices and tariffs available (2007/08 prices). Therapy costs, administration costs and indirect costs were included in the analysis. *Results*. A typical course of CHL, CVP and CHOP cost £340, £1,012 and £4,639 respectively. An eight cycle induction course of RCVP or RCHOP, including administration costs, was estimated to be £13,056 and £14,418 respectively. For a six cycle course, the cost was estimated to be £9,792 and £10,814. Rituximab alone as a maintenance treatment was estimated to cost £12,691. The cost of consolidation with Yttrium-90 (90Y)-ibritumomab tiuxetan was estimated to be £10,996. When combined with induction CVP, this cost was £13,073, including administration. Health care administration costs accounted for between 12% and 73% of overall therapy costs. Transplantation (peripheral blood, autologous) was the most expensive therapy, costing an additional £25,000 per patient after induction therapy. In addition to direct health care costs, indirect costs associated with time off work for administration was estimated. All patients incur an opportunity cost of their time when receiving treatment. For those patients of working age, this can include lost productivity costs. Such costs were found to be higher for patients undergoing more complex administrations (e.g. CHOP or rituximab) and transplantation. *Summary and Conclusions.* Rituximab based induction therapies are most commonly used in the UK. The overall cost of these therapies was sensitive to the time taken to administer the treatments as well as the number of cycles given. Using regimens that help reduce the burden on health care resources, such as administration time, and reduce the burden on patients, can provide important cost offsets. Consolidation therapy, such as Yttrium-90 (90Y)-ibritumomab tiuxetan, also provides an additional treatment choice to clinicians, at a similar cost to usual therapy and at significant cost savings to transplantation.

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CXCR7 IS EXPRESSED ON CD34$^+$ CELLS AND VARIOUS HEMATOPOIETIC CELL LINES

M. Majka, K. Miekus

Jagiellonian University Medical College, CRACOW, Poland

Background. SDF-1 and CXCR4 was a unique pair among chemokines and chemokine receptor since SDF-1 was shown to bind only CXCR4 and CXCR4 was shown to respond only to SDF-1 stimulation. However, recently CXCR7 has been discovered as a second receptor for SDF-1. It also binds I-TAC (interferon-inducible T cell alpha chemokine). The function of this new receptor has been first studied in tumor cells. Very recently expression of CXCR7 has been detected in renal progenitor cells. It has been shown that SDF-1 exerts different actions on both tumor and primary cells. Activation of CXCR7 stimulated adhesion, proliferation and inhibited apoptosis in these cells, but in opposite to activation of CXCR4 by SDF-1 it did not stimulated cell migration or Ca+2 flux. *Aims.* In this study we analyzed the expression of CXCR7 on normal hematopoietic cells and hematopoietic cell lines. *Methods.* Bone marrow CD34 positive cells were used for direct assessment of CXCR7 expression and for serum free expansion toward erythroid (BFU-E), granulocyto-monocytic (CFU-GM) and megakaryocytic (CFU-Meg) lineages. We used both real time RT-PCR detection strategy and flow cytometry staining to study the CXCR7 expression on the surface of hematopoietic cells. *Results.* High expression of mRNA for CXCR7 was detected in CD34 positive bone marrow cells. After 12 days serum free expansion toward erythroid, megakariocytic and granulocytic-monocytic lineages the expression of mRNA for this receptor diminished. We have also studied the expression of the receptor on established hematopoietic cells lines. CXCR7 was present on Jurkat cells (T cell lines), THP-1 (pro monocytic cell line). Cells of NK origin (YT cells) and U266 (multiple myeloma cell line) turned out to be negative for the receptor. *Summary*. This is the preliminary study in which only the expression of CXCR7 was evaluated. The functional study to check the response pattern of human hematopoietic cells to CXCR7 stimulation is ongoing in our lab.

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HIGH-RESOLUTION COPY NUMBER ANALYSIS OF INFANT LEUKEMIA WITH MLL REARRANGEMENT USING SNP-GENOTYPING MICROARRAYS

J. Takita,[1] M. Kato,[2] Y. Chen,[2] G. Yamamoto,[3] Y. Nannya,[3] M. Sanada,[3] A. Kikuchi,[4] T. Igarashi,[2] Y. Hayashi,[5] S. Ogawa[3]

[1]Tokyo University, TOKYO, Kazakhstan; [2]Dept. of Pediatrics, Univ. of Tokyo, TOKYO; [3]The 21 century COE programe, Univ. of Tokyo, TOKYO; [4]Div. of Hematology/Oncology, Saitama Children's Med. Ctr., MAGOME; [5]Gunma Children's Med. Ctr., MAEBASHI, Japan

Background. MLL rearrangement-positive leukemia is one of the most aggressive types of leukemia. It is diagnosed predominantly in infants and typically shows a multilineage phenotype. Since current chemotherapy fails in more than 50% of infantile leukemia with MLL rearrangement, a better understanding of biological features of the disease is importantly in order to develop more specific and successful treatment strategies. *Aim.* To identify genetic changes and thus to get a better understanding of the pathogenesis of MLL rearrangement-positive leukemia, we performed comprehensive analysis of copy number alterations as well as allelic imbalances in MLL rearrangement-positive leukemia genomes. *Methods.* We analyzed 20 MLL rearrangement-positive infant leukemia specimens and 8 cell lines of MLL rearrangement-positive acute lymphoblastic leukemia (ALL) using high-density single nucleotide polymorphism (SNP)-genotyping microarrays (Affymetrx® GeneChip® 100K/500K arrays). In this platform, use of large numbers of SNP-specific probes showing linear hybridization kinetics allows not only for high-resolution copy number analysis, but also for accurate determination of the loss of heterozygosity (LOH) regions. Unfortunately, however, the sensitivity of the currently available algorithm for LOH detection using SNP arrays may be greatly reduced when they are applied to primary tumor specimens frequently suffering from significant normal cell components. Thus, to overcome this limitation, we developed a novel algorithm, CNAG/AsCNAR, that enables accurate determination of allele-specific copy numbers and allelic compositions in a genome-wide fashion even in the face of up to 70-80% normal cell contamination. The algorithms calculate allele-specific copy numbers without depending on the availability of matched control DNA, enabling sensitive detection of LOH as well as copy number alterations in a wide spectrum of primary tumor specimens, which are in combination, termed *molecular allelo-karyotyping*. *Results.* While unbalanced translocation involving chromosome 11q23 is the most frequent genetic alterations, no other common genetic alterations were observed in our series. A number of other genetic changes including uniparental disomy (UPD) of chromosome 9p, LOH on chromosome 12p and homozygous deletion on chromosome 14p were identified but these were mostly found in a single case. In seven specimens from primary cases, no genetic changes were detected, in which a possibility of very low tumor contents could not be excluded. *Summary and Conclusions.* Molecular allelo-karyotyping profile revealed that unbalanced translocation involving chromosome 11q23 was exclusive unique patterns of MLL rearrangement-positive leukemia genomes. Thus, other chromosomal alterations such as LOH, gain, high-grade amplification and homozygous deletion were rare events in MLL rearrangement-positive leukemia. These results indicated that high-resolution analyses of genetic aberrations using microarray techniques are powerful and useful for detection of new findings in the pathogenesis of infant leukemia with MLL rearrangement.

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ASSOCIATION BETWEEN DEFB1 GENE HAPLOTYPES AND HERPES VIRUSES SEROPREVALENCE IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

R. Tesse, N. Santoro, P. Giordano, G. Arcamone, F. De Leonardis, V. Cecinati, D. De Mattia, L. Armenio

University of Bari, BARI, Italy

Background. Recent studies investigated the role of an inappropriate immune response to infective agents in the etiology of acute lymphoblastic leukemia (ALL) in children. It has been also hypotisized that inherited variations in immune genes that influence responses to infections probably play a role in susceptibility to leukemia. Human β-defensin-1 (hBD-1) is an antimicrobial peptide of the innate immune system, which is constitutively expressed in several epithelial tissues, monocytes, macrophages and dendritic cells. *Aims.* We tested for an association between genetic variants of hBD-1 and seroprevalence of antibodies for herpes viruses in ALL patients. *Methods.* The study included 40 Caucasian patients [26 male, 14 female, median age 4.9 years, (range 1-15 years)] who were diagnosed with ALL at the Pediatric Department of the University of Bari, between March 2005 and October 2007. A total of 40 unselected healthy subjects [23 male, 17 female, median age 4.5 years, (range 1-14 years)], acted as controls. Informed consent was provided according to the Declaration of Helsinki. We genotyped three polymorphisms, -52G/A, -44C/G and -20G/A, of DEFB1gene, coding for hBD-1, by use of polymerase chain reaction and restriction fragment length polymorphism. Serum samples from all enrolled subjects were tested for herpes simplex viruses 1 and 2 (HSV) and cytomegalovirus (CMV) antibodies, by enzyme linked immunosorbent assay.

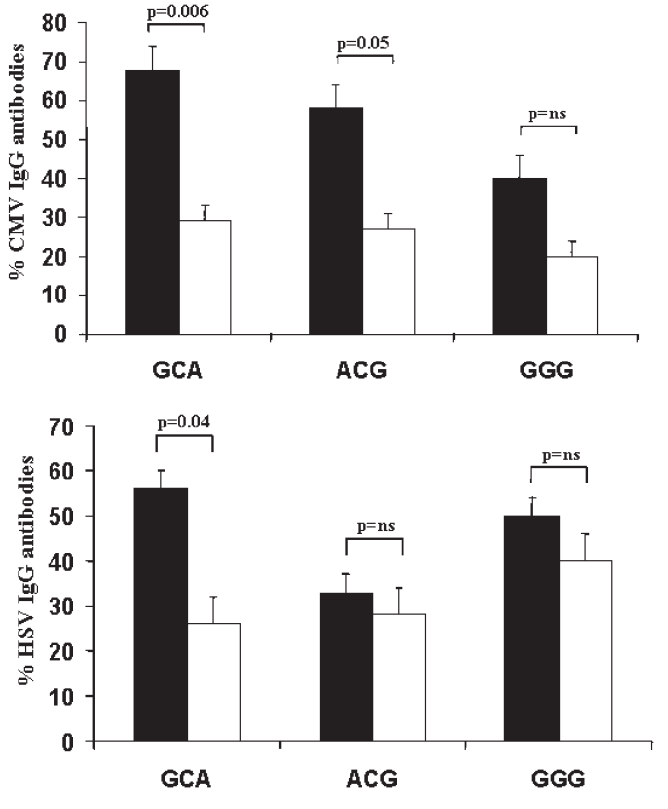

Figure 1.

Results. We found that the seroprevalence of HSV and CMV IgG antibodies in leukemic children was significantly higher than that in controls [HSV: 50% *vs* 24.2%, odds ratio (OR) 3.1, 95% confidence interval (CI) 1.01-9.6, *p*=0.04; CMV: 61.5% *vs* 27.3%, OR 4.2, 95% CI 1.4-12.8, *p*=0.008]. The SNPs generated three main haplotypes in our population: GCA, ACG, and GGG. Leukemic children carrying the polymorphic A allele at the -52 locus site of the DEFB1 gene had significantly increased seroprevalence of CMV and HSV IgG antibodies than healthy children with the same polymorphic variant [CMV: GA/AA *vs* GG 61% *vs* 25%, OR 5.23, 95% CI 1.40-19.51, *p*=0.01; HSV: GA/AA *vs* GG 44.4% *vs* 15%, OR 4.08, 95% CI 1.11-14.88, p=0.03]. Likewise, patients homozygous and heterozygous for the A allele at the -20 locus site presented an increased seroprevalence of CMV IgG antibodies than controls carrying the same polymorphic A allele (GA/AA *vs* GG: 60% *vs* 27%, OR 4.00, 95% CI 0.99-16.14, p=0.05). Among ALL patients, carrying the -44CC genotype was related to an increased seroprevalence of CMV IgG antibodies as compared to healthy carriers of the same genotype (CC *vs* CG/GG: 63% *vs* 28%, OR 4.41, 95% CI 1.22-15.80, p=0.02). Haplotype analysis revealed that patients carrying the GCA haplotype had a significantly higher seroprevalence of CMV and HSV IgG antibodies than healthy children bearing the same haplotype (CMV: 68% *vs* 29%, OR 5.29, 95% CI 1.53-18.29, p=0.008; HSV: 56% *vs* 26, OR 3.69, 95% CI 1.03-13.21, *p*=0.04), Figure 1. *Conclusions.* We conclude that leukemic patients carrying untraslated variants of hBD-1 have a higher susceptibility to herpes viruses infections than controls, presumibly due to chromosome molecular abnormalities predisposing to inappropriate expression of the antimicrobial protein.

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INCIDENCE AND SIGNIFICANCE OF JAK2 V617F MUTATION IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISORDERS FROM THE SULTANATE OF OMAN

S. Alkindi,[1] A. AlRiyami,[1] S. AlZadjali,[1] A. AlMadhani,[2] D. Dennison,[1] A. Pathare[1]

[1]Sultan Qaboos University Hospital, MUSCAT; [2]Sohar Hospital, SOHAR, Oman

Background. The myeloproliferative disorders are a heterogeneous group of clonal haemopoietic disorders, characterized by bone marrow proliferation of one or more of blood cell lineages. Amongst these, chronic myeloid leukemia[CML] has a distinct underlying molecular mechanism, whereas, the other three disorders namely polycythemia rubra vera[PRV], essential thrombocythemia[ET], and idiopathic myelofibrosis[MF] are considered to be clinically and pathologically related, with tyrosine kinases being implicated. *Aims.* To investigate the incidence and significance of tyrosine kinase JAK2 V617F in patients with chronic myeloproliferative disorders from Oman. *Methods.* We obtained DNA samples from patients with PRV, ET and MF after an informed consent according to the proposal which was previously sanctioned by our institutional review board. Genomic DNA was extracted from whole blood by using the semi-automated ABI 6100 nucleic extractor. A genomic DNA fragment containing the exon 14 of the JAK2 gene which bears the V617F mutation site was amplified by direct sequencing using ABI 3100 Genetic analyzer. Allele-specific polymerase chain reaction was additionally performed to validate the results obtained by direst sequencing. *Results.* The study enrolled a total of 103 subjects, namely 24 ET patients, 5 MF patients, 19 PRV patients, 25 CML patients and 30 ethnic normal Omani blood donors. A single point mutation (Val617Phe) was identified in JAK2 in 5(20.83%) of 24 patients with ET; 5(100%) of 5 patients with MF and 13(69%) of 19 patients with PRV (Table 1).

Table 1. Demographic & Lab. Data on ET, MF & PRV patients.

	ET [n=24]	MF [n=5]	PRV [n=19]
Male : Females	11:13	0:4	13:6
JAK2 V617F [%Pos]	20.83%	100%	69%
Age, yrs Median [Range]	56 [19-82]	58 [34-67]	60 [22-64]
Hb g/dl Mean ± SD [Range]	12.1 ± 1.4 [9.6-15]	10.5 ± 2.8 [8.9-14.8]	14.7 ± 2.1 [11.9-17.5]
HCT L/L Mean ± SD [Range]	0.37 ± 0.03 [0.29-0.44]	0.34 ± 0.08 [0.28-0.47]	0.46 ± 0.05 [0.38-0.53]
RBC $X10^{12}$/L Mean + SD [Range]	4.25 + 0.63 [2.64-5.21]	4.3 + 0.86 [3.59-5.56]	5.9 + 1.6 [3.86-9.13]
WBC $X10^{9}$/L Mean ± SD [Range]	6.57 ± 2.1 [4.1-12.4]	12.4 ± 7.2 [7.3-23.1]	9.8 ± 5.2 [4.5-20.9]
Plats X109/L Mean ± SD [Range]	936 ± 296 [896-1712]	437 ± 430 [162-311]	450 ± 290 [148-510]

It was not identified in any of the 25 patients with CML and 30 normal ethnic Omani blood donor controls. Statistical analysis of the subgroups with ET and PRV patients between those who were JAK2 positive and negative did not reveal any discriminating variables, especially the platelet count and the hemoglobin levels respectively. However, In the PRV group who were on regular phlebotomy [n=14], 93% were JAK2 positive. *Summary and Conclusions.* A single acquired mutation of JAK2 was noted in almost half of the patients with myeloproliferative disorders.[48%] The incidence of JAK2 V617F mutation in myeloproliferative disorders from the Sultanate of Oman is similar to that reported by other groups. Early screening of suspected PRV patients for JAK2 V617F mutation rapidly identifies nearly all those patients who will ultimately need definitive treatment without invasive investigations.

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HOLOTRANSCOBALAMIN (HOLO-TC) FOR DIAGNOSING EARLY VITAMIN B12 DEFICIENCY

G.B. Lobreglio, A. Gatto, R. Cardinali, D. Fiorentino, G. Rizzo, L. Pappaccogli, M. Sparascio

A.O. Card G. Panico, TRICASE, Italy

Background. Vitamin B12 deficiency is a frequent problem, particularly among older persons. Early diagnosis of vitamin B12 deficiency is crucial because of the latent nature of this disorder and the possible risk of irreversible neurological damage and hematologic diseases. The standard screening test for vitamin B12 deficiency, measurement of total serum vitamin B12, has limitations of sensitivity and specificity. For low concentrations of total vitamin B12 there is likely to be misclassification of B12 status if relying on total serum B12 alone. Serum vitamin B12 bound to transcobalamin II (Holo-TC), constitutes only 6%-20% of total vitamin B12, and is the fraction of total vitamin B12 available for tissue uptake. Serum concentrations of Holo-TC has been proposed as a potential and alternative marker of early vitamin B12 deficiency. *Aims.*We investigated the usefulness of Holo-TC compared with total serum vitamin B12, in diagnosing of early vitamin B12 deficiency. *Methods.* We compared the performance of Holo-TC with total vitamin B12 to screen for metabolic vitamin B12 deficiency. The study included 54 serum samples from patients with concentrations of total vitamin B12 in the range of 150-300 pg/mL (gray-zone). Total serum vitamin B12 concentrations were determined by chemiluminescent microparticle immunoassay (CMIA) (Architet Abbott); serum Holo-TC concentrations were determined by microparticle enzyme immunoassay (MEIA) (Axsym Abbott). *Results.* Low levels of Holo-TC (<35 pmol/L) were observed in 19 samples (35%) with total vitamin B12 levels of 150-300 pg/mL. Linear regression analysis and Pearson correlation were used to analyse the association between the biochemical variables. Regression analysis shows that there is only a poor correlation between vitamin B12 and Holo-TC (r=0,495) for values of vitamin B12 falling in the gray-zone Figure 1.

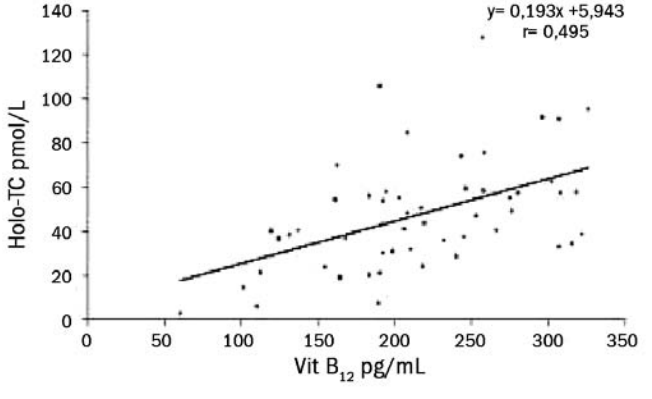

Figure 1.

Summary and Conclusions. Low values of Holo-TC was observed in a considerable number of patients with normal level of total vitamin B12.Since it can take months, even years, for a significant fall in total vitamin B12 levels, the more rapid decline in Holo-TC may be masked when measuring serum total vitamin B12. Therefore, total cobalamin concentration in the range of 150-300 pg/mL, cannot exclude a deficiency of the vitamin. Our results suggest that serum concentrations of Holo-TC is a more sensitive marker in diagnosing vitamin B12 deficiency when compared with serum total vitamin B12. In conclusion, our study demonstrated that Holo-TC can be used in screening studies as a first line parameter for detecting early deficiency before the development of clinical symptoms such as macrocytic anemia and neurological damages. Nevertheless, large scale clinical studies are warranted in order to clarify the usefulness of Holo-TC in the clinical setting.

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BETA-THALASSEMIA: PREVALENCE IN ALGARVE, PORTUGAL

M. Cabral,[1] I. Baião,[1] I. Valente,[1] M. Eloy,[2] M. Freitas[1]

[1]Escola Superior de Saúde de Faro, Universidade do Algarve, FARO; [2]Departamento de Saúde Pública, Administração Regional de Saúde do Algarve, IP, P, FARO, Portugal

Background. Thalassemia is a genetic disease that makes part of an heterogeneous group of mendelian disturbs characterised by a diminution or absence in the production of one of the α or β Haemoglobin A chains (α2 β2). β-thalassemia is a variance of the thalassemic disease. This disease is found in many different ethnic groups and is more incident in some geographic areas, like Mediterranic zones where Algarve is insert. The migration can be an important factor on this disease prevalence once that occur immigration in Algarve provided from others geographic areas where β-thalassemia prevalence is high too. The detection of this disease is very important because is a way of prevention once that with this information it's possible to detect and inform the carriers, identify and give genetic counselling to the risk couples and an eventual prenatal diagnostic. *Aims.* The aim of this study is to determine the prevalence of this disease in population resident in Algarve (dependent variable), analysing its dependence of nationality and ethnicity (independent variables). *Methods.* The studied sample is formed by 28800 individuals, residents in Algarve. Data related to sex, age, nationality, ethnicity, and haemoglobinopathy type were collected between 1986 and 2006, among the National Program of the Haemoglobinopathies Control carried out by the Public Health Department of the Regional Health Administration of Algarve. Statistic data treatment was made with the program SPSS v. 15.0. *Results.* Most of the tested individuals are non carriers of any kind of haemoglobinopathy (90.7%) being β-thalassemia the most prevalent haemoglobinopathy in Algarve (7.5%) followed by Haemoglobin S (1.6%). This study allowed us to verify β-thalassemia as the most prevalent in people original from Algarve (9.3%), followed by the individual from other parts of the country (6.5%) and is higher in caucasians (8.1%) followed by black people (1.4%). *Summary and Conclusions.* Previous studies have shown that β-thalassemia presented values between 2.0% and 15.0% in Mediterranic areas. The result found in this study, 7.6%, shows the importance of this kind of study.

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SPECTRUM OF β-THALASSEMIA MUTATIONS IN THE KERMANSHAH PROVINCE OF IRAN

Z. Rahimi,[1] H. Mozafari,[1] R.L. Nagel,[2] A. Muniz[2]

[1]Kermanshah University of Medical Sciences, KERMANSHAH, Iran; [2]Albert Einstein College of Medicine, NEW YORK, USA

Background. β-thalassemia is the most common single gene disorder in Iran. The presence of around 25000 affected individuals and two million carriers of β-thalassemia, requires an extensive study for the type of β-globin gene mutations in different ethnic groups of Iran. *Aims.* To find the spectrum of β-thalassemia mutations in the Kermanshah province of Iran with ethnic background of Kurd we studied 366 chromosomes from 183 unrelated homozygous β-thalassemia patients. *Methods.* Polymerase chain reaction (PCR), amplification refractory mutation system (ARMS) and direct sequencing were used to identify the type of β-thalassemia mutations. *Results.* As many as 20 different mutations (15 β0 and 5 β+ mutations) were identified. The most prevalent mutation was IVSII.1 G:A accounting for 33.3% mutations in patients. The frequency of the common mutations were [CD8/9 (+G), 13.7%], [IVSI.110 (G:A), 8.5%], [CD 36/37 (-T), 7.9%], [FSC 8 (-AA), 6%], [CD 15 (G:A), 4.9%], [IVSI.1 (G:A), 4.6%)], [IVSI.6 (T:C), 3.8%)], [CD 39 (C:T), 3%)], [IVSII.745 (C:G), 2.5%], [CD 44 (-C), 2.5%], [IVSI.5 (G:C), 2.2%], [IVSI, 3end(-25 del), 2.2%], [CD 83 (-G), 1.1%], [FSC 22/23/24 (-AAGTTGG), 1.1%]. Rare mutations were identified to be [IVSII. 2,3 (+ACGTTCTCTGAA), 0.6%], [IVSI.128 (T:C), 0.6%], [CD 6 (-A), 0.3%], [CD 37 (G:A), 0.3%], [CD 9/10 (+T), 0.3%]. The unknown alleles comprised 0.6% of the mutations. Around 82.5% of patients carried β0 type of mutations. *Conclusions.* The results of present study can help to establish prenatal diagnosis programs leading to lower medical cost.

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AN ASSOCIATION OF APLASTIC ANEMIA WITH LOW SOCIOECONOMIC STATUS IN NORTHERN INDIA

G. Gella, P. Malhotra, N. Varma, V. Suri, S. Kumari, S. Jain, S. Varma

Postgraduate Institute of Medical Education and Research, CHANDIGARH, India

Background. Aplastic anemia (AA) is more common in Asian countries than in the West. The etiology of higher incidence of this disease in these countries is believed to be related to environmental factors rather than the genetic factors. *Aims.* To explore the association of AA with socioeconomic status in a tertiary care hospital in northern India. *Methods.* This was a case control study in which 102 patients of AA (diagnosed by standard criteria) and 201 controls of other blood disorders (OBD) selected from the adult hematology clinic were studied. The OBD group consisted of 100 patients of idiopathic thrombocytopenic purpura (ITP group) and 101 patients of malignant hematological disorders (leukemia group). A detailed structured questionnaire used in National Family Health Survey (NFHS) of India was filled up for every patient and control. Besides socio-demographic data, patients were evaluated clinically and investigated according to standard guidelines. The socioeconomic status score was calculated based on 10 item score. Subjects in the score group of 0-14 were classified in low standard of living (SLI), 15-24 in medium and 25-67 in high SLI group. *Results*. Patients with AA were significantly younger than patients in both control groups (table). The mean SLI score was significantly lower in AA group than the control group. Patients of AA with lower SLI had 3 times (odds ratio 3.41, 95% CI 1.72-6.79, $p<0.0001$) higher odds of having the disease when compared with patients of high SLI. The mean monthly family income was significantly lower in AA group than the control group (Table 1). There was a trend of increasing risk of having AA with decreasing monthly family income with a odds ratio of 4.35 (95% CI 1.76-10.7, chi-square value 13.68, p-value 0.001) for those with monthly family income less than 8,000 INR (€ 133.3) when compared to those with monthly family income greater than 15,000 INR (€ 250). The study did not find association of educational status with occurrence of AA. *Conclusions*. The finding of high prevalence of AA in low socioeconomic status group is surrogate for presence of infectious aetiology or toxic exposure as a cause of AA in this population. It would be interesting to know in future the prevalence of AA with rising socioeconomic status in this population.

Table 1.

Variable	AA group	Leukemia group	ITP group	P value
Age (median) years	24	40	28	<0.0001
SLI score (Mean)	26.76	29.54	35.48	<0.0001
Monthly income INR (€) (Mean)	6023 (€100.4)	10150 (€ 169.1)	23650 (€394.1)	<0.0001

AA=Aplastic Anemia; ITP=immune thrombocytopenic purpura; SLI=Standard of living; INR=Indian National Rupees; 1€ (Euro) = INR 60.00

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OXYHAEMOGLOBIN SATURATION, ASTHMA AND CHEST CRISIS IN PAEDIATRIC SICKLE CELL

J. Kirkham,[1] J. Dingle-Gavlak,[1] J Krings,[2] D.K.M. Hewes,[1] I. Dundas,[3] R. Lane,[1] S. Carr,[3] J.P.M. Evans,[1] B. Kaya,[3] P. Telfer,[3] R.S. Bucks[4]

[1]UCL Institute of Child Health, LONDON, UK; [2]Notre Dame university, NOTRE DAME, INDIANA, USA; [3]Royal London hospital, LONDON, UK; [4]University of Western Australia, PERTH, Australia

Background. Children with Sickle Cell Anaemia (SCA) have lower resting and overnight oxygen saturations than normal populations. Additionally, obstructive sleep apnoea syndrome (OSAS) and asthma are more common in those of African ancestry.[1] We have previously shown that low mean overnight oxyhaemoglobin saturation (SpO2) predicts central nervous system events and increased pain frequency in the East London cohort. As oxygen supplementation has previously been considered detrimental, before interventions for nocturnal oxyhaemoglobin desaturation can be designed, more data are needed about whether low SpO2 predicts a higher or lower risk of other complications. *Aims.* In this secondary analysis, we have looked at the prediction of another complication of Sickle Cell, chest crisis, from overnight pulse oximetry data in the East London cohort, with the hypothesis that chest crisis would be more common in those with low mean overnight oxyhaemoglobin saturation. As doctor-diagnosed asthma has recently been shown to predict chest crisis,[2] we included this in the analyses. *Methods.* Overnight pulse oximetry was recorded between 1991 and 2002 in 141 unselected patients with SCA from the East London Cohort who regularly attended a single hospital. The endpoint was chest crisis, defined as a new pulmonary infiltrate in a patient with respiratory symptoms. *Results.* Nocturnal oxyhemoglobin desaturation was common in children with SCA, with mean overnight oxygen saturation (SpO2) ranging between 84-99.7% (median 95.9%). Twenty-one (15%) had doctor-diagnosed asthma, very similar to the previously reported prevalence of 17%.[2] Seventy-nine of 141 (56%) had a total of 290 chest crises and the median length of stay after that diagnosis was 8 (range 1-42) days. In the 41 children <5 years, asthma predicted an increased risk of chest crisis (hazard ratio, hr, 3.0, 95% confidence interval, ci, 0.96, 9.2, $p=0.056$), while persistent SpO2 desaturation (mean overnight SpO2 <95%) predicted a non-significant reduced risk subsequent to the sleep study (hazard ratio, hr, 0.64, 95% ci, 0.21, 1.9, $p=0.4$). However, in the 100 children >5 years, asthma was a less powerful predictor of subsequent chest crisis (hr 1.2, 95% ci 0,48, 2.9) and mean SpO2 <95% predicted increased risk of chest crisis subsequent to the sleep study (hr 2.0; 95% ci 1.02, 3.9; $p=0.045$). *Summary and Conclusions*. The data suggest differences between young and older children, perhaps because the pathophysiology of chest crisis is different in these age groups. In addition, sustained and/or chronic intermittent hypoxia may promote adaptations, possibly in the pulmonary vascular bed, which increase the chance of chest crisis in older children with SCA. The underlying mechanisms may be similar to those underpinning chronic adaptation to high altitude in native populations such as Ethiopians, Tibetans and Bolivian Aymara. Further exploration of the definition and pathophysiology of chest crisis, particularly in young children, the effect of oxyhaemoglobin desaturation and the interaction with asthma in a larger population (of around 500 from our power calculations) should inform the design of randomized controlled trials of interventions in SCA.

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AN UNSUAL CASE OF JUVENILE MYELOMONOCYTIC LEUKEMIA ASSOCIATED WITH ROSAI-DORFMAN DISEASE

G. Menna,[1] N. Marra,[1] R. Parasole,[1] C. De Fusco,[1] V. D'Onofrio,[1] G. Pollio,[2] F. Cecere,[2] R. Russo,[2] M. Zecca,[3] D. Longoni,[4] F. Locatelli,[3] V. Poggi[1]

[1]Santobono-Pausilipon Hospital, NAPLES; [2]Ruggi D'Aragona Hospital, SALERNO; [3]Policlinico San Matteo, PAVIA; [4]San Gerardo Hospital, MONZA (MI), Italy

Background. Sinus Histiocytosis with massive lymphoadenopathy known as Rosai-Dorfman disease (RDD) is a rare benign systemic histio-proliferative disorder, characterised by massive lymphoadenopathy of head and neck, often associated to extranodal involvement. Juvenile myelomonocytic leukaemia (JMML) is a malignant proliferation of myelomonocytic cells, characterised by prominent hepatosplenomegaly, skin infiltration, marked peripheral monocytosis and thrombocytopenia. *Aims.* We describe an exclusive case of juvenile myelomonocytic leukemia in a 2 year-old boy, who had received a previous diagnosis of

Rosai-Dorfman disease. *Patient and Results.* In the august 2006, at the age of 8 months the patient presented with fever and massive bilateral laterocervical, sovraclavear and mediastinal adenopathy, cutaneous nodules, severe anemia (Hb 5.4 g/dL), leukocytosis with neutrophilia (WBC 31.220/mm^3; neutrophils 27.800/mm^3, monocytes 700/mm^3), normal platelet count (147.000/mm^3). Bone marrow aspirate showed a normal trilinear marrow. Lymphonodal biopsy showed histiocytic and plasmacellular infiltration, erythro-lymphophagocytosis; the histiocytes were S-100 and CD 68 positives and CD1a,CD30, CD3, CD20, ALK and EMA negatives. A cutaneous biopsy confirmed the histiocytic infiltration. A diagnosis of RDD was made and a treatment with Prednisolone 2 mg/kg/die for 4 weeks plus Vinblastine 6 mg/m^2/weekly for 6 weeks was started. Clinical and haematological remission was documented. Manteinance therapy with Vinblastine and prednisolone was started. In the august 2007 the patient showed hepatosplenomegaly, thrombocytopenia and increasing monocytosis (>1000/mm^3). A diagnosis of JMML was suspected and confirmed by atypical monocytes on blood smear, elevated HbF (13,9%), monosomy of chromosome 7 with absence of Ph-chromosome at karyotype, spontaneous growth of GM-CFC, K-RAS mutation. According to AIEOP-EWOG MDS 2006 Protocol, therapy with 6-mercaptopurine and cis-retinoic acid was started, with disappearing of fever, mild reduction of spleen enlargement, decrease of monocyte count and improvement of thrombocytopenia. He is still waiting for suitable matched unrelated donor (MUD). *Conclusions.* To the best of our knowledge, this is the first case report of a JMML associated with Rosai-Dorfman disease. If JMML was concurrent or secondary to Rosai-Dorfman disease is still under investigation. Further studies are going on to clarify a possible underling disease, such as FAS deficiency, that can explain the occurrence of both diseases.

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EVALUATION OF ERYTHROPOIESIS IN MYELODISPLASTIC PATIENTS ON AZACITIDINE TREATMENT

P. Danise, F. Rivellini, A. Di Palma, V. Belsito, D. Avino, G. Amendola, A.M. D'Arco

Umberto I, Hospital, NOCERA INFERIORE, Italy

Background. Azacitidine (AZA) treatment of myelodisplastic patients (MDS) has been associated to a significant decrease of transfusional requirement, together with other haematological modifications. In the literature there are only few studies that evaluated the characteristics of the erythropoiesis during AZA treatment. *Aims.* The aims of the study was the evaluation of the erithrocyte and reticulocyte parameters in order to better characterize the erythropoiesis of MDS patients during the AZA therapy.

Table 1.

	BASELINE MEAN RANGE	4 CYCLES	6 CYCLES	P VALUE (0-4)	P VALUE (0-6)
RBC	2.13 (1.61-2.64)	2.89 (2.23-3.22)	3.03 (2.51-3.84)	0.0018	0.0411
Hb	6.97 (6.2-8.3)	9.34 (7.4-10.2)	9.5 (7.9-12.2)	0.0010	0.0469
MCV	100.6 (96.9-107.7)	103.7 (97.0-113.2)	107.0 (100.5-111.8)	0.4261	0.4994
MCH	32.9 (29.2-38.5)	33.5 (30.0-37.9)	35.6 (33.9-36.4)	0.5793	0.0928
MCHC	34.1 (32.6-35.7)	33.7 (33.2-35.7)	34.3 (32.8-36.2)	0.3464	0.9655
RET#	38.8 (7.0-105.1)	93.3 (23.4-158.9)	74.7 (49.1-95.0)	0.0084	0.1059
MCVr	121.1 (94.5-138.3)	127.6 (110.4-137.7)	129.4 (118.3-142.2)	0.2833	0.4865
MCHr	34.4 (30.2-40.0)	37.3 (30.3-41.8)	39.1 (34.4-41.1)	0.1783	0.2266
MCHCr	28.5 (25.7-32.0)	29.2 (26.8-31.7)	30.8 (28.4-33.2)	0.3459	0.0849

Methods. 14 MDS patients (8 males, 6 females), mean age 70 years (range 54-82), including 2 ARSA, 4 AR, 1 AREB-I,1 AREB-II, 6 RCMD, were treated with six cycles of AZA; in each cycle AZA was administered at the dose of 75 mg/m^2/day subcutaneous x 6 days, followed by 1 day without treatment, and by another day of treatment, every 28 days. The erithrocytes and reticulocytes parameters including, Erythrocytes (RBC), Haemoglobin (Hb), Mean Corpuscolar Volume (MCV), Mean Haemoglobin Content (MCH), Mean Corpuscolar Haemoglobin Concentration (MCHC), Reticulocyte absolute count (RET#), Reticulocyte MCV(MCVr), Reticulocyte MCH (MCHr), Reticulocyte MCHC (MCHCr), were evaluated using the analyser Siemens Advia 2120. The data were collected before the therapy and after 4 and 6 months of treatment. The patients were defined responders according to International Working Group response criteria in Myelodysplasia (Cheson BD *et al.* Blood, 2006). *Statistical analysis.* The Student's t test was applied in the responders to evaluate the differences observed during the treatment. *Results*. 9/14 patients showed a decrease of transfusion need and were considered responders. In Table 1 the mean values of parameters of this group, observed at starting point and at 4 and 6 months of the treatment period, are reported together with the statistical evaluation of the differences. Only RBC, RET and Hb showed a significant difference; the qualitative eritrocyte and reticulocyte parameters showed no significant difference. *Conclusions*. AZA treatment can decrease the transfusional requirement in some MDS patients, as confirmed by the present study. The persistence of increased MCV and MCH of reticulocytes associated with decrease of transfusion requirement in the responders could suggest that the maior effect of AZA treatment is not the recovery of a normal erytropoiesis but the expansion of the displastic clone.

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31P MRS *IN VITRO* ASSAY OF PHOSPHOLIPID CHANGES IN RED BLOOD CELLS (RED) FROM PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

M. Kuliszkiewicz-Janus,[1] M. Tuz,[1] M. Kielbinski,[1] B. Jazwiec,[1] J. Niedoba,[1] S. Baczynski[2]

[1]*Wroclaw Medical University, WROCLAW;* [2]*University of Wroclaw, Faculty of Chemistry, WROCLAW, Poland*

Background. 31P magnetic resonance spectroscopy (MRS) *in vitro* is a convenient and analytical tool for phospholipid analysis of extracts from biological samples (tissues, cells and body fliuds). It is a powerful method for investigations aiming at explanation of phospholipid mechanisms in cancer. Our previous studies concerned on phospholipids from plasma and mononuclear cells - from peripheral blood (PBMC), and bone marrow (BMMC), of patients with hematological cancers and healthy volunteers. *Aims.* The aim of this investigation was the application of 31P MRS to determine the phospholipids concentration in erythrocytes from patients with MDS in comparison with healthy volunteers as a control group. *Methods.* 31P MRS spectra of phospholipids were obtained from 15 patients with MDS and 15 healthy volunteers. Phospholipids extracts from RBC (5×10^9 cells) were obtained according to the modified Folch's method employing methanol-chloroform extraction. Studies were carried out on AMX 300 Bruker (7.05 T) spectrometer. The calculation of concentrations was based on integral intensity of the phospholipids compared to methylenediphosphonic acid (MDPA), serving as an external reference substance. *Results.* 31P MRS spectra of phospholipids' extracts from RBC consisted of 7 peaks due to phosphatidylocholine (PC), plasmalogen of phosphatidylocholine (CPLAS), sphingomyelin (SM), phosphatidylinositol and phosphatidylethanolamine (PI+PE), phosphatidylserine (PS) and cardiolipin (CL). We observed these peaks in RBC of both healthy volunteers and patients with MDS. In patients suffering from MDS concentrations of all phospholipids were significantly decreased in comparison with healthy volunteers. The same peaks were noticed previously in 31P spectra of phospholipids extracts from PBMC and BMMC patients with acute leukemia and healthy volunteers. *Summary and Conclusions*. Our investigation shoved that not only phosphatidylinositol, but also the other six phospholipids are decreased in RBC of MDS patients. This disturbance may explaine functional and structural changes observed in erythoryte cell membranes in MDS.

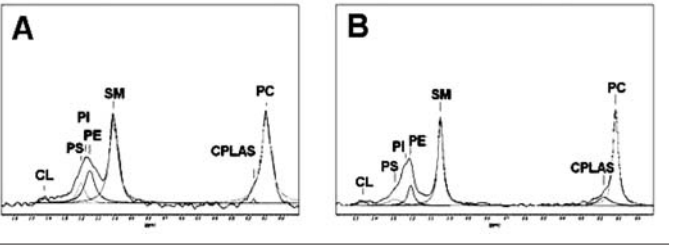

Figure 1. 31P NMR spectra: healthy volunteer (A) and MDS (B).

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EXPRESSION OF C3ORF9 GENE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

A.-V. Karadonta,[1] T. Kourelis,[1] A. Manola,[1] I. Karadonta,[1]
K. Garlemou,[1] D. Kyriakou,[1] N. Stathakis,[1] M. Alexandrakis[2]

[1]University Hospital of Larissa Thessalia, LARISSA; [2]University Hospital of Heraklion Crete, HERAKLION, Greece

Myelodysplastic syndromes (MDS) are clonal disorders of haemopoietic stem cells, characterized by ineffective haemopoiesis and increased probability of transformation to acute leukemia. MDS cells present genetic instability and multiple chromosomal abnormalities. Many genes are disturbed or dysregulated so as an abnormal cell phenotype and function is expressed. C3ORF9 is a gene isolated from an MDS cDNA library and codes for a putative protein of 46.2kDa called X010. We studied the expression of C3ORF9 in various (MDS) syndromes. Eighty-one patients 52 men and 29 women aged 33-85 years (median 69) and 17 healthy counterparts 9 men and 8 women aged 33-85 years (median 71), were involved in our study. RNA extraction was performed from bone marrow aspirates and from isolated CD 34$^+$ bone marrow cells. Gene expression was estimated by Real time PCR. C3ORF9 expression was found downregulated in patients with CMML compared to the normal controls (p<0.01). There was no difference between RARS and the normals (p=0.1), while increased expression was found in RA, RAEB and RAEB-T compared to the normals (p<0.01 for all). No mutations or polymorphism of the gene were detected in our population. CD34$^+$ cells expressed higher levels of C3ORF9 (p<0.01). The increased expression correlated to the proportion of CD34$^+$ in RAEB and RAEB-T(r 2 =0.64). In conclusion the increased C3ORF9 expression was possibly due to either different gene regulation in these patients or the increased CD34$^+$ cells.

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CYTOPENIA PARTICULARLY THROMBOCYTOPENIA AT DIAGNOSIS AS AN IMPORTANT NEGATIVE PROGNOSTIC MARKER FOR PATIENTS WITH ISOLATED 5Q- ABNORMALITY AND NO BLASTS IN BONE MARROW

A.T. Jonasova,[1] R. Neuwirtova,[1] I. Hochova,[2] M. Siskova,[1]
E. Kadleckova,[3] E. Polonyova[4]

[1]Charles University General Hospital Prague, PRAGUE,; [2]Faculty Hospital Motol, PRAGUE; [3]Bata Hospital Zlin, ZLIN; [4]Hospital Karlovy Vary, KARLOVY VARY, Czech Republic

Patients with 5q- isolated abnormality and no blasts in bone marrow (BM) are usually considered as the best prognostic group among MDS patients with survival often much longer than 100 months. Yet there are low risk 5q- MDS patients with much shorter survival. Other chromosomal aberrations or increased BM blasts are well known negative prognostic markers for 5q- patients. We suggest that cytopenia and especially thrombocytopenia at the time of diagnosis is an important and easily obtained marker that can help to identify patients with worse prognosis. *Patients.* We have collected data from 331 MDS patients and identified 39 patients with isolated 5q-abnormality and low BM blasts percentage (<5%) according to aspirate and FACS studies (all BM samples were submitted to second reading). All patients had bi- or trilineage dysplasia in BM. All had sole 5q-abnormality diagnosed by routine chromosome banding and in majority confirmed by FISH. Median age was 70.5 years. Sex ratio F/M was 23/15. Twenty one patients had platelet (PLT) count 150-400×10^9/L (group A), 8 patients had thrombocytosis >400×10^9/L (group B). Only 5 patients in group A had leucopenia <3.0×10^9/L, none had neutropenia <1.0×10^9/L. Among 8 thrombocytemic patients there were 7 females and 1 male. All exhibited classic 5q- syndrome features, with typical macrocytic anemia and typical MGC changes. In thrombocytopenic group with PLT below 150×10^9/L (group C) there were 9 patients; 5 males and 4 females. Two patients had hypoplastic MDS (h-MDS) with pancytopenia and overlap AA features. In this group 4 patients had also leucopenia but only two h-MDS patients had neutropenia <1,0×10^9/L. All patients were treated by standard supportive therapy, some received at some point mostly unsuccessful treatment with GF. Only 2 h-MDS patients treated with immunosuppressive therapy had longer lasting good response (HI - IWG criteria). *Results.* Median survival for group A patients was 64 months. Median survival in group B with PLT>400 was not reached, 5 patients are still alive, 3 survived 96-162 months. Interestingly in several patients in group B thrombocytosis progressed during the course of disease and was inversely related to increasing need of transfusion. Surprisingly all had very high MPV (in comparison to other myeloproliferative disorders with thrombocytosis). None transformed to AML. However, 4 patients (14%) from group A progressed to AML. Median survival in thrombocytopenic group C (PLT<150) was 27 months. One patient progressed to RAEB-T, others died due to infection, during BMT or complications with comorbidities. Only 2 with h-MDS had a longer survival. (Table 1). *Summary.* Cytopenia and mainly thrombocytopenia inversely to thrombocytosis is an important negative prognostic marker for patients with isolated 5q- abnormality without increased BM blasts. However, progression in thrombocytosis in our 5q- syndrome patients was often accompanied by worsening anemia and transfusion dependency.

Table 1. Isolated 5q- patients characteristis.

5q-	All	Group A PLT 150-400	Group B PLT >400	Group C PLT <150
No. of patients	39	21	8	9
Male	15	10	1	4
Female	23	11	7	5
Age median	70.5	70	66	73
Leucopenia	8	4	0	4
AML transform.	5	4	0	1 REAB-T
Median survival months	70	64	Not reached min. 96-162	27

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FACTORS INVOLVED IN RESPONSE TO THALIDOMIDE IN MYELODYSPLASTIC SYNDROME: IMPACT OF HLA AND PROGNOSTIC FACTORS

S.M. Bakanay, K. Dalva, P. Topcuoglu, S. Bozdag-Civriz, M. Beksac, G. Gurman

Ankara University, ANKARA, Turkey

Background. Myelodysplastic Syndrome (MDS) is a clonal disease of hematopoiesis characterized by dysplasia in one or more series. Since the syndrome is clinically heterogenous, treatment options are complex and not well-defined. Thalidomide (T) is the first agent tested in MDS for its immunomodulatory and anti-angiogenic effects. Twenty to 59% response rates, which is mainly hematological improvement, have been reported. However, because of excessive toxicity and low response rates, thalidomide is not generally recommended as an effective treatment option for MDS. Factors invoved in response to T are not well defined. Better response to immunosupression (IS) in HLA-DRB1*15$^+$ patients with aplastic anemia and some MDS subgroups have been reported. *Aims.* Factors which might be important in response to T are investigated. *Methods.* The retrospective medical reports of a total of 54 MDS patients to whom T was prescribed were screened. Responses could be evaluated in 47 patients with median age at diagnosis 59 (15-84) and E/K: 29/18. For response evaluation, patients should have used the drug for ≥ 3 months. According to WHO classification, the subgroups were consisted of 7 refractory anema (RA), 9 refractory anemia with ringsideroblasts (RARS), 5 Refractory cytopenia with multilineage dysplasia (RCMD), 2 RCMD-RA, 13 RCMD-RS, 6 Hypoplastic MDS, 1 RAEB, 4 MDS/MF. International Prognostic Scoring (IPSS) risk groups consisted of 42 low/intermediate-1 and 5 intermediate-2/high risk patients. Abnormal karyotypes were found in 16 patients (5 trisomy 8, 3 monosomy 7, 2 delY, 2 increased chromosomal breaks, 1 del5q, 1 monosomy 15 and 1 del21q). Thalidomide was administered at a dose of 100-400 mg/day and for a range of 3-48 months. *Results.* Twenty-one patients had hematological improvement (10 major and 11 minor). Seven patients among responders had received erythropoietin(EPO) combined with T. There was no statistically significant difference between responders and non-responders in terms of age (median 53 *vs* 60.5 years), sex (M/F: 12/9 *vs* 17/26) and IPSS risk groups (Low risk/high risk: 19/2 *vs* 23/3). Response rates were 44% in RA; 50% in RCMD-RA; 56% in RARS; 55% in RCMD-RS; 20% in RCMD ; 20% in hypoplastic MDS; 75% in MDS/MF. and none in RAEB patient. The EPO levels did not differ significantly between the responders and non-responders (EPO miu/mL <500 in 93% *vs* 83%). However, compared with non-responders, higher ratio of responders had ferritin levels <1000 mcg/L (83% *vs* 50%,

$p<0.05$). A total of 21 patient had their HLA type screened. HLADRB1*15 was detected in 44% of responders (4/9) and 16% of non-responders (2/12) ($p<0.05$). Interestingly, among 5 patients who had trisomy 8, 4 responded to T. *Conclusions*. Fourty-five percent of mainly low risk-MDS patients responded to T. Patients with MDS/MF and RARS/RCMD-RS and patients with low iron burden seem to respond better. Increased ratio of HLADRB1*15 and the presence of trisomy 8 in responders to T should be pointed. Selection of patients will improve the response rates to T decrease the unnecessary exposure to toxicity in others.

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INCIDENCE OF BAALC GENE OVER-EXPRESSION IN ACUTE MYELOID LEUKEMIA SUBTYPES AS DEFINED BY THE WORLD HEALTH ORGANIZATION CLASSIFICATION

G. Balatzenko, A. Stoimevov, M. Guenova, V. Nikolova, S. Toshkov

National Center of Hematology, SOFIA, Bulgaria

Background. The over-expression of the Brain and Acute Leukemia, Cytoplasmic (BAALC) gene is considered as one of the most important prognostic factors in acute myeloid leukemia (AML) patients, particularly in cases with normal cytogenetics. A correlation between BAALC expression level and AML subtypes defined by French-American-British classification has been previously demonstrated. However, the frequency of BAALC over-expression in the AML subtypes as defined by the World Health Organization (WHO) classification has not been reported yet. *Aims.* To determine the distribution of BAALC over-expression among the AML subtypes according to WHO classification. *Methods.* BAALC expression in bone marrow cells was evaluated in 91 adult AML patients [42 females; 49 males] with a mean age of 51.6±6.5 years. The gene expression was studied by a multiplex reverse transcription polymerase chain reaction with co-amplification of BAALC and b-actin mRNA as an internal control. Semi-quantitative characterization of BAALC expression was done using 3 grade score system: negative (–) - strong β-actin reaction, no product of BAALC amplification; weak positive (±) - strong β-actin reaction, faint *band* of BAALC amplification with intensity lower than that of the β-actin; strong positive (+) - clearly visible product of BAALC amplification with intensity equivalent or higher than that of b-actin. *Results*. RT-PCR revealed $BAALC^-$ in 38 pts [41.7%], BAALC(±) in 15 pts [16.5%] and $BAALC^+$ results in 38 pts [41.8%]. Interestingly, an inverse association between the level of BAALC expression and age was observed: mean age of 56.6±4.1 years in $BAALC^-$; 52.9±16.8 years in BAALC(±); and 46.1±17.6 years in $BAALC^+$ (p=0.019). The incidence of $BAALC^+$ in different AML subtypes was as follows: AML with AML1/ETO - 4/5 [80%]; AML with CBFb-MYH11–1/4 [25%]; acute promyelocytic leukaemia with PML-RARA–1/6 [17%]; AML with multilineage dysplasia 1/10 [10%]; therapy related AML - 2/5 [40%]; AML minimally differentiated - 8/10 [80%]; AML without maturation - 6/12 [50%]; AML with maturation - 5/11 [45%]; acute myelomonocytic leukaemia - 4/12 [33%]; acute monocytic leukaemia - 4/9 [44%]; acute erythroid leukaemia - 1/4 [25%]; AML unknown - 1/3 [33%]. No correlation between BAALC expression status and white blood cells count, presence of FLT3 internal tandem duplication and MDR1 gene over-expression was found. *Conclusions*. The incidence of $BAALC^+$ differs among WHO AML subtypes with the highest incidence in AML with AML1-ETO and AML minimally differentiated, while the lowest in AML with PML-RARA and in AML with multilineage dysplasia.

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IDENTIFICATION OF SOMATIC JAK3 MUTATIONS IN ACUTE MYELOID LEUKEMIA

L. Riera,[1] L. Bonello,[1] F. Sismondi,[1] F. Tondat,[1] F. Marmont,[2] L. Godio,[2] P. Francia di Cell,[2] R. Chiarle,[1] G. Inghirami[1]

[1]University of Torino, TORINO; [2]S. Giovanni Battista Hospital, TORINO, Italy

Background. The activating Janus kinase 2 mutation (JAK2V617F) is seen in most Polycythemia Vera (PV) as well as in one third of Essential Thrombocythemia and Primary Myelofibrosis (PMF) patients. Notably, additional JAK2 and MPL (thrombopoietin receptor) mutations have also been reported, some of which can induce a PV-(JAK2) or PMF-like (MPL) phenotype in mice. Thus JAK-STAT pathway appears to play a central pathogenetic role in myeloid malignancies and JAK tailored therapies may represent novel modalities for the treatment of these processes. The Janus kinase genes encode non-receptor tyrosine kinases (JAK1, JAK2, JAK3, and TYK2) and gain-of-function (GOF) mutations have been described for JAK2 and JAK3. Rare JAK3 GOF mutations (JAK3A572V, JAK3V722I [JH2 domain] and JAK3P132T [JH6 domain]) have been recently described in primary and cell lines derived from patients with acute megakaryocytic leukemia (AMKL). *Aims.* Here, we sought to further evaluate the frequency and the possible leukemogenesis role of JAK3 mutations in *de novo* acute myeloid leukemia (AML). *Methods and Results.* We sequenced the JAK3 JH2 domain, (including A572V and V722I) and the exon 3 of the JH6 domain spanning the amino acid site P132T, of 134 *de novo* AML and 191 control DNA samples. All AML and control DNA were wild-type for the A572V substitution (119/119). Moreover, we found two other putative activating substitutions [V722I (2/134) or P132T (1/119)] in AML samples, however at the same frequency observed in our control set, suggesting that these putative mutations may simply represent Single Nucleotide Polymorphism (SNP, V722I 5% and P132T 1%). Interestingly, in a single AML-M7 (1 of 28), a novel homozygous mutation at 132 position, which leads to a transversion of C to G (proline at 132 to alanine, P132A) was documented. To examine the pathogenetic role of JAK3 mutations, we transfected the wild type (JAK3WT) and JAK3V722I, JAK3P132A, JAK3A572V, into IL-3 dependent Ba/F3 cells and cell growth was measured after IL-3 withdrawal. Only JAK3A572V positive Ba/F3 cells grew in the absence of IL-3. These findings were also confirmed using NIH 3T3 transfection/focus formation assays and murine xenograft models, *in vivo*. *Conclusions*. Overall, our results indicate that only JAK3A572V has a transforming potential. Thus additional studies may be required to dissect the precise pathogenetic contribution of JAK3 mutations in myeloid proliferative disorders.

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ACTIVATION OF SETBP1 AS A NOVEL LEUKEMIC MECHANISM IN A PATIENT WITH AML-M5 AND A T(12;18)(P13;Q12) INVOLVING ETV6

I. Cristobal,[1] L. Garcia-Orti,[1] P. Aranaz,[2] N. Marcotegui,[1] M. Maicas,[1] J. Rifon,[3] F.J. Novo,[2] M.J. Calasanz,[2] M.D. Odero[1]

[1]CIMA, University of Navarra, PAMPLONA; [2]University of Navarra, PAMPLONA; [3]Clinica Universitaria, University of Navarra, PAMPLONA, Spain

The ETV6 gene encodes an ETS family transcription factor that is specifically required for hematopoiesis within the bone marrow. Disruption of ETV6 plays a critical role in both myeloid and lymphoid leukemias. At present, more than 15 ETV6 fusion gene partners have been described. Most translocations involving ETV6 generate fusion genes that lead to the activation of either unrelated transcription factors or kinases. However, in a number of cases functionally significant fusions could not be identified and an alternative mechanism consisting in the ectopic expression of genes located close to the breakpoints has been described. Here, we report a novel t(12;18)(p13;q12) involving ETV6 that lead to SETBP1 (18q12) overexpression as a consequence of the translocation, in a patient with acute monocytic leukemia. The patient did not response to the treatment and died three months after diagnosis. FISH and 3'-RACE-PCR experiments showed that the fusion was between exon 2 of ETV6 followed by an intergenic sequence on 18q12 located upstream and close to the SETBP1 start site. This predicts a truncated ETV6 protein that is unlikely to play an important role in the oncogenic process because it lacks both the PTN domain and the ETS DNA binding domain. A 5´-RACE was also carried out but reciprocal transcripts were not observed. Real time PCR showed SETBP1 was overexpressed at diagnosis and in the post-treatment samples. SETBP1 specifically interacts with the acute undifferentiated leukemia-associated protein SET, a potent inhibitor of protein phosphatase 2A (PP2A). Our finding suggests that SETBP1 overexpression could play a role in the leukemogenesis of this patient, perhaps by conferring a growth advantage to leukemic blasts or by affecting PP2A function via SET. SETBP1 has been only reported to be fused to NUP98 in a patient with T-ALL. The function of SETBP1 protein is not well known yet but it has been found an immortalized line containing a Setbp1 integration, which can engraft and induce myeloid leukemia in mice. Nevertheless, it has also been proposed that SETBP1 might require more additional cooperating mutations to be able to induce leukemia. At diagnosis the patient had a trisomy 19 in one clone, indicating that SETBP1 overexpression as a consequence of position effects could cooperate with other additional aberrations to the development of AML in this patient. This case confirmed that deregulation of the expression of surrounding genes could be an alternative leukemogenic mechanism for translocations lacking functionally significant fusion transcripts in AML. Further functional characterization of this translocation is in progress.

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CHANGES IN LEUKEMIA STEM CELL FREQUENCY IN ADULT ACUTE MYELOID LEUKEMIA DURING REMISSION INDUCTION CHEMOTHERAPY

J.-W. Cheong,[1] H.J. Hwang,[2] H.W. Lee,[1] A.J. Choi,[1] H.Y. Choi,[1] J.W. Song,[1] J.S. Kim,[1] Y.H. Min[2]

[1]Yonsei University College of Medicine, SEOUL; [2]BrainKorea21 Research Team of Nanobiomaterials for the Cell-Based Implants,YUMC, SEOUL, South-Korea

Background. To eradicate leukemia stem cells (LSCs) characterized by their self-renewal property is important for long-term cure of acute myeloid leukemia (AML). It has been known that LSCs have CD34+ and CD38- immunophenotype, and several novel molecules such as CD96 and C-type lectin-like molecule-1 (CLL1) have been recently reported as another specific LSC markers. Early detection of LSCs at hematologic complete remission (CR) status may contribute to complete control of the disease. However, the clinical significance of the LSC marker is not yet defined clearly. *Methods.* We evaluated retrospectively the characteristics of bone marrow mononuclear cells and clinical aspects of AML patients who underwent remission induction chemotherapy between 2000 and 2007 in Severance hospital. Bone marrow study was performed at diagnosis, complete remission and relapse. All patients were received induction chemotherapy with cytarabine and idarubicin. FACS analysis was done to assess the expression of CD96 or CLL1 on $CD34^+CD38^-$ cell. *Results.* Forty samples at diagnosis and 26 at CR were obtained from 46 patients. CR rate was 93% and 3 cases were refractory. Seventeen patients were relapsed after 1st CR. Twenty-one were underwent allogeneic hematopoietic stem cell transplantation. The mean frequency of $CD34^+CD38^-CD96^+$ cell at diagnosis was 4.14% and that of $CD34^+CD38^-CLL1^+$ cell was 2.11%. Both $CD34^+CD38^-CD96^+$ and $CD34^+CD38^-CLL1^+$ cell frequencies were decreased to 1.53% and 0.83% (p=0.02, p=0.016) after achievement of 1st CR. Initial CLL1 positvity fraction on $CD34^+CD38^-$ cells was 11.63% and decreased to 2.68% after treatment (p=0.006). CD96 positivity fraction on $CD34^+CD38^-$ cells was not significantly changed from 39.02% to 42.85% (p=0.696). All of 4 available relapsed cases showed that both $CD34^+CD38^-CD96^+$ and $CD34^+CD38^-CLL1^+$ cell frequencies increased than before. Subgroup analysis between low and high LSC frequency at CR was done. High $CD34^+CD38^-CD96^+$ cell group showed shorter CR1 duration (p=0.492) than lower group. However, there was no significant correlation between $CD34^+CD38^-CLL1^+$ cell frequency at CR and CR duration (p=0.89). Overall survival of each group showed no difference statistically (p=0.512, p=0.753). *Conclusions.* LSC seems to correlate with the leukemic burden during remission induction chemotherapy, and the frequency of LSC marker, CD96 or CLL-1, may be one of predicting factors for outcomes of chemotherapy. Further studies with large population to define their clinical implication as a prognostic index or a marker of minimal residual disease are needed.

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JAK2V617F ALLELIC BURDEN QUANTIFICATION IN PH-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES. A NEW METHOD: RESULTS AND CLINICAL CORRELATIONS

M.C. Alonso-Fuentes,[1] E. Salido,[1] J.M. Raya,[1] M. Tapia,[2] B.J. Gonzalez-Gonzalez,[1] M.J. Rodriguez-Salazar,[1] R.F. Rodriguez-Sanchez,[1] M.T. Hernández-García,[1] Y. Barrios,[1] G. Gonzalez-Brito,[1] A. Jimenez,[1] M.A. Guijo,[1] L. Hernandez-Nieto[1]

[1]Hospital Universitario de Canarias, LA LAGUNA (TENERIFE); [2]Hospital General de La Palma, LA PALMA (TENERIFE), Spain

Background. Ph-negative chronic myeloproliferative diseases (CMPD) are a heterogeneous set of entities characterized by uncontrolled clonal proliferation of multipotential hemopoietic cells, which until 2005 lacked a clear genetic marker. Somatic mutation JAK2V617F at the exon 14 has been recognised as a decisive pathogenetic event in these diseases, with a key role in the phenotypic clinical-haematological features of the patients. Several studies have shown a dose-dependent effect of the allelic burden of JAK2V617F, with homozygosity being mainly associated to polycythemia vera (PV), and a lower incidence in essential thrombocythemia (ET) and primary myelofibrosis (PM). *Aims.* To introduce a relatively simple method to quantify allelic frequencies of JAK2V617F mutation in patients with Ph-negative CMPD, and to test eventual associations between the allelic burden, diagnosis, and clinical and haematological data. *Methods.* We have developed a method based on regression analysis to obtain the allelic ratios in heterozygous patients previously genotyped for JAK2V617F. WHO criteria were applied for diagnosis and the patients were clinically followed at our Hospital Department. DNA extraction was made from isolated blood granulocytes of patients previously classified as heterozygous. The region of interest was amplified by PCR, and a 289 bases pairs fragment was obtained by means of specific primers for the JAK2 gene. This purified and selected product was cloned in the vector with a high number of copies (pGEM®-T Easy Vector II), according to manufacturer instructions (Promega). The resulting construct was transfected in competent JM 109 cells. A selection of transformants was made on plates with LB / ampicillin / IPTG / X-Gal. Confirmation of wild-type and mutant alleles was performed by sequencing and a PCR-RFLP (restriction fragment long polymorphism) specific technique. An estimation of the frequency of the mutation was obtained by image analysis of DNA gels using a standard curve generated from plasmid cloned alleles with known proportions. This method was used in a pilot study on 16 patients with Ph-negative CMPD (6 PV; 10 ET). *Results.* With this method we were able to quantify allelic ratios in 16 patients heterozygous for the mutation (range 24-92%). The higher allelic percentages of JAK2V617F allele were seen in PV patients (mean 64.7%), and the lower ones in ET (mean 37%; p=0.002). Higher allelic burdens were related, just over the limits of significance, with thrombosis (p=0.06) and splenomegaly (p=0.06), and significantly related to hematocrit (p=0.01), LDH (p=0.01), and PRV-1 overexpression (p=0.01). *Conclusions.* The allelic burden of JAK2V617F mutation was higher in PV than in ET patients. Higher allelic burden was seen in patients with higher hematocrit and LDH, and near the limits of significance in relation with thrombosis and splenomegaly. Although our PCR-RFLP based method is more laborious than RT-PCR, we consider that it is useful, specific and less costly. We therefore recommend it for allelic quantification in order to reinforce clinical information.

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BASELINE HOMOCYSTEINE LEVELS AND POLYMORPHISMS IN HOMOCYSTEINE METABOLIZING ENZYMES: ASSOCIATION WITH DEEP VEIN THROMBOSIS IN ASIAN INDIANS? AIIMS EXPERIENCE

A. Biswas,[1] A. Meena,[2] R. Ranjan,[2] M. Akhter,[2] B. Yadav,[2] J. Bajaj,[1] R. Saxena[2]

[1]All India Institute of Medical Sciences, NEW DELHI; [2]Department of Hematology. All India Institute of Medical Sciences, NEW DELHI, India

Background. Elevated homocysteine levels have been seen to be associated with the presence of thrombotic events. These may include arterial thrombotic events like stroke and myocardial infarctions or venous events like deep vein thrombosis, more so arterial as its association with thrombosis is well established. *Aims.* Lowering of homocysteine through administration of appropriate vitamin supplements has been documented in Cardiovascular disorders in India. These are primarily arterial conditions. The role of homocysteine in venous thrombotic conditions is less well documented and very little data is available in Deep Vein Thrombosis in Indian patients in this regard. Our present study takes a retrospective look at Deep Vein Thrombosis patients attending the Hematology Department of our hospital, which apart from looking into issues of Vitamin supplementation and Homocysteine, includes data on polymorphisms as well. *Methods.* 120 consecutive patients with deep venous thrombosis, confirmed by Doppler ultrasonography, were the subjects of study. All patients and equal number of healthy age and sex-matched controls were investigated for Homocysteine levels and also screened for MTHFR C677T, A1298C, MTR A2756G and MTRR G66A polymorphisms. Patients who were seen to have high levels of homocysteine were prescribed vitamin supplementation (Folic acid 5mg O.D, Vitamin B12 5µg O.D, Vitamin B6 2mg O.D) and were asked to report for a follow-up homocysteine check up post 3 months. 61 patients were found to have high levels of homocysteine of which only 23 reported for a follow-up homocysteine checkup. Differences between allelic and genotypic frequencies of individual polymorphisms between cases and controls and Hardy-Weinberg equilibrium (HWE) were tested using chi square tests. Statistical tests were done using SPSS version 12. *Results.* Most cases (97) were of lower limb thrombosis, 11 cases were of upper limb thrombosis. Thrombosis at unusual sites was present in 12 cases. These included the cerebral (5), abdominal (3), subclavian(2), renal(1) and retinal vein(1). High levels of baseline homocysteine were seen to be significantly associated with Deep Vein Thrombosis (p<0.001). All of the patients responded to the Folic acid dosage and showed reduction in the levels of plasma homocysteine(of the order of 13.1 umol/L i.e. 60.6% of the mean baseline plasma homocysteine of responders). How-

ever in some of these (7 out of 23) only a partial response was seen (plasma homocysteine levels reduced but not normalized i.e. between 5-15 umol/L). In these partial responders none of the patients were seen to carry the mutant homozygous forms of the MTHFRC677T, MTRA2756G or MTRRG66A polymorphisms. Only one patient amongst them carried the CC (mutant) genotype of the A1298C polymorphism. None of the complete responders carried the mutant homozygous forms of any of the four polymorphisms studied. The partial response to Folic acid dosage may be explained by the short duration of follow-up time(3-6 months) involved. Only MTHFR C677T & A1298C polymorphisms were seen to be associated with homocysteine levels ($p<0.01$). However none of the investigated four polymorphisms showed any association with Deep Vein Thrombosis. *Summary and Conclusions.* Nutritional deficiency and not genetic variation maybe the primary contributor to the prevalent hyperhomocysteinemic condition in Deep Vein Thrombosis patients of Asian Indian origin.

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HIGH LEVELS OF FACTOR VIII IN ASIAN INDIAN PATIENTS SUFFERING FROM ACUTE ONSET NON-CARDIOEMBOLIC STROKE

A. Biswas,[1] R. Ranjan,[2] A. Meena,[2] M. Akhter,[2] B. Yadav,[2] R. Saxena,[2] M. Behari[3]

[1]All India Institute of Medical Sciences, NEW DELHI; [2]Department of Hematology, All India Institute of Medical Sciences, NEW DELHI; [3]Department of Neurosciences. All India Institute of Medical Sciences, NEW DELHI, India

Background. Factor VIII is uniquely placed as a risk factor. It is well established as an acute phase reactant and therefore one could argue that high levels of Factor VIII post a thrombotic event may be more as a result of the acute phase rather than Factor VIII level itself being the cause for the event. However there have been reports, which do establish it to be predictive for thrombosis and not just the result of an acute phase event. Therefore the dichotomy still remains. *Aims.* We conducted the present study to see if there is a significant association of high levels of Factor VIII and stroke even in a post acute phase situation. *Methods.* This study was conducted on 120 stroke patients and 120 healthy age and sex matched controls with ages below 40 years. The subjects of this study were patients of acute onset non-cardioembolic stroke. The diagnosis of stroke was confirmed by imaging methods like CT/MRI. Samples were collected in the acute phase as well as 3 months post onset of stroke. Ethical Clearance for the above study was taken from the local ethic committee. Factor VIII levels were performed using the one stage aPTT based assay and expressed as percentage of Normal Pool Plasma (% NPP). Statistical analysis was performed on SPSS version12. A p-value <0.05 was considered statistically significant. Calculations were done at 95% Confidence interval and all p values were two tailed. *Results.* The distribution of Factor VIII levels in the control population was seen to be normal (Kolmogorov-Smirnov test, $p=0.283$). Post-acute phase mean Factor VIII levels [125.6 + 21.1%NPP for controls and 136.2+28.8% NPP for patients; Figure 1.] were significantly raised in patients compared with controls ($p=0.001$).

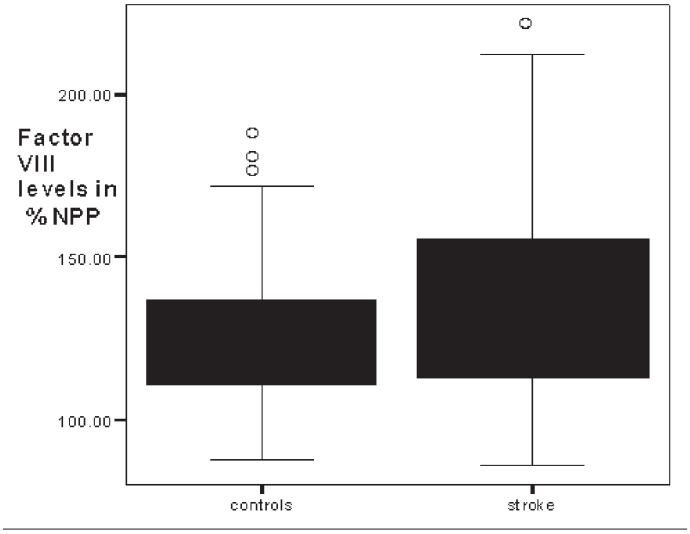

Figure 1. Factor VIII levels patients *vs* controls.

However when the number of patients and controls [12(10%) and 4(3.3%) respectively] with high Factor VIII levels i.e. >95th percentile of the control population] in our study were compared the difference was not seen to be significant ($p=0.070$). We did not find any significant difference in the levels of Factor VIII seen in strokes of venous or arterial origin from our study ($p=0.396$), In our study 5.8%(7 out of 120) of patients and 0.8%(1 out of 120) of controls with high Factor VIII level showed Activated Protein C resistance. *Conclusions.* Factor VIII level is not just an acute phase reactant and may have a predisposing prothrombotic role in stroke patients although it may not act in an independent manner. The predisposing role of high Factor VIII level may have its origins in natural genetic variation or inability to clear Factor VIII or increased vWF half-life amongst other reasons. The effect of Activated Protein C resistance in 5(4.1%) patients may be attributed to high Factor VIII level. However, since 4(3.3%) patients with high Factor VIII levels were not positive for Activated Protein C resistance indicates that Activated Protein C resistance may not be the only prothrombotic mechanism for Factor VIII action.

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Withdrawn by the authors

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CORRELATION OF COAGULATION MARKERS, PLATELET PARAMETERS AND RESPIRATORY INDEXES IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

A. Lekka,[1] M. Dalamaga,[2] M. Triantafilli,[1] G. Sotiropoulos,[1] D. Poulou[1]

[1]NIMTS General Hospital, ATHENS; [2]Attikon General University Hospital, ATHENS, Greece

Background and Aims. The clinical course of chronic obstructive pulmonary disease (COPD) has been associated with thromboembolic events. The aim of the present was to investigate the changes in platelet parameters (mean platelet volume-MPV, platelet distribution width-PDW) and coagulation markers such as (PT, aPTT, fibrinogen, ATIII, Pr C, plasminogen-PL, D-Dimer and lupus anticoagulant-LA) in COPD as well as to correlate the above markers with respiratory function indexes (FEV1, pO2 and pCO2). *Methods.* We studied 56 patients with COPD (39 males and 17 females) with a mean age of 68, matched on gender and age with 56 healthy controls. Blood samples were collected. Platelet number (PLT), MPV, PDW, PT, aPTT, fibrinogen, ATIII, PrC, PL, D-Dimer and LA were determined. Respiratory function was assessed by determining forced expiratory volume in one second-FEV1, pO2 and pCO2. Statistical analysis of the data was performed using SPSS version 10 for Windows statistical package. *Results.* Statistical analysis of the data showed: 1) only fibrinogen from the coagulation markers was significantly higher in patients with COPD than in controls ($p=0.001$), 2) ATI-II was significantly lower in patients with COPD than in controls ($p=0.002$), 3) 6 patients with COPD presented positive LA and 4) Platelet mass was significantly higher in patients with COPD than in controls ($p=0.03$). In patients with COPD, we found that: 1) fibrinogen presented significant negative correlation with FEV1 ($p=0.04$, r=-0.362) and pCO2 ($p=0.013$, r=-0.43), 2) ATIII was negatively and significantly correlated with MPV and PDW ($p=0.038$, r=-0.362 and $p=0.028$, r=-0.383 respectively). On the contrary, ATIII didn't present any significant correlation with respiratory function indexes ($p>0.05$). *Conclusions.* Patients with COPD present an enhanced prothrombotic process characterized by a higher platelet mass and fibrinogen as well as a lower ATIII. Higher fibrinogen is associated with respiratory dysfunction. Further studies are needed to explore underlying mechanisms associating coagulation markers and platelet parameters with respiratory function in patients COPD.

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INCIDENCE OF ALTERATIONS OF COAGULATION ON THE RISK OF THROMBOSIS IN ESSENTIAL THROMBOCYTEMIA

N. Padron Rodriguez, N. Fernandez Mosteirin, C. Salvador Osuna, P. Mayayo, M. Torres, P. Giraldo, M. Giralt

Hospital Universitario Miguel Servet, ZARAGOZA, Spain

Background. Essential Thrombocythemia (ET) is a myeloproliferative disorder (MPD) characterized by an hyperplasia of the megakaryocytic cells in bone marrow resulting in a persistent increase of platelet count in peripheral blood. Thrombosis and bleeding complications are the main causes of morbidity and mortality in ET. There is an increas-

ing number of studies that shown the influence of thombophilic states in the occurrence and severity of thrombotic events. *Aims.* To analize the incidence of thrombotic events in ET patients and the influence of thrombophilia as a thrombotic risk factor in our patients. To determine the incidence of inherited thrombophilia in patients with ET diagnosed in our centre. *Patients and Methods.* We have reviewed clinical files and thrombophilic parameters including cardiovascular risk factors in patients diagnosed of ET between 1994 and 2007. The studies included: APTT, Protein C (PC), Protein S (PS), Antithrombin (AT), Plasminogen (Pl), lupic anticoagulant (LA), anticardiolipin antibodies (ACA), Protein C Activated Resistance (PCAR), FV Leiden, Prothrombin 20210A and MTHFR mutation. JAK2 V617 mutation was investigated too. Occurrence and clinical characteristics of thrombotic events before diagnosis and during patients follow up. *Results.* We have analized 53 patients (32 female/21 male). Mean age at diagnosis 57.7 years (range: 28-85). Median time of follow up 75.6 months (range: 4.8-216). 15 patients (27.7%) developed thrombotic events, and 24 (44.4%) developed thrombotic and/or vasomotor symptoms (headache, pruritus, erythromelalgia, paresthesias). 93.3% of the thrombotic events where arterial thrombosis. 1 patient experienced venous and arterial thrombosis, 2 patients experienced thrombotic events once cytorreductive and antiaggregant therapy had started, both of them with abnormal thrombophilic parameters (positive LA and low levels of Pl). The rest of the patients developed thrombotic events before starting treatment. Presence of cardiovascular risk factors didn't appear to significantly increase the incidence of thrombotic complications 79% of thrombosis happened in patients with one or more alterations in the study of thrombophilia. 30 patients (55.5%) showed normal results of the thrombophilic parameters analized. In patients with abnormal results the most frequent thrombophilia alteration was presence of LA present in 8 patients (7 of them showed arterial thrombosis). 10 patients showed MTHFR mutation (8 heterozygous/2 homozygous), 5 of them showed non significant increased levels of homocystein. 5 patients showed low levels of Pl, 3 low levels of PC, 1 low levels of PS and 1 low levels of AT. 9 of the 24 patients with thrombosis had been studied for the presence of JAK2 mutation and was present in 5 of them. In Table 1 we describe clinical characteristics of thrombotic events and thrombophilic parameters in these patients. *Conclusions.* According to the results of our centre thrombosis risk in ET is increased in patients with alterations of thrombophilia parameters, mainly presence of LA. Thus, thromobophilia studies could be considered as an useful tool in risk stratification of these patients at diagnosis. However, more studies are necesarious to stablish the independent predictive value of each one of these alterations.

Table 1.

	A	V	Vasomotor	Thrombophilia		A	V	Vasomotor	Thrombophilia
1	-	-	Paresthesias	-	13	Stroke	-	-	LA MTHFR/MTHFR
2	IC	-	-	-	14	Stroke	-	-	Low Plasminogen
3	AMI	-	-	-	15	-	-	Pruritus	Low Plasminogen
4	TIA	-	Angina	-	16	-	-	Paresthesias	Low PC MTHFR/N
5	-	-	Paresthesias	-	17	Stroke	-	Paresthesias	MTHFR/N
6	PI	-	-	LA	18	-	TVP	-	MTHFR/N
7	-	-	Paresthesias	LA MTHFR/N	19	-	-	Paresthesias	MTHFR/N
8	IHD	-	-	LA	20	-	-	Angina	MTHFR/N
9	TIA	-	-	LA	21	TIA	-	-	MTHFR/N
10	Stroke	DVT/PE Espleno portal	-	LA	22	TIA		-	MTHFR/MTHFR
11	-	-	Paresthesias Pruritus	LA	23	IHD	-	Erythromelalgia	MTHFR/MTHFR
12	-	-	Headache	LA MTHFR/N	24	TIA	-	Paresthesias	Low AT MTHFR/N

IC: ischemic colitis, AMI: acute myocardial infarction, TIA: transient ischemic attack, PI: peripheral ischemia, IHD: ischemic heart disease, DVT: deep vein thrombosis, PE: pulmonary embolism.

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COMBINED TROMBOPHILIC DEFECTS IN PATIENTS AFTER AN INCIDENT OF ACUTE IDIOPATHIC VENOUS THROMBOEMBOLISM

K.M. Zawilska,[1] E.W. Wojtasinska,[2] Z.T. Turowiecka,[2] A.K. Lehmann-Kopydlowska,[3] M.Z. Zytkiewicz,[4] K.C. Ciepluch[2]

[1]University of Medical Sciences in Poznan, POZNAN; [2]University of Medical Sciences, POZNAN; [3]J.Strus Hospital, POZNAN; [4]City Hospital, POZNAN, Poland

Background. Venous thromboembolism is a multifactorial disease that manifests when a person with an underlying predisposition to thrombosis (i.e. inherited thrombophilia) is exposed to clinical risk factors. *Aim of the study*. Laboratory assessment of the frequency of combined trombophilic defects in patients with idiopathic venous thromboembolism. *Methods.* In 136 patients, 74 female and 62 males, with an age range of 18-74 years (mean 39,4 years), at least 6 weeks after an incident of acute idiopathic venous thromboembolism, following laboratory evaluation has been performed: antithrombin activity, protein C activity, free protein S antigen level, APC-resistance ratio, factor VIII activity, factor V Leiden mutation and prothrombin G20210A mutation genotyping. *Results.* 45% of patients had inherited thrombophilia, most frequently it was the factor V Leiden mutation (24% of all patients, 40% of patients aged 25 years or less). Other thrombophilic defects: antithrombin deficiency in 11%, protein C deficiency in 10%, protein S deficiency in 8%, prothrombin G20210A mutation in 6% of patients. We found 5,9% of combined carriers for more than one familial thrombophilia defect. 15% of patients heterozygous for the factor V Leiden had also prothrombin G20210A mutations. In the group of patients with antithrombin deficiency 20% had a diminished activity of protein C, and 30% of patients - a diminished free protein S antigen level. 18% of patients with protein C deficiency and 30% of patients with protein S deficiency had antithrombin deficiency as well. *Conclusions.* Factor V Leiden mutation was the most frequent thrombophilic defect, which occured in 24% of all patients tested because of an incident of acute idiopathic venous thromboembolism and in 40% of subgroup of patients aged ≤25 years. 5,9% of patients from the whole study group were carriers for more than one familial thrombophilia defect.

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ARTERIAL THROMBOSIS IN PATIENTS WITH ANTIPHOSHOLIPID SYNDROME

A. Matyja-Bednarczyk, J. Swadzba, T. Iwaniec, J. Musial

Jagiellonian University Medical College, KRAKOW, Poland

Background. Arterial thrombosis in antiphospholipid syndrome (APS) is less common than venous thrombosis but results in greater disability and morbidity. *Aims.* The aim of the study was to find characteristic laboratory and/or clinical features which would distinguish APS patients with arterial thrombosis from the other APS patients. *Methods.* We retrospectively studied 121 patients with definite APS (updated classification criteria of APS -2006; 88 APS secondary to SLE and 33 without any features of other autoimmune diseases). Laboratory determinations included: lupus anticoagulant (LA), anticardiolipin (aCL) and anti-beta2-glycoprotein I (anti-β2GPI) antibodies (Abs) [IgG and IgM class]. Clinical features concern symptoms included in the classification criteria as well as antiphospholipid antibody-associated symptoms (e.g. livedo reticularis, thrombocytopenia). *Results.* Among our 121 patients, 52 suffered from arterial thrombosis (total of 84 episodes; 31 ischemic stroke, 21 transient ischemic attack -TIA, 14 acute myocardial infarction, 15 peripherial arterial thrombosis, 1 splenic infartion and 2 renal thrombotic microangiopathy) were registered. A significant association was found between the level of aCL IgG and arterial thrombosis as compared to other APS patients. *Conclusions.* Some authors suggest that we can limit antiphospholipid antibody determinations to anti-β2GPI only. Association of aCL IgG levels and arterial thrombosis argues against such limitations.

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PRIMARY MYELODYSPLASTIC SYNDROME WITH NORMAL CYTOGENETICS: VALUE OF INTERPHASE FISH FOR THE DETECTION OF CRYPTIC CHROMOSOME 7 ABNORMALITIES

M.-J.P.L. Stevens-Kroef, A. Simons, R. Kuiper, S. Langemeijer, D. Olde Weghuis, T. de Witte, A. Geurts van Kessel

Radboud University Nijmegen Medical Centre, NIJMEGEN, Netherlands

Background. Myelodysplastic syndrome (MDS) constitutes a heterogeneous group of hematopoietic disorders. In the past, cytogenetic abnormalities have been proven to serve as valuable markers for the prognosis of MDS, both with respect to survival time and progression risk to acute myeloid leukaemia. Poor risk factors (as proposed by IPSS) include defined abnormalities of chromosome 7, i.e., monosomy 7 and partial losses of the long arm of chromosome 7. The accurate detection of these abnormalities is imperative for the prognosis of patients with MDS. *Aims.* Since, conflicting results have been published regarding the added value of interphase FISH, we have evaluated the effectiveness of interphase FISH for the detection of chromosome 7 abnormalities in MDS patients with a normal karyotype. *Methods.* For cytogenetic analysis fresh bone marrow cells were cultured during 24 hrs using standard procedures. In order to consider a patient cytogenetically normal, at least 20 metaphases had to be evaluated extensively and found to be normal. FISH was performed with the LSI D7S486 (7q31) / CEP 7 probe-set (VYSIS) on the same bone marrow preparations as used for cytogenetic analysis, and at least 200 interphase nuclei were scored by two independent investigators (cut-off value 2%). *Results.* We have tested 43 MDS patients, and failed to observe any discrepancies between the cytogenetic and FISH analyses. In addition, three of these cases were subjected to high-resolution SNP-based array CGH. Again, in none of these cases chromosome 7 abnormalities were detected. *Conclusions*. Taken together, we conclude that additional FISH for identification (partial) loss of chromosome 7 in MDS patients with a normal karyotype, as demonstrated by standard karyotyping, has limited added value.

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JUMPING TRANSLOCATIONS IN HEMATOLOGICAL MALIGNANCIES: MOLECULAR CYTOGENETIC STUDY OF FOUR CASES

K. Manola,[1] V. Georgakakos,[1] C. Stavropoulou,[1] A. Spyridonidis,[2] M. Angelopoulou,[3] I. Vlachadami,[4] P. Roussou,[5] G. Pantelias,[1] C. Sambani[1]

[1]NCSR Demokritos, ATHENS; [2]Division of Hematology, University of Patras, PATRAS; [3]1st Department of Internal Medicine, Medical School, Laikon Hospital, University, ATHENS; [4]Department of Pathophysiology, Medical School, Laikon Hospital, University of At, ATHENS; 53rd Department of Internal Medicine, Sotiria Hospital, Medical School, Universit, ATHENS, Greece

Backround: Jumping translocations (JTs) are rare cytogenetic aberrations in hematological malignancies which include unbalanced translocations involving a donor chromosome segment that has fused to multiple recipient chromosomes. Although they have been associated with poor clinical outcome, the origin and the pathogenesis of JTs remain obscure. *Aims.* We present a molecular cytogenetic study of four cases with different hematological disorders and JTs in order to contribute in the investigation of the origin and the pathogenesis of JTs. *Patients and Methods.* Chromosome studies were performed on unstimulated bone marrow cells, derived from one 30-year-old female patient with AML-M1, one 22-year-old male patient with Burkitt lymphoma (BL), one 66-year-old male patient with AML-M5, and one 55-year-old male patient with undifferentiated leukemia. FISH studies were performed on the same bone marrow cytogenetic specimens using the following commercial DNA probes: whole chromosome paints specific for chromosomes 1, 11, 12, and 13, alpha satellite probes for the centromeres of chromosomes 1, 7, 11, 12, 13/21, 14/22, and 15, C-MYC/IGH t(8;14), and LSI MLL dual color break apart rearrangement probe. *Results*. Patient 1 with AML-M1, showed clonal JT of 1q as a sole abnormality jumping on to 7q, 11q, 12q and 15p, resulting in trisomy of 1q. Patient 2 with BL demonstrated a JT of 1q as a secondary abnormality, in addition to t(8;14)(q24;q32), jumping on to 13q, 14p, 18q and 21q, resulting in partial trisomy of 1q. None of these two patients demonstrated increased constitutional heterochromatin of chromosome 1, or pericentromeric heterochromatin decondensation. Patient 3 with AML-M5, showed JT of 13q, as a secondary change in addition to trisomy 8, jumping on to 1p, 14q, 10p, and Yq, leading to a partial trisomy of 13q. Patient 4 with undifferentiated leukemia, revealed JT of a duplicated segment of 11q, jumping on to two recipients chromosomes in each cell line. FISH analyses confirmed the cytogenetic findings in all patients. FISH proved the dicentric nature of the derivative recipient chromosomes in patient 1, in whom JT was disappeared four months later. In patient 4, FISH showed that the donor of JT was a duplicated segment of 11q containing two copies of the MLL gene, resulting in partial exasomy of 11q and six copies of MLL. All patients had an aggressive clinical course. *Summary and Conclusions*. This study presents the first case of JT associated with AML-M1, the first case of JT involving 13q as a donor chromosome and the first report of JT involving a duplicated segment of 11q, containing two copies of the MLL gene, jumping on to two recipients chromosomes in each cell line, resulting in six copies of the MLL gene. Moreover, it demonstrates that chromosome band 1q10 as a breakpoint of the donor chromosome 1q, is also implicated in AML and not only in multiple myeloma as it has been known until now. The transient nature of JT in patient 1, indicates that JT may have not contributed to the progression of disease but may have been a simple reflection of chromosomal instability.

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COMPARATIVE STUDY OF CONVENTIONAL CYTOGENETICS AND FISH FOR EGR1 IN PATIENTS WITH PRIMARY MYELODYSPLASTIC SYNDROME AND 5Q DELETION

C. Stavropoulou, V. Georgakakos, K. Manola, G. Pantelias, C. Sambani

NCSR Demokritos, ATHENS, Greece

Background. Deletion of the long arm of chromosome 5 (del(5q)) and loss of a whole chromosome 5 (monosomy 5) are the most frequent recurring abnormalities arising *de novo* in 10% to 15% of patients with primary myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). They are present either as the sole karyotypic abnormality or in combination with other chromosomal abnormalities. The deletion 5q can be detected either by conventional cytogenetic methods analyzing mitotic cells in bone marrow, or by fluorescent *in situ* hybridization in non-proliferating interphase cells. *Aims.* To verify 5q31 deletion by the use of FISH on a series of patients with primary MDS in which conventional cytogenetics showed chromosome 5 abnormalities at diagnosis, and to compare the efficacy of both karyotyping and FISH to detect 5q deletion. *Methods.* Twenty eight consecutive patients with primary MDS and chromosome 5 abnormalities were enrolled in the present study. For each patient, karyotyping was done on up to 20 metaphases from bone marrow specimens at diagnosis. FISH studies were carried out on the same fixed material used for conventional cytogenetics, by the use of probes specific for EGR1 (5q31.2) and D5S23/D5S721 (5p15.2). *Results.* Patients were subdivided according to the complexity of their karyotype. Among the 28 patients studied, 10 (35.7%) showed an isolated 5q deletion, 4 (14.3%) had deletion 5q accompanied by one additional abnormality and 14 (50%) had -5/del(5q) as part of a complex karyotype, including ≥3 numerical and/or structural abnormalities. Seven out of 28 patients (25%) exhibited monosomy 5, all of them showing the abnormality as part of a complex karyotype. Twenty six out of 28 patients presented 5q31/EGR1 deletion. All 7 cases with monosomy 5 showed two copies of the signal corresponding to the 5p15.2 region and one copy for EGR1. The mean percentage of cells carrying 5q deletion was higher in the karyotypic evaluation as compared to the corresponding percentage in FISH analysis (63.1±31.8, range 10-100 *vs* 47.7±22.9, range 2-75.8). Discordance between FISH and conventional cytogenetics was observed in 1/21 cases with 5q deletion. The patient showed unusual breakpoints (del(5)(q13q22)) without loss of the hybridization site for EGR1/5q31. *Summary and Conclusions.* Our findings indicate that true monosomy 5 is a rare cytogenetic aberration in primary MDS which is in line with recent reports. Interestingly, percentage of cells with 5q deletion was lower by FISH than conventional cytogenetics. This observation may be related to a higher mitotic rate of cells with 5q deletion than normal cells. For this reason, it is important to avoid alternating between the two techniques in order to quantify the tumour clone. In conclusion, our results support the use of conventional cytogenetics for the detection of 5q deletion at diagnosis and the follow up of patients undergoing lenalidomide treatment. Thus, FISH analysis should be used as a complement rather than a substitute for conventional cytogenetics in the detection of 5q deletion.

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EXPRESSION OF BONE MORPHOGENETIC PROTEINS (BMPS) IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

J. Dzietczenia, T. Wróbel, G. Mazur, R. Poreba, A. Butrym, B. Jazwiec, K. Kuliczkowski

Wroclaw Medical University, WROCLAW, Poland

Background. Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-TGFβ superfamily. About 20 BMPs have been identified. These multifunctional cytokines regulate proliferation, differentation, morphogenesis and apoptosis in a variety of cell types including hematopoietic cells. To initiate their cellular response BMPs bind to two types of serine/threonine kinase receptors:BMP type I receptors-RIA (ALK3-activin like kinase-3), RIB (ALK6-activin like kinase-6) and BMP type II receptor-RII. Autophosphorylation of the BMP recep-

tors complex causes phosphorylation of intracellular signal transducers SMAD-s. BMPs may be involved in protection against several types of cancer. Irregulation of BMP signaling pathways has been reported in some malignancies such as pancreatic, biliary tract and colorectal carcinomas. Increased levels of BMPs have been detected in prostate and breast cancers.The role and significance of BMPs and their receptors in hematopoietic malignancies remains unclear. *Aims.* The aim of our study was to analyze the expression of BMP type I and BMP type II receptors in untreated patients with B-cell chronic lymphocytic leukemia. *Materials and methods*. 30 patients with B-chronic lymphocytic leukemia (B-CLL) were evalueted (17 males and 13 females). The median age of patients was 68 years (range: 42-81 years). 15 patients (50%) had stage I, 7 patients (24%) had stage II, 4 patients (13%) had stage III and 4 patients (13%) had stage IV acc. Rai Staging System. All patients were examinated before treatment. The healthy control group includes 10 persons. Expression of BMP-receptors: BMP RIA, BMP RIB and BMP RII was determined by 2-color flow cytometry. Antibodies against BMP RIA, BMP RIB and BMP RII (R&D System) were used. For statistical analysis U-Mann Whitney Test and ANOVA rang Kruskal-Wallis Test were used. $p<0,05$ was considered statistically significant. *Results*. In B-CLL patients expression level of BMPRIA, BMPRIB and BMPRII were significantly higher than in the controls (BMPRIA 5,33 *vs* 0,18; BMPRIB 5,20 *vs* 0,18; BMPII 6,97 *vs* 0,19 respectively). Furthemore, the expression level of BMPRIA and BMPRIB were higher in patients with III and IV stage of disease than in patients with stage I or II (BMPRIA 14,51 *vs* 1,85; BMPRIB 13,40 *vs* 2,07 respectively). We found that increased level of lactate dehydrogenase correlate with higher expression of BMPRIA and BMPRIB in comparison with normal level of lactate dehydrogenase (BMPRIA 11,55 *vs* 1,52; BMPRIB 11,41 *vs* 1,40)($p<0,05$). *Conclusions.* These results suggest that BMPs and their receptors are increased in active CLL and is closely related with the stage of the disease. It is also possible to use BMP-receptors as prognostic markers in B-CLL patients.

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INCREASED EXPRESSION OF VASCULAR ENDOTHELIAL FACTOR RECEPTOR-1 AND THE LOSS OF ESTROGEN RECEPTOR BETA IN PEDIATRIC ACUTE MYELOID LEUKEMIA PATIENTS

F. Atalar,[1] A. Tekiner,[2] U. Ozbek[3]

[1]Istanbul University DETAE, ISTANBUL; [2]Istanbul University, Institute of Experimental Medical Research Genetics Dept, ISTANBUL; [3]Istanbul University,Institute of Experimental Medical Research (DETAE, ISTANBUL, Turkey

PI3K/Akt/mTOR cascade is one of the possible activation mechanisms in Acute Myeloid Leukemia (AML). Constitutive PI3K/Akt/mTOR signaling is upregulated by the activating mutations of receptor tyrosine kinases (RTKs), autocrine/paracrine secretion of growth factors (FGF, IGF-1,VEGF) and estrogens triggering the binding of estrogen receptors alpha (ER?) to PI3K and estrogen receptor beta (ERβ) to AKT. The FLT3 internal tandem duplication (FLT3/ITD) as well as the FLT3 activation loop mutation (FLT3/D835) lead to constitutive activation of the RTK. Other members of this class of receptors such as vascular endothelial growth factor [VEGF] receptors have also been implicated in the pathogenesis of AML as it was previously shown that FLT-1 activation by VEGF or PLGF stimulation results in a significant increase of AML migration. We performed mutational analysis of FLT3 together with the expression analysis vascular endothelial growth factor (VEGF) receptors (Flt-1, KDR [kinase domain receptor]) and estrogen receptors (ERα and ERβ) in a group of 50 pediatric patients with AML. FLT3/ITD and FLT3/D835 mutations were performed on DNA material isolated from peripheral blood and /or bone marrow cells of 50 pediatric AML patients by PCR and enzyme digestion *Methods.* Total RNA was also isolated from patients and the expressions of FLT-1, KDR, ERα and ERβ were analyzed by RT-PCR. FLT3/ITD and FLT3/D835 mutations have been identified in 12% and 2% of our study group, respectively. Flt-1 and KDR are expressed in 57.5% and 48.5% of pediatric AML patients, respectively. The decreased expression of KDR led us to investigate the expressions of ERα, which is known to downregulate the expression of KDR, and ERβ. Although we observed diminished ERβ expression in pediatric AML patients, ERα expression was observed in 54.5% of the study group. Activating mutations in the RTK/ras signaling pathway are common in pediatric AML, and their presence may identify a population at higher risk of poor outcome. Our data shows the Flt-1 expression is increased in pediatric AML patients compare to KDR expression. To our knowledge it is the first data representing the loss of ERβ expression in pediatric AML paients. ERβ was previously demonstrated to have important regulatory functions in the control of the proliferation various cancer. The loss of ERβ in AML, would shed the restrictive properties of this steroid receptor in the regulation of cell growth, death and motility. This might appear to be an important event leading to the development of hematological malignancies. Our ongoing studies would highlight its possible role in leukomogenesis.

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FOETAL/NEONATAL ALLOIMMUNE THROMBOCYTOPENIA (FNAIT) IN 2 CHILDREN OF A HUMAN PLATELET ANTIGEN (HPA)-1A AND HPA-5A IMMUNIZED MOTHER

S. Schouwers,[1] C. Vandenabeele,[2] J. Hoegaerts,[2] K. Vanneste,[2] L. Verdonck,[2] I. Van Haute[3]

[1]University Hospital Ghent, GHENT; [2]AZ Alma Hospital, EEKLO; [3]BTC Vlaanderen, GHENT, Belgium

Foetal or neonatal alloimmune thrombocytopenia (FNAIT) results from maternal alloimmunisation against foetal platelet antigens inherited from the father and not present on maternal platelets. Anti-HPA 1a antibodies are the most common cause of FNAIT in the Caucasian population (80%); anti-HPA5a antibodies on the contrary are a very rare cause of FNAIT since only 2% of Caucasians are homozygous HPA5b/5b and express no HPA5a. The importance of anti-HPA5a antibodies in the development of FNAIT has not been established. Until now the combination of anti-HPA1a and anti-HPA5a antibodies has not been reported. The boy was born through vaginal delivery after 41 weeks of gestation, he appeared completely healthy (Apgar 7/9/9) with no signs of bleeding. After a few hours he developed petechiae and hematochezia. A platelet count was performed and revealed severe thrombocytopenia (15000 plt/microL). Since maternal platelet count was normal and no other abnormalities were found, FNAIT was the most probable diagnosis. Transfusion with single donor HPA-1a negative platelets in combination with IV immunoglobulins (500 mg/kg/day) was started and samples for detection of maternal antibodies and for platelet typing of mother, father and child were collected. The diagnosis of NAIT was confirmed since both anti-HPA1a and anti-HPA5a maternal antibodies were present. Platelet typing revealed maternal homozygosity for both HPA1a and HPA5a; paternal heterozygosity for HPA1a and homozygosity for HPA5a; whereas the infant was heterozygous for both platelets antigens. The boy received 3 single donor platelet transfusions (day 1, 2 and 3) in combination with IV immunoglobulins during 4 days. On day 1 and 2 platelets were HPA1a negative and only on day 3 the platelets were both HPA1a and HPA5a negative. After day 4 a normal platelet count was reached. Approximately 1 year later the mother presented at the hospital with a second pregnancy. Since the probability recurring neonatal thrombocytopenia is 85% and severity of thrombocytopenia increases in subsequent pregnancy, maternal treatment with IV immunoglobulins (8×10 g every 3 weeks) was started at 32 weeks of gestation. An elective sectio was performed at 38 weeks and a healthy baby girl was born (Apgar 8/9/10). There were no signs of bleeding. Immediately after birth a platelet count was performed, which revealed a slight thrombocytopenia (63 000 plt/microL). Based on the history of the brother one platelet transfusion with single donor HPA1a negative platelets was given in combination with IV immunoglobulins during 4 days. On day 5 a normal platelet count was reached. To date both children are healthy and show no sequellae of the neonatal thrombocytopenia.

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EFFICACY AND SAFETY OF RITUXIMAB FOR ADULTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA: A SINGLE CENTER EXPERIENCE

S. Tavera, M. Bonferroni, M. Grasso, R. Raviolo, C. Gazzera, M.A. Pistone, A. Gallamini

S. Croce e Carle Hospital, CUNEO, Italy

Background. Rituximab is increasingly used to treat idiopathic thrombocytopenic purpura (ITP); however, the evidence to support the use of Rituximab in ITP is uncertain. *Aims.* To evaluate efficacy and safety of Rituximab for the treatment of adults with ITP. *Methods.* We reviewed 14 patients with ITP treated with Rituximab at our institution between March 2002 and January 2008. Patients were 34 to 84 years of age (mean age: 57ys), had had ITP for 2 to 348 months and had a platelet count that ranged from 5 to 45×10^9 cells/L before Rituximab treatment (9/14 patients had platelet count $<10\times10^9$/L). Time to response was not evaluable because of concomitant therapy with corticosteroids. All patients had received corticosteroids as first line therapy and 9/14 pts underwent splenectomy. Other previous treatments were immunosuppressants, including cyclosporine, azathioprine, cyclophosphamide, vinca alkaloids

and danazol. Complete remission (CR) was defined by a platelet count >150×10^9/L; partial remission (PR) was defined by a platelet count >50 ×10^9/L; minimal platelet count response was defined by a platelet count >30×10^9/L. One patient received Rituximab plus Dexa as first-line therapy; 1 pts Rituximab as second-line, 4 pts as third-line and 8 pts received Rituximab after more than 3 lines therapy. Rituximab was administered as a weekly infusion of 375 mg/mq for 4 consecutive weeks (standard dose) in 13/14 pts; one old patient received a low dose of Rituximab (100 mg x 4 weeks). Three pts were re-treated with Rituximab: 1 patient received 2 course of Rituximab at standard dose achieving CR for 2 ys and 1 y respectively and one course with Rituximab 100 mg for 4 weeks achieving PR (+ 8 weeks);1 patient received three course of Rituximab achieving CR lasting respectively 15, 17 and 12 months; one patient received standard dose of Rituximab achieving PR of 8 months and Rituximab 100 mg x 4 weeks achieving CR (+10 weeks). *Results.* 2/14 pts were not evaluable because of short follow up; 3/12 pts achieved a partial response (PR) and maintain a response at +17, +18, + 3 months; 3/12 pts achieved a complete response (CR) and maintain normal platelet counts at +48, +4, +32 months; 1/12 achieved a minimal platelet count response mantein at +72 months; 2/12 pts were non responder. No toxicities were observed. *Conclusions.* Rituximab was associated with a platelet count overall response in 10/12 patients without toxicity. Our preliminary good results in 2 patients on the use of low dose of Rituximab in ITP need additional studies. Three patients re-treated with Rituximab experienced no adverse events, suggesting efficacy and safety of Rituximab in relapsed patients.

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SEVENTEEN-YEARS EXPERIENCE WITH ATRA BASED THERAPY IN APL: LONG TEERM FOLLOW UP OF PATIENTS TREATED AT S.MARTINO HOSPITAL (GENOVA)

M. Clavio, A. Ghiso, A. Albarello, C. Ghiggi, L. Vignolo, M. Spriano, S. Aquino, N. Colombo, R. Grasso, R. Varaldo, F. Olcese, I. Pierri, M. Miglino, S. Biasco, A.M. Carella, M. Sessarego, R. Ghio, M. Gobbi

Hematology Inst., GENOVA, Italy

Background. and Aims. since ATRA was introduced in induction therapy, prognosis of APL patients has dramatically improved. We present here the final analysis of retrospective study on 91 consecutive, newly diagnosed APL patients who have been treated and followed -up in a period of 17 years at the Department of Hematology and Oncology of the S. Martino Hospital (Genova, Italy). *Methods and patients.* Cytogenetic demonstration of t(15;17) and or detection by RT-PCR of PML-RAR-alfa was required for the confirmation of diagnosis. In brief, the median age of patients was 41 years (range 17-83). Seventy-five patients were below 60, 16 older than 60. FAB subtypes were found to be M3 in 82 patients (90%) and M3v in 9 patients (10%). The vast majority of patients had *de novo* APL. One patient developed APL after NHL , while two other patients after radiochemotherapy for breast carcinoma. According to the PETHEMA scoring system the prognostic risk was low in 28 patients (31%), intermediate in 41 (45%) and high in 22 (24%).Three patients died of brain haemorrhage before the beginning of therapy. Nine patients were not treated initially with ATRA as the drug was not available yet. Four of them were treated with ATRA containing regimens due to relapse or refractory disease. Seventy-nine patients were enrolled in multicenter GIMEMA trials, all of which included ATRA. *Results*. Among the 79 patients who had been initially treated with ATRA containing regimens, there were 3 haemorrhagic deaths during the first period of therapy (4%) and one in consolidation, which was due to infection. Symptoms of retinoic acid syndrome (RAS) were reported in 8 patients (10%). Seventy-six of 79 patients (96%) were evaluated for response and CR was achieved by 75 patients (95%). Sixty-one patients underwent molecular evaluation of response at the end of induction, and 47 (62%) of them achieved molecular remission. Fifty-seven (76%) patients completed the 3 consolidation courses. Following consolidation, molecular assessment of response was performed on 56 patients, and 55 of them were found to have achieved cytogenetic and molecular remission (98%). Fifty-four patients (68%) completed maintenance therapy. The median follow up for living patients is 100 months (10-192).Ten of the 76 patients who achieved haematologic response relapsed (13%). Eighty-one per cent of patients achieving CR after ATRA containing therapy are alive and disease free at 14 years. Seventy-eight per cent of patients are expected to be alive at 14 years from diagnosis. Median survival is 76 months (range 1-159) and median length of CR is 68 months (range 3-157). The expected survival at 14 years is 90% and 48% in patients with intermediate-low risk and high risk at presentation, respectively (p=0.0009), thus highlighting the prognostic relevance of the PETHEMA score. *Conclusions.* a review of our long term results in the treatment of APL in the ATRA era largely confirms that this *targeted* therapy has profoundly modified the clinical outcome of this severe disease, even thoug several problems still persist and need to be specifically addressed with more tailored therapeutic strategies.

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PROGNOSTIC VALUE OF THYMIDINE KINASE LEVEL IN ACUTE MYELOID LEUKEMIA PATIENTS WITH KARIOTIPE ABNORMALITY

N.M. Tretyak,[1] N.V. Goryainova,[1] O.A. Kyselova,[1] O.M Vakulchuk,[1] O.V. Myronova[2]

[1]Institute of Hematology and Transfusiology of UAMS, KYIV; [2]National medical university, KYIV, Ukraine

Background. Some cytogenetics abnormality in acute myeloid leukemia (AML) patients are prognostic unfavourable. On the other hands, thymidine kinase (TK) is a enzyme, which level in blood serum corresponds with the number of dividing leukaemia's cells, being sensible marker of tumor gross and one level has the prognostic value. Lover TK level in blood serum in the time of diagnosis can predict the upper probability of remission obtaining. *Aims.* The purpose of the current investigation is determination of correlation between TK level in blood serum and cytogenetics abnormality in AML patients and its prognostic value. *Methods.* Activity of TK in blood serum was measured in 296 AML patients by radioimmunoassay using 5-125 I-iodedeoxy uridine as a substrate. TK levels were observed before chemotherapy starting. Cytogenetics abnormalities were detectable by banding techniques. *Results*. Upper probability of remission obtaining in standard chemotherapy treatment was observed if TK level had been lower than 20,0 U/L. Similar level of TK (13,65±1,271 U/L) was in M2 AML patients, who has translocation (8; 21). These patients had also the upper remission rate (92%). M3 AML patients with translocation (15; 17) had TK level in the time of diagnosis lower than 15,0 U/L (11,89±1,679 U/L). We can observe favorable course of decease and long term of remission in this group of patients. TK level in blood serum upper than 20 U/l predicted the chemotherapy resistance. We can observed similar TK level in AML patients (M2, M4, M5) with trisomy 8 (average TK level was 28,33±5,287 U/L). The most pessimistic prognosis was in AML patients, who has TK level in blood serum upper than 30,0 U/L (average TK level was 43,946±8,46 U/L). The patients had such cytogenetics abnormality as monosomy 7, deletion 7q,3q-, 7q-. *Conclusions*. Correlation between TK level and chromosomal abnormality in AML patients can be used in prognosis of remission obtaining and the course of disease.

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RESULTS OF EMA THERAPY IN RELAPSED AND REFRACTORY ACUTE MYELOBLASTIC LEUKEMIA

S. Civriz, P. Topcuoglu, B. Ceydilek, S.M. Bakanay, M. Arat, O. Arslan, G. Gurman, M. Ozcan

Ankara University, Faculty of Medicine, ANKARA, Turkey

We aimed to show the long-term effect of EMA (Etoposid, Cytosine arabinosid, Mitoxantron) in 44 patients with refractory or relapsed acute myeloblastic leukemia (AML). The median age was 32 years (range: 17-57ys). Forty-four patients (27 Male/17 Female) were evaluated in this retrospective study. The distribution of the patients according to FAB classification; AML M0-1(n=10), AML-M2 (n=7), AML-M4-5 (n=20) and AML-M6 (n=2); biphenotypic leukemia (n=2), and secondary AML (n=3). The disease situation prior to EMA was primary refractory (n=12) or relapsed (n=28) (22 patients relapsed before 12months;6 relapsed after 12 months). Treatment regimen consisted of Mitoxantron 12 mg/ m2/d, day 1-3, Etoposide 200 mg/m^2/d, day 8-10 and Cytosine Arabinoside 500 mg/m^2/12 hours infusion day 1-3 and day 8-10. G-CSF, the daily dose of 5 mcg/kg was started on day 12 and continued until absolute neutrophil >0,5×10^9/L for 3 consecutive days. *Results.* Since 6 patients died at the aplasia period due to bacterial and fungal infections, 38 patients could be evaluated: 57.9% of the patients achieved complete remission after EMA therapy and 42.1% of them remained in refractory to the therapy. Total 13 patients after EMA therapy underwent allogeneic hematopoietic stem cell transplantation (Allo-HSCT) as consolidation (n=7) or salvage therapy (n=6). Thirteen out of the refractory patients (n=16) after EMA induction therapy were received EMA with ciclosporine and two patients received other salvage therapies; six of 13 patients who received EMA with ciclosporine achieved complete remission (46%). Median follow-up of all the patients was 13.2 months. Two-year overall survival (OS) was 47.4±9.1%. OS and event-free survival

after salvage EMA therapy was 40.4±%10.1,2 and 23.4±8.4, respectively. When the patients were divided into two groups according to the indication of EMA treatment as relapsed and refractory, Both OS from the diagnosis and EFS in the refractory group were shorter than relapsed group (median 13.7 months *vs* 81.7 months, p=0,006; median 18.3 months *vs* 0,0 months, *p*=0.011). But we did not find any difference of OS after EMA therapy in refractory patients compared with relapsed ones (26.7±13.8 months *vs* 10.7±7.6 months, p=0.326). In the conclusion, EMA therapy led to significant differences of OS from the diagnosis and EFS in relapsed patients compared with refractory patients. In our cohort analysis, only 34% of the patients underwent allo-HSCT after EMA therapy. Other postremission therapies should be analyzed in further studies.

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Withdrawn by the authors

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CD34+ AND CD34- LEUKEMIC SUBPOPULATIONS DIFFER IN ABILITY TO APOPTOSIS, PROLIFERATION AND EXPRESSION OF MULTIDRUG RESISTANCE ASSOCIATED GENES IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

T.V. Shman, V.P. Savitski, U.U. Fedasenka

Belarusian Research Center for Pediatric Oncology and Hematology, MINSK, Belarus, Republic of Belarus

The main obstacle in the treatment of childhood acute lymphoblastic leukemia (ALL) is a relapse. Nowadays, conventional prognostic factors can not fully predict differences in clinical outcome between patients. Thus, searching factors responsible for disease progression is of current importance. We suppose that phenomenon of heterogeneity (coexistence of several leukemic cells subpopulations) may be the cause for such differences in leukemia treatment results. Coexistence of several cells subpopulations with different biological properties may result in different sensitivity of leukemic subpopulations to chemotherapy in vivo and can be the probable cause of relapse. With regard to controversial data about prognostic relevance of CD34 expression in acute leukemias the objective of this study was to investigate the efficacy of apoptosis, cell cycle distribution, expression of multidrug resistance associated genes in $CD34^+$ and $CD34^-$ leukemic cells subpopulations in childhood ALL. ALL samples with CD34 heterogeneous expression (n=16) were separated into $CD34^+$ and $CD34^-$ leukemic fractions using fluorescence activated cell sorting. Cell cycle distribution, ability to spontaneous apoptosis, expression of BCRP, MDR1, LRP and BCL-2 genes of sorted subpopulations were estimated by flow cytometry and real-time PCR. $CD34^+$ subpopulations of most patients (twelve of sixteen) after 20h incubation showed lower ability to apoptosis than $CD34^-$ fractions. For all analyzed ALL cases mean level of spontaneous apoptotic cells was 41.3±4.6% and 56.1±3.6% for $CD34^+$ and $CD34^-$ subpopulations correspondently (*p*=0.002). Mean level of cells in S+G2M-phases for $CD34^+$ versus $CD34^-$ subpopulations did not differ significantly. Then we analyzed B- and T-lineage ALL separately. In B-lineage ALL the level of proliferating cells did not differ between subpopulations, however we revealed increased level of proliferating cells in $CD34^+$ fractions in all analyzed T-lineage ALL cases. Mean level of proliferating cells in T-lineage ALL for $CD34^+$ fractions was 22.5±6.2% whereas for $CD34^-$ subpopulations it was 8.9±4.1% (*p*=0.02). Quantitative analysis of the expression levels of MDR1, BCRP, LRP and Bcl-2 genes did not differ significantly in $CD34^+$ and $CD34^-$ leukemic fractions for B-lineage ALL samples. However, CD34- leukemic fractions of T-lineage ALL patients had higher expression level of Bcl-2 gene in four of six analyzed cases (from 1.2 to 30.2-fold), BCRP gene in five of six cases (from 1.6 to 8.1-fold) and LRP gene in all six patients (from 1.2 to 3.9-fold, *p*=0.007) than those of $CD34^+$ subpopulations. These results does not allow us to safely conclude that $CD34^+$ or $CD34^-$ fractions have more resistant phenotype because of significant differencies in the expression levels of apoptosis and multidrug resistance genes between patients. However, we found that $CD34^+$ leukemic fractions were more resistant to apoptosis than $CD34^-$ as for B-lineage and for T-lineage ALL as well. In T-lineage ALL $CD34^+$ leukemic subpopulations revealed higher proliferation rate and predominantly lower Bcl-2, BCRP and LRP genes expression compared to $CD34^-$ fractions. Nevertheless, distinctions between $CD34^+$ and $CD34^-$ cells exist and may lead to different chemosensitivities between leukemic subpopulations *in vivo* and may determine the alteration of the leukemic immunophenotype during treatment and in relapse.

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PEDIATRIC HYPEREOSINOPHILIC SYNDROME

R. Caruso,[1] L. Trotta,[2] D. Funaro,[1] V. Coletti,[1] V. Pansini,[1] C. Rapanotti,[3] F. Lo Coco,[3] G. Derossi[1]

[1]Children Hospital Bambino Gesù, ROME, VATICAN CITY; [2]Campus Biomedico, ROME; [3]Tor Vergata University, ROME, Italy

Background. Hypereosinophilic Syndrome (HES) includes an heterogeneous group of disorders characterized by unexplained peripheral blood (eosinophils >1.5×10^9/L) and bone marrow hypereosinophilia persisting for at least 6 months, and increased serum IgE levels leading to tissue and organ damage. Beside secondary forms reactive to allergic disease, we distinguish: Hydiopathic Eosinophilic Syndromes, Clonal Hypereosinophilic Syndromes and HES hiding and preceeding lymphoproliferative diseases. The differential diagnosis between Secondary or Clonal Hypereosinophilic Syndrome is still controversial and subject of several studies. This distinction influences the choice of treatment, sometimes suggesting a variable observational period. *Aims*. The aim of this study was to evaluate clinical and biological heterogeneity of pediatric HES in order to evidenciate parameters able to define early the clinical outcome and the therapeutical approaches. *Methods.* We studied ten children (six males and four females) showing clinical and haematological features of HES. We analysed B-cell clonality profile amplifying CDRI, CDRII and CDRIII regions of heavy chain immunoglobulin gene (IgH-PCR). Amplification of CDRIII region was performed using CA1 and CA2 primers respectively *consensus-primers* of Variable and Joining-heavy chain region. Amplification of CDRI and CDRII were performed using oligo-degenerate sequence primers mapping the frameworks FR2 and FR3. *Results*. One of the ten patients, detected positive for the gene fusion PDGFRA/FIP1L1 was classified as affected by Clonal Hypereosinophilia: it was started therapy with Imatinib achieving molecular and haematological remission. Four of the ten cases showed an IgH clonality (documented by FR2-IgH-PCR). In particular, one of these cases six months later developed a lymphoblastic B-cell lymphoma showing the same molecular monoclonality amplification. One patient, twelve months later developed a lymphoblastic B-cell leukaemia. During the follow-up, six patients showed a spontaneous regression of hypereosinophilia associated in two cases with a reversion to IgH polyclonal profile. *Summary and Conclusions.*The level of peripheral blood eosinophils and IgE levels are not helpful to identify clonal myeloproliferative disorders, as we observed in our two patients, also the presence of a clonal expansion of B lymphocyte is not a significative diagnostic marker of hypereosinophilia secondary or preceeding a lymphoproliferative disease. Only the presence of the fusion gene PDGFRA/FIP1L1 is able to define certainty the clonal myeloproliferative feature of the hypereosinophilia giving the possibility to start early specific treatments. For prognosis, long term folow up with a wait and see strategy is mandatory in the majority of asimptomatic cases.

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CHRONIC EOSINOPHILIC LEUKEMIA ASSOCIATED WITH T(5;16)(Q32;P13) SENSITIVE TO IMATINIB

P. Belohlavkova, J. Voglova, L. Kucerova, P. Balicek, L. Jebavy, J. Maly

Faculty Hospital, Charles University, HRADEC KRÁLOVÉ, Czech Republic

Background. The World Health Organization (WHO) defines chronic eosinophilic leukemia (CEL) in the presence of blood eosinophil count that is 1,5.10/9/L or higher accompanied by either presence of myeloblast excess (either >2% in the peripheral blod or 5%-19% in the bone marrow) or evidence of myeloid clonality. Reported cytogenetic abnormalities in CEL include trisomy 8 (the most frequent), t(10;11)(p14;q21) and t(7;12)(q11;p11). The successful empiric treatment of patients with tyrosine kinase inhibitor imatinib suggested the presence of an imatinib-sensitive tyrosine kinase inhibitor. The identification of a specific chromosome deletion del(4)(q12;q12) creating the FIPL1/PDGFRA fusion gene confirmed this hypothesis. The involvement of another chimeric gene PDGFRB was also reported in CEL. The PDGFRB gene is located on chromosome 5q33 and can fuse to many different partner genes. Most patient have t(5;12)(q31-33;p12-13) with ETV6-PDGFRB. To date, more 35 different fusion genes have been identified in myeloproliferative disorders with eosinophilia. *Methods.* We report a case of 70-year-old female with CEL. A complete blood count were: a white blood cell count of 13,5.10/9/l with 41% eosinophils, normal hemoglobin and platelet. FIP1L1/PDGFRA by RT-PCR were nega-

tive. No evidence of organ damage was detected. Bone marrow aspirates and biopsy showed normocelular bone marrow with 22% eosinophils and clonal cytogenetic abnormality 46,XX,t(5;16)(q32;p13). Fluorescence *in situ* hybridization (FISH) identified breakpoints close to 5q31.1 and 16p13.1 loci. The locus for genus EGR1 and MYH11 were unchanged. We supposed that the changes correspond to fusion PDGFRB/NDEl. The treatment has been started with imatinib 100 mg daily. Complete haematological remission has been achieved after two weeks and cytogenetic remission after 3 month. The patient is still in remission after 12 months. *Conclusions.* Our results identify a second case of CEL patient with the karyotype 46,XX, t(5;16)(q32;p13) and illustrates the importance of searching for PDGFRB rearrangements. It is possible that the successful use of imatinib or alternative inhibitors may lead to identification of the other unknown abnormalities.

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MRNA ELECTROPORATION IS AN EFFICIENT GENE TRANSFER METHODOLOGY IN CLL

J. Philippé,[1] F. Van Bockstaele,[1] V. Pede,[1] E. Naessens,[1]
S. Van Coppernolle,[1] V. Van Tendeloo,[2] B. Verhasselt[1]

[1]Gent University, GENT; [2]Antwerp University, ANTWERP, Belgium

Background. Chronic lymphocytic leukemia (CLL) consists of diverse prognostic subgroups, characterized by different cellular and molecular markers, such as the IgVH mutational status and the ZAP70 expression level. Eventually, the function and clinical importance of these markers may be questioned. In order to address these questions, an efficient and reliable method for gene transfer is most informative. For several practical and technical reasons, the number of reported functional *in vitro* studies in CLL cells using viral vectors or nucleofection for the introduction of genetic material is limited. *Aims and Methods.* In this study, we compared efficiency and utility of different gene transfer techniques in CLL. Propidium iodide (PI) staining and expression of the marker gene enhanced green fluorescent protein (EGFP) were determined by flow cytometry in order to quantify viability and transgene expression levels. *Results.* Lenti-, retro- and adenoviral transduction was disappointing and did not yield appreciable numbers of $EGFP^+$ CLL cells, despite various pre-stimulation protocols. Efficient transgene expression was observed after nucleofection of CLL cells with plasmid DNA, but results were highly variable, depending on the program used for nucleofection. Increased cell death was observed, probably due to DNA-related cytotoxicity and to the co-introduction of bacterial components remaining in the plasmid preparations. Moreover, expression levels were dramatically different between promoter types: use of the phosphoglycerate kinase promoter did not result in $EGFP^+$ cells, whereas the cytomegalovirus promoter yielded efficient transgene expression. Efficient gene transfer was obtained through mRNA electroporation. After optimization, electroporation of *in vitro* transcribed EGFP mRNA yielded up to 90% $EGFP^+$ CLL cells, without affecting viability. Transgene expression was detectable from 1 hour after electroporation, and remained present for at least two weeks after electroporation. Efficiency was not affected by freezing and thawing the cells before or after electroporation. Furthermore, we could demonstrate overexpression of ZAP70 and of a ZAP70-EGFP fusion protein after electroporation of ZAP70 or ZAP70-EGFP mRNA in CLL cells. *Conclusions.* mRNA electroporation is a novel and straightforward method for highly efficient gene transfer in CLL. It is estimated that mRNA electroporation can be easily extended to other genes related to prognosis or pathogenesis. The application of this technique should facilitate functional studies in CLL cells, as well as clinical research related to CLL.

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NO CONVINCING EVIDENCE FOR A ROLE OF CD31-CD38 INTERACTIONS IN THE PATHOGENESIS OF CHRONIC LYMPHOCYTIC LEUKEMIA

S.H. Tonino, R. Spijker, D.M. Luijks, M.H. van Oers, A.P. Kater

Academic Medical Center, AMSTERDAM, Netherlands

Background. A high expression level of CD38 on chronic lymphocytic leukemia (CLL) is associated with an unfavorable prognosis. Although CD38 is primarily known as an ecto-enzyme, it has also been ascribed a role as a receptor, whose interactions with its proposed ligand CD31 result in proliferative and survival-signals. The aggressive clinical course in patients with $CD38^{high}$ CLL was suggested to result from binding of the leukemic cells to nurse-like cells in the bone marrow, which express high levels of CD31. However, CD31 is expressed on multiple cell types, including endothelial cells and PBMCs from healthy donors. Moreover, also CLL cells express variable levels of CD31. Therefore, CD31-CD38 interactions between CLL cells and the environment are rather expected to be ubiquitous. *Aims.* The aim of the present study was to analyze whether CD38-CD31 interactions result in proliferative and anti-apoptotic signals in CLL. *Methods.* The purity of the CLL samples was >90%. $CD38^{high}$ cells were defined as >30% $CD38^+$ and $CD38^{low}$ cells as <30% $CD38^+$. Co-culture experiments were performed with the endothelial cell-line ECRF24 or 3T3 cells (control or transfected with a human CD31 or CD40-ligand-construct). The blocking anti-CD31-mAbs HEC65 or HEC170 and anti-CD38-mAb AT1 were used. Apoptosis was assessed by Annexin-V/ Propidium Iodide staining and proliferation was determined by CFSE labeling and dilution. Expression of apoptosis regulating genes was assessed by reverse transcription-multiplex ligation-dependent probe amplification assay (RT-MLPA). *Results.* The expression of CD31 on CLL cells of all patients tested (n=23) was high. Culture of CLL cells of 4 $CD38^{high}$ patients at high density for 5 days, with or without addition of blocking antibodies, did not result in modulation of apoptosis or proliferation. To analyze heterotypic interactions between CLL cells and endothelium, CLL cells of 6 $CD38^{high}$ patients were co-cultured with ECRF24 cells. $CD38^{low}$ cells and addition of blocking antibodies were used in control experiments. Also in these experiments no significant differences in apoptosis or proliferation were observed after up to 72 hours. To perform analyses beyond this time-point, CLL cells of 12 $CD38^{high}$ and 6 $CD38^{low}$ patients were co-cultured with either CD31 transfected or control 3T3 cells, with or without addition of blocking antibodies. Co-culture for up to 7 days did neither result in proliferation, nor modulation of apoptosis in any condition. In sharp contrast, stimulation with CD40-ligand-transfected 3T3-cells rescued cells from apoptosis (difference seen after 48 hours), and addition of the oligo-dinucleotide CpG induced marked proliferation (after 4 days). Furthermore, MLPA analysis of RNA obtained after 3 days of co-culture with 3T3 cells, with or without CD31, revealed no significant difference in the expression profile of apoptosis regulating genes, whereas co-culture with CD40-ligand expressing feeder cells induced characteristic changes in this profile (upregulation of BCL-XL, A1 and Bid). *Conclusions.* In conclusion, although survival and proliferation of CLL cells can indeed be influenced by external stimuli via receptor triggering (CD40, Toll-like receptor 9), our data do not support an important role for CD38-CD31 interactions in the pathogenesis of CLL.

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UNMUTATED IGHV3-11 CASES CHARACTERIZED BY HIGH FREQUENCY OF AUTOIMMUNE HEMOLYTIC ANEMIA DEVELOPMENT

I. Abramenko, N. Bilous, A. Chumak, D. Bazyka, V. Bebeshko

Research Centre for Radiation Medicine, KIEV, Ukraine

Background. It was shown that IgHV gene repertoire in CLL was biased and several subtypes with stereotypic heavy chain complementarity determining regions 3 (HCDR3s) were characterized. Some of them were shown to have specific clinical features. For example, IgHV3-21 positive cases with short ARDANGMDV motif experienced progressive disease more frequently in comparing with heterogeneous ones; while IgHV1-69/HD3-10(3)/HJ6 cases (CLL subset #5) were associated with a more indolent disease despite unmutated Ig status. In our group of 218 CLL patients with productive IgHV/HD/HJ gene rearrangement we identified increased frequency of IgHV3-11 expressing cases (8 cases; 3.7%). The aim of this work was their detail characterization. *Methods.* IGHV gene configuration was analyzed using reverse transcription, polymerase chain reaction, and direct sequence. *Results.* All IgHV3-11 positive cases were unmutated (7 had 100% homology with germline gene) and preferentially expressed IgHJ6 gene (6 of 8 cases). Three cases showed specific IgHV rearrangement patterns with stereotyped HCDR3 described previously (subsets #22, 25, 32), 2 cases - with antibodies described in patients with X-HIgM syndrome, and 1 case showed closely similar HCDR3 with anti-streptococcus pneumonia polysaccharide 4 (PPS4) antibodies.In comparison with the other unmutated cases (n=147), IgHV3-11 positive cases did not differ significantly by mean age of patients at the diagnosis (56 *vs* 57 years), CD38 expression (25% IgHV3-11 positive cases with >30% CD38 expression on $CD5^+/CD20^+$ cells *vs* 39.1% among the other unmutated cases), initial WBC counts (64.2×10^9/L *vs* 79.9×10^9/L), stage at diagnosis (7 IgHV3-11 cases were diagnosed at BII stage, one - at AI stage), duration of non-treatment period (median 7 and 8 months, respectively), progression-free survival (median 34 and 42 months, respectively), overall survival (median 86 and 101 months, respectively). However, CLL course in the majority of IgHV3-11 expressed patients was accompanied by severe complications. First of all, 4 patients developed autoimmune hemolytic anemia, AIHA

(50% in comparison with 10.8% among the other unmutated cases, p=0.001; and 9% among the other unmutated + mutated cases, p=0.0002). Only IgHV4-59 expressed patients showed the same high frequency of autoimmune disorders (3 of 7 patients, 42.8%, p>0.05), while for the other cases, it was much lower (for example, 11% among 45 IgHV1-69 positive cases, p<0.01; 7.7% among 13 IgHV1-02 positive cases, p<0.001). Then, 2 IgHV3-11+ patients developed prostate cancer (one patients - additional recurrent basal cell carcinoma), and one IgHV3-11+ patient relapsed with Richter transformation. *Conclusions.* Unlike the other CLL cohort we revealed increased frequency of IgHV3-11 positive unmutated cases (3.7%), most of which showed HCDR3 homology with CLL or other Ig sequences. These IgHV3-11 cases may present prognostically unfavorable group with high frequency of development of AIHA and the other severe complication of CLL.

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DOCK10, A NOVEL CZH PROTEIN SELECTIVELY INDUCED BY INTERLEUKIN-4 IN HUMAN B LYMPHOCYTES

A. Parrado, E. Yelo, M.V. Bernardo, L. Gimeno, M.J. Alcaraz, M.C. González, M.J. Majado

Hospital Virgen de la Arrixaca, MURCIA, Spain

Background. Interleukin-4 (IL4) affects cell growth and differentiation in a wide range of hematopoietic and non-hematopoietic cells, including B lymphocytes. The Rho-family proteins constitute a branch of the Ras superfamily of small GTPases. The best-known members are RhoA, Rac1 and Cdc42. The Dock or CZH proteins are a family of non classical guanine exchange factors (GEF) for Rho GTPase proteins, defined by the presence of a GEF domain called CZH2. Within the CZH family, the Zizimin subfamily, characterized structurally by the presence of an N-terminal PH domain, a central CZH1 domain, and a C-terminal CZH2 domain, is composed of 3 members: Dock9, Dock10, and Dock11. We have identified Dock10 as an IL4-inducible gene in CLL cells. However, the coding sequence of Dock10 in the databases was incomplete. *Aims.* To clone the full length coding cDNA of human Dock10. To study Dock10 tissue expression, and the effect of IL4 on Dock10 expression in CLLs and peripheral blood (PB) lymphocyte subsets. *Methods.* The 5' end of Dock10 cDNA was obtained by RACE-PCR. The full length coding cDNA of Dock10 was obtained by PCR cloning. Tissue distribution and modulation by IL4 in CLLs and normal PB-B cells were studied for the three Zizimin genes by Northern Blotting. A polyclonal antibody raised against a synthetic peptide comprising amino acids 4 to 24 of Dock10 was generated, and used to study Dock10 expression and its modulation by IL4 in CLLs, normal PB-B cells and PB-T cells, by Western Blotting. *Results.* The N-terminus of Dock10 protein was revealed in the form of a 5'-RACE-PCR fragment of 2 kb in size. The full-length cDNA sequence of Dock10 encodes a 2180 amino acid protein. Dock9 (2069 amino acids) and Dock11 (2073 amino acids) are 58% identical. Dock10 is 52% identical to Dock9, and 50% identical to Dock11. The PH and CZH2 domains of the Zizimin proteins are more conserved than the CZH1 domain. Among normal human tissues, Dock10 mRNA was mainly expressed, like Dock11, in PB leukocytes. IL4 increased Dock10 mRNA levels in CLLs, but neither those of Dock9 or Dock11. Dock10 protein levels were heterogeneous in CLLs. As expected from mRNA studies, IL4 induced Dock10 protein expression in CLLs. Dock10 protein was expressed at similar levels in normal PB-B and PB-T cells. Like in CLLs, Dock10 expression was potentiated by IL4 in normal PB-B cells. However, IL4 did not change the levels of Dock10 expression in PB-T cells. Dock10 protein distributed in the cytoplasm and nucleus of CLL cells, and IL4 increased its expression in both cellular compartments. *Summary and Conclusions.* Dock10 shares more structural homology to Dock9 than to Dock11. However, Dock10 and Dock11 have in common their prominent expression in PB leukocytes, where Dock9 is not expressed. The rapid and distinctive induction of Dock10 expression by IL4 in CLL and normal PB-B cells, which was exclusive for this Zizimin factor, suggests a role for Dock10 in IL4-induced B-cell activation. Dock10 could represent a point of convergence for IL4 signalling and Rho GTPase function in B cells.

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CONCOMITANT Z SCORE AND ZAP 70 ALONG WITH ATM DELETION IDENTIFIES PATIENTS WITH POOR SURVIVAL FROM THE RPCI CHRONIC LYMPHOCYTIC LEUKEMIA SERIES: A CORRELATION OF TREATMENT AND SURVIVAL OF B-CLL PATIENTS WITH PROGNOSTIC VARIABLES SUCH AS FISH, ZAP70 EXPRESSION, CGH

S. Padmanabhan, A.W. Block, P. Wallace, W. Tan, N. Nowak, P. Varadarajan, M. Barcos, F. Hernandez, S. Ketepalli, G. Wilding, M. Czuczman, A. Chanan-Khan

Roswell Park Cancer Institute, NY, USA

Background. B-CLL, the most common leukemia in adults is a malignant disorder characterized by progressive accumulation of functionally incompetent B-lymphocytes. Clinical staging provides prognostic information; however cannot predict outcomes of individual patients. Independent factors that predict disease progression, response to treatment and outcomes include chromosomal aberrations and somatic mutation status in the expressed immunoglobulin heavy chain variable region (IgVH), and its surrogate marker Zeta associate protein or ZAP70. *Aims*. Even though the latter marker ZAP70 expression has shown to be of prognostic significance several variables have not been standardized including internal controls and normal B cell controls. In order to take into account the background expression by normal T and NK cells we developed a *Z score* and investigated the concordance with high risk cytogenetics in B-CLL patients diagnosed at Roswell Park Cancer Institute. ZAP70 expression in greater than 20% of gated B-CLL cells along with a Z score ratio of >1.4 were considered ZAP positive. *Methods.* Prospective Fluorescence *in situ* hybridization (FISH) and flow cytometric analyses were done on all B-CLL patients (n=230) in Roswell Park Cancer Institute from 2002-2007. Using a FISH panel from Abbott Molecular/Vysis that identified specific numerical and structural abnormalities (+12, del (13q), del (11q), del (17p), 14q32 abn), we studied peripheral blood and bone marrow from unselected patients (pts) to investigate the concordance of high risk markers in these patients. For the del(13q) two probes were used;D13S25 which identifies the 13q 14.3 and LSI13 which identifies the Retinoblastoma (Rb) region. ZAP70 expression in CLL cells was determined by flow cytometry according to the methods detailed by Crespo *et al.* ZAP 70 values were similarly obtained for normal T cells, NK cells from the patient and B cell from healthy volunteers. The *Z* scores were the ratio of ZAP70 expression in CLL-B/normal B cells and CLL-B/T cells. Statistical assessment of observed differences in the survival distributions of different groups of interest was done using the log-rank test and analysed using SAS (version 9.1). *Results*. Males formed 63%(n=144) of the cohort, 73.8% were smokers, 42% had high Risk Rai Stage and 68% had elevated β-2 microglobulin; the median baseline variables are as follows: age of diagnosis 65, WBC values- 18×10^3 and LDH-580. Seventy patients showed del(13q), of which 48/70 were monoallelic for the 13q14.3 using the D13S25, 14/70 were bi-allelic; 39/70 had also deletion of Rb while 1/70 had the Rb alone; High risk genomic aberrations were detected in 82 patients: 25 patients showed +12, 23 showed del(11q), 18 showed del(17p), 16 showed 14q32 abn, while 26 had multiple FISH abnormalities. Using criteria defined above for ZAP, 78(34%) patients were deemed ZAP70 positive. Around 65% of patients subsequently received treatment (mostly fludarabine based). Fisher's exact test was used to study the association between categorical variables and the two Zap groups. Only del(11q) had a significant difference (p=0.0264) between the two ZAP groups in terms of predicting inferior survival. *Conclusions*. To develop risk-adapted strategies in CLL, concordant prognostic factors are needed to allow the prediction of individual patient course. In this ongoing prospective study poor risk patients were defined by NCI-WG criteria. While there a concordance in the predominantly male population who exhibited the ZAP-70 and high risk cytogenetic abnormalities only the presence of positive ZAP70 and del(11q) statistically predicted for inferior survival. Further correlation analysis between these and array CGH will be presented at the EHA annual meeting.

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ANALYSIS OF CD44 EXPRESSION IN MONOCLONAL GAMMOPATHIES: CORRELATION BETWEEN GENE EXPRESSION LEVEL AND FLOW CYTOMETRY FINDINGS

J.M. Raya,[1] K. Todoerti,[2] L. Morabito,[1] L. Agnelli,[2] P. Ponzo,[2] T. Martin,[1] F. Morabito,[3] G. Lambertengui-Deliliers,[2] M.L. Brito,[1] L. Hernández-Nieto,[1] A. Neri[2]

[1]Hospital Universitario de Canarias, LA LAGUNA; [2]Ospedale Maggiore IRCCS Milano, MILANO; [3]Azienda Ospedaliera di Cosenza, COSENZA, Italy

Background. Adhesion molecule CD44 (HCAM) is a cell surface transmembrane glycoprotein encoded by single gene, involved in lymphocyte activation, recirculation and homing, adhesion of extracellular matrix, angiogenesis, cell proliferation, cell differentiation and cell migration, as a receptor for hyaluronic acid. CD44 is highly expressed in many tumors, and correlated with the tumor biological behaviour including tumorigenesis, growth, metastasis and prognosis. It is a reliable indicator of tumor load and disease activity, and also called metastasis-associated protein. Recent studies have shown that CD44 overexpresses on hematopoietic cells and has been implicated in the interactions between bone marrow stromal layers and hematopoietic progenitors. Its overexpression is associated with poor prognosis in a number of haematological malignancies. *Aims.* To compare the expression of CD44 in patients with different type of plasma cell disorders, at two levels: evaluation of CD44 gene transcript (high-density oligonucleotide microarrays), and plasma cell-surface CD44 expression (flow cytometry). *Methods.* We studied separately two groups of patients affected by monoclonal gammopathies: an Italian group which included 153 patients (12 MGUS, 132 multiple myeloma and 9 plasma cell leukemia) and four healthy controls, and a Spanish group with 31 patients (5 MGUS, 18 multiple myeloma, 2 plasma cell leukemia and 6 reactive plasmocytosis). In the Italian group, we examined the presence of CD44 in highly purified plasma cells at gene expression level using high-density oligonucleotide microarrays (data reported as normalized expression values). In the Spanish group, we studied the phenotypic CD44 expression (EPICS XL-MCL cytometer) on $CD138^+$ gated plasma cells, and results were expressed as percentage, fluorescence mean intensity (FMI, which is related with cell-membrane antigen density) or isotypic control-related FMI. Mann-Whitney and Krukall-Wallis tests were used to statistical analysis. *Results.* Although plasma cell leukemia (PCL) patients showed the highest values, we do not find significant differences concerning CD44 gene expression level when the whole Italian group is considered (p=0.092). Nevertheless, patients with PCL exhibits significantly higher values when compared with MM (p=0.014) and MGUS (p=0.05). Phenotypic expression of CD44 was significantly different in the Spanish group of patients in terms of percentage of positive cells (p=0.001), FMI (p=0.025) and isotypic control-related FMI (p=0.008). Specifically, FMI was four-fold higher in PCL when compared with myeloma patients, and this difference was even more pronounced between both diseases (x7) when isotypic control-related FMI was analyzed. *Conclusions.* We find a correlation between CD44 gene expression level and plasma cell-surface CD44 expression in monoclonal gammopathies. Patients with PCL exhibit the highest values of CD44 gene level and CD44 cell-membrane antigen density (measured by FMI) among plasma cell disorders. Of interest, marked differences between PCL and myeloma patients are found. Although further studies need to be made on this phisiopathological aspect, a high CD44 expression (both at gene and antigenic level) seems to be a specific feature of PCL, a disease with a well-known poor prognosis.

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NATURAL POLYPHENOLS ANTAGONIZE THE ANTI-MYELOMA ACTIVITY OF PROTEASOME INHIBITOR BORTEZOMIB BY DIRECT CHEMICAL INTERACTION

T. Kim,[1] J. Park,[2] B. Oh,[1] H.J. Min,[1] T.-S. Jeong,[3] J.H. Lee,[4] C. Suh,[5] J.-W. Cheong,[6] H.J. Kim,[7] S.-S. Yoon,[8] S.B. Park,[2] D.S. Lee[9]

[1]Cancer Reseach Institute, Seoul National University College of Medicine, SEOUL; [2]Department of Chemistry, Seoul National University, SEOUL; [3]National Research Laboratory of Lipid Metabolism and Atherosclerosis, KRIBB, DAEJEON; [4]Department of Internal Medicine, Gachon University Gil Medical Center, INCHEON; [5]Department of Internal Medicine, Asan Medical Center, University of Ulsan Colle, SEOUL; [6]Department of Internal Medicine, Yonsei University College of Medicine, SEOUL; [7]Department of Internal Medicine, College of Medicine, Hallym University, SEOUL; [8]Department of Internal Medicine, Seoul National University College of Medicine, SEOUL; [9]Department of Laboratory Medicine, Seoul National University College of Medicin, SEOUL, South-Korea

Background. Bortezomib is the therapeutic proteosome inhibitor with antimyeloma activity and polyphenols are well known compounds with antiproliferative effect against tumors. Aims. We attempted cotreatment of myeloma cells with bortezomib and polyphenols, expecting a synergistic effect. *Methods.* We performed cell viability assay in the presence of bortezomib alone or combination with 6 polyphenols (rutin, quercetin, caffeic acid, gallic acid, EGCG, and tannic acid) in both genetically different MM cell lines (U266, RPMI8226, and MC/CAR) and $CD138^+$ primary myeloma cells from patients. Antioxidant potential assay, western blotting, and flow cytometry analysis were done to determine blocking effect of polyphenols on bortezomib. Direct chemical interaction of bortezomib with polyphenols was confirmed by 11B NMR spectroscopy. *Results.* The anticancer activity of bortezomib was blocked by polyphenols. The structural features of polyphenols correlated strikingly with their antagonistic effect; particularly, the presence or absence of vicinal diol moiety was the key element for effective blockage of the anticancer function of bortezomib. We infer that the vicinal diols in the polyphenols interact with the boronic acid of bortezomib and convert the active triangular boronic acid of bortezomib to inactive tetrahedral boronate, which abolishes the antimyeloma activity of bortezomib. *Summary and Conclusions*. The anticancer function of bortezomib was blocked by polyphenols. Based on this, restriction of the intake of natural polyphenols in foods or vitamin supplements should be considered during bortezomib treatment in MM patients.

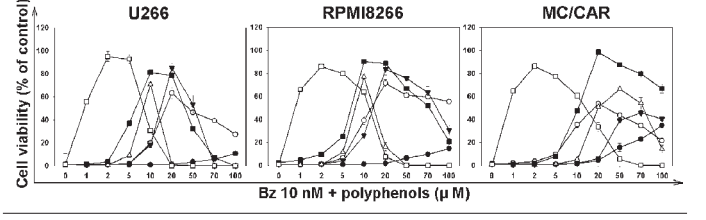

Figure 1.

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THE ANGIOGENESIS-RELATED POLYMORPHISMS: ROLES IN MULTIPLE MYELOMA RISK

E. Faber, G. Lourenco, M. Ortega, P. Silva, C. De Souza, I. Lorand-Metze, C. Lima

State University of Campinas, CAMPINAS, SÃO PAULO, Brazil

Background. Angiogenesis (AG) is an important step in tumour development, including multiple myeloma (MM). The enzymes of the glutathione S-transferases (GSTs) system are likely to facilitate the hypoxia-inducible factor activity, also stimulating the AG. On the other hand, the vascular endothelial growth factor (VEGF) plays a crucial role in the initiation of AG. The genes coding for GST mu1 (GSTM1), theta1 (GSTT1) and VEGF are polymorphic in humans. In breast cancer, the GSTM1 null-type genotype was associated to a lower angiogenic phenotype in comparison with the wild genotype. In addition, the C936T polymorphism, T allele was linked to cancer risk in solid tumours. Therefore, in theory, carriers of the variant alleles could be protected from tumours development. *Aims.* We tested in study whether the GSTM1, GSTT1 and VEGF genotypes altered the risk for MM in south eastern region of Brazil. *Meth-*

ods. Genomic DNA from 80 MM patients (mean age: 56, range: 32-86; 65 Caucasians; 15 African-Americans) and 203 controls (mean age: 49; range: 24-62; 175 Caucasians; 28 African-Americans) was analysed by polymerase chain reaction (multiplex PCR) and PCR followed by enzymatic digestion with the NlaIII enzyme for identification of gene genotypes. Statistical significance of the differences between groups was calculated by chi-square or Fischer exact test. Crude odds ratios (ORs) were calculated and were given within 95% confidence intervals (CI). *Results.* Both the patient and control samples were in Hardy-Weinberg equilibrium for VEGF C936T (X2=0.74, *p*=0.39; X2=2.15, P=0.14). Similar frequencies of the GSTM1 (35.0% *vs* 40.4%, P=0.13) and GSTT1 (30.0% *vs* 21.2%, *p*=0.26) null genotype were seen in patients and controls, respectively. No significant difference in frequencies of the GSTM1 and GSTT1 null combined genotype was seen in either group of individuals (8.0% *vs* 7.4%; *p*=0.67). Similar risks for disease was seen in individuals with the distinct genotypes of the GSTM1 (OR=0.62, CI95%: 0.34-1.14), GSTT1 (OR=1.45, CI95%: 0.75-2.80), and GSTM1 and GSTT1 combined (OR=1.24, CI95%: 0.46-3.32) genotypes. In addition, similar frequencies of VEGF CT+TT genotype (17.5% *vs* 23.6%, *p*=0.31) were seen in patients and controls. Individuals with the distinct genotypes of the C936T of the VEGF were under similar risks for MM (OR=0.68, CI95%: 0.33-1.43). Moreover, no difference in frequencies of the GSTM1 null and VEGF CT+TT (7.5% *vs* 12.8%, *p*=0.11), GSTT1 null and VEGF CT+TT (5.0% *vs* 3.45%, *p*=0.53) and GSTM1 null, GSTT1 null and VEGF CT+TT (1.2% *vs* 2.0%, *p*=0.49) combined genotypes were seen in patients and controls enrolled in study. Individuals with the distinct GSTM1 and VEGF (OR=0.41, CI95%: 0.14-1.21), GSTT1 and VEGF (OR=1.68, CI95%: 0.32-8.75), and GSTM1, GSTT1 and VEGF (OR=0.42, CI95%: 0.37-4.88) combined genotypes were under similar risks for disease. *Conclusions.* Our data suggests that the GSTM1, GSTT1 and VEGF genotypes, isolated or combined, do not influence the risk for MM in individuals from south eastern region of Brazil, but requires further investigation with larger number of individuals.

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SERUM FREE LIGHT CHAINS IN WALDENSTROM'S MACROGLOBULINAEMIA (WM) PATIENTS AT DIAGNOSIS

M.-C. Kyrtsonis,[1] T.P. Vassilakopoulos,[2] T. Tzenou,[1] N. Kafasi,[3] S. Sachanas,[2] E. Koulieris,[1] D. Maltezas,[4] M. Dimopoulou,[2] S.I. Kokoris,[2] E. Dimitriadou,[2] M.P. Siakantaris,[2] M.K. Angelopoulou,[2] P. Panayiotidis,[1] G.A. Pangalis[2]

[1]1st Dpt of Propedeutic Int. Medicine, Univ. of Athens, Laikon Hospital, ATHENS; [2]Dpt of Hematology, Athens Med. School, Laikon Hosp, ATHENS; [3]Dpt of Immunology, Laikon Hospital, ATHENS; [4]Dpt of Hematology, Metaxa Cancer Hospital, PIRAEUS, Greece

Background. Serum free light chains' (sFLC) values and their ratio (sFLCR) are useful for the diagnosis and/or the evaluation of response and prognosis in plasma cell dyscrasias such as multiple myeloma (MM) and amyloidosis, as well as for the prediction of possible disease evolution in MGUS and smoldering MM. *Aims.* To evaluate the contribution of sFLC and sFLCR in WM. Patients and *Methods.* sFLC were determined nephelometrically with the Freelite serum free light chain assay (The Binding Site, Ltd, Birmingham, UK) in 29 patients at diagnosis and in 29 healthy individuals (HI). Patients' absolute sFLC values and their ratio were compared to HI values and possible correlations with disease parameters were evaluated. sFLCR was calculated, accordingly as kappa/lambda or lambda/kappa, depending on the light chain type restriction of the patient. *Results.* In HI kappa sFLC ranged from 1.9 to 12.7 mg/dL (median 8.9 mg/dL) and lambda from 12.7 to 37.1 mg/dL (median 20.4 mg/dL). The resulting normal median kappa/lambda sFLCR was 0.96 and lambda/kappa sFLCR 7.55. Of the 29 patients, 19 presented kappa light chain restriction and in them kappa sFLC ranged from 6.62 to 895 mg/dL (median 36.6 mg/dL); the median sFLCR was 2.60 (0.82-18.97). In 10 patients with lamda restriction, the median lambda sFLC was 47.6 mg/dL (range 10.5-498) and the median sFLCR was 3.10 (0.44-51.50). 72% of patients had increased sFLCR values (superior than the higher value of HI). In addition patients' sFLC strongly positively correlated with serum beta-2 microglobulin and negatively with haemoglobin. In WM patients with kappa restriction, the presence of lymphadenopathy correlated with sFLC values. A borderline positive correlation was found with bone marrow lymphoplasmacytic infiltration and no relationship was observed with platelet counts, serum LDH levels or the presence of splenomegaly. *Conclusions.* sFLC and sFLCR are increased in WM and correlate with beta-2 microglobulin, the presence of lymphadenopathy, bone marrow infiltration and inversely with haemoglobin.

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BONE MARROW IMMUNOHISTOCHEMICAL FINDINGS INCLUDING MORPHOLOGY, MAST CELL PRESENCE, SYNDECAN-1 AND CYCLIN D1 EXPRESSION IN WALDENSTROM'S MACROGLOBULINAEMIA (WM)

T. Tzenou,[1] G. Levidou,[2] P. Korkolopoulou,[2] T.P. Vassilakopoulos,[3] N. Stavropoulos,[3] V. Salpeas,[2] V. Bartzis,[1] A. Gassiamis,[1] X. Papanikolaou,[4] M. Moschogiannis,[3] E. Koulieris,[1] C. Kalpadakis,[3] M.K. Angelopoulou,[3] P. Panayiotidis,[1] G.A. Pangalis,[3] M.-C. Kyrtsonis[1]

[1]1rst Dpt of Propedeutic Int. Medicine, Athens Med.School, Laikon Hosp., ATHENS; [2]Dpt of Pathology, Athens Med. School, Laikon Hosp., ATHENS; [3]Dpt of Hematology, Athens Med. School, Laikon Hosp, ATHENS; [4]Dpt of Hematology, Metaxa Cancer Hospital, PIRAEUS, Greece

Background. WM is characterised by a clonal proliferation of neoplastic lymphocytes, lymphoplasmacytes and few plasma cells in the bone marrow (BM) and, less frequently, in other nodal or extranodal sites. Monoclonal B-cells secrete IgM and may overexpress syndecan-1 (CD138) on the cell surface. In some instances, differential diagnosis with other low-grade lymphomas with plasmacytic differentiation may be difficult. *Aims.* To present and discuss BM immunohistochemical findings in WM patients. Patients and *Methods.* BM paraffin embedded sections of 32 newly diagnosed WM patients were studied. Infiltrating monoclonal B-cells morphology, the expression and intensity of syndecan (CD138) in neoplastic cells, the percentage of mast cell infiltration and cyclin D1 staining were evaluated. Correlation of findings with parameters of disease activity was attempted. *Results.* The median percentage of neoplastic B-cells infiltrating the BM was 40% of which the median percentage of cells presenting lymphoplasmacytic differentiation was 37% and of cells expressing syndecan-1 30%. Syndecan-1 expression was strong in 1/3 of cases. In 59% of studied sections a percentage of 2% or more mast cells was seen. Expression of cyclin D1 in few cells was observed in 32%. No correlation was found between these findings and parameters of disease activity. Conclusions: In this series, the majority of WM B-cells looked like small lymphocytes. The presence of mast cells was prominent. Syndecan-1 was frequently expressed by the monoclonal cells, strongly in 1/3 of cases. As already reported in marginal zone lymphoma and multiple myeloma, cyclin D1 expression may be observed.

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EVALUATION OF EARLY 18FDG-PET IN ADVANCED HODGKIN LYMPHOMA'S PATIENTS (PTS) TREATED WITH CHLVPP/ABVVP REGIMEN

E. Cocorocchio, S. Bassi, L. Nassi, F. Gigli, A. Alietti, P. Bertazzoni, M. Negri, F.A. Peccatori, H.A. Azim Jr, L. Travaini, G. Martinelli

European Institute of Oncology, MILAN, Italy

Background. Negative 18FDG-PET after 2 cycles of ABVD chemotherapy (early 18FDG-PET) has been associated with better prognosis in advanced HL pts. No data are available with more intensified regimens. *Aims.* To evaluate the prognostic value of early 18FDG-PET in advanced HL pts treated with a high dose intensity regimen. *Methods.* 33 consecutive pts received 6 cycles of ChlVPP/ABVVP chemotherapy regimen (D1 Vinblastine 6 mg/sm; D1-7 Procarbazine 80 mg/sm/d, Chlorambucil 6 mg/sm/d, Prednisone 50 mg/d; D8 Doxorubicin 30 mg/sm, Vincristine 1 mg, Bleomycin 7.5 IU/sm; D8-10 VP16 100 mg/sm/d; D11 pegfilgrastim 6 mg; every three weeks) for stage II (18 pts) III (6 pts) IV (9 pts) disease. Median age was 33 yrs (range: 16-68), with classic HL in 32 pts and lymphocyte predominant in 1. All pts had an 18FDG-PET scan before treatment and after 3 and 6 cycles. 18FDG-PET scan was considered positive if persistent uptake was present after treatment in previously involved sites. *Results.* Median follow-up was 17 months from diagnosis. After six cycles of ChlVVP/ABVVP, 30/33 pts were considered in complete remission (CR) and three in partial remission (PR). Early 18FDG-PET was positive in 4 cases (12%), becoming negative in 1 case at the end of therapy. The 3 pts with persistent 18FDG-PET uptake at the end of therapy were considered in PR and were subjected to high dose chemotherapy, achieving CR in two cases, while the other patient had disease progression and died shortly. Two pts (with negative early 18FDG-PET) relapsed after 5 and 27 months from the end of therapy and are still alive. *Conclusions.* early 18FDG-PET status seems to be related to CR rate after ChlVPP/ABVVP chemotherapy (100% CR for negative early 18FDG-PET pts vs. 25% CR for positive early 18FDG-PET), but failed to predict relapse in 1 out of 4 (25%) pts with early positive 18FDG-PET and in 2 out of 29 (6%) pts with early negative 18FDG-PET. Considering the incidence of false positive and false negative results, the prognostic value of early 18FDG-PET in HL must be reviewed.

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CARDIOTOXICITY ASSESSMENT IN HODGKIN'S LYMPHOMA USING BIOCHEMICAL MARKERS

A. Giordano,[1] F. Gaudio,[1] T. Perrone,[1] A. Guarini,[1] C. De Risi,[1] M. Sorino,[2] V. Ruggieri,[3] A. Scorca,[1] V. De Luca,[2] F. Di Serio,[3] V. Liso,[1] G. Specchia[1]

[1]Hematology, BARI; [2]Cardiology Department, BARI; [3]Clinic Pathology, BARI, Italy

Dose-dependent anthracycline-induced cardiotoxicity has been observed in long term survivors of Hodgkin Lymphomas (HL). However, it is not yet possible to identify patients at high risk of developing clinically important cardiotoxicity and no commonly accepted guidelines have been established for monitoring heart function in adults receiving chemotherapy. Recent data suggest that circulating biomarkers such as N-terminal pro-brain natriuretic peptide (NT-proBNP) and Troponin I (TROPI) are sensitive and specific predictors of cardiotoxicity. Elevated secretion of NT-proBNP has been associated with both left ventricular systolic dysfunction and diastolic filling abnormalities in patients treated with anthracycline. TROPI has also been shown to be elevated prior to changes in left ventricular ejection fraction and before the appearance of cardiac symptoms. In the present study 49 patients (21 females and 28 males) with *de novo* Hodgkin's Lymphomas, median age 29.5 (range 16-64), who were scheduled to receive up to 6 cycles of ABVD at a mean cumulative doxorubicin dose of 275 mg/sqm (range 200-300 mg/sqm), were examined to analyze the value of serial TROPI and NT-proBNP measurements and echocardiographic parameters in the early detection of doxorubicin-induced cardiotoxicity. Plasma levels of NT-proBNP and TROP I were measured at baseline and before and after each cycle of therapy. Echocardiography was performed at baseline and after the fourth and the sixth cycle of chemotherapy. In all patients, baseline ECHO parameters and biomarkers were normal. The TROPI value did not change significantly during the therapy, no significant impairment in left ventricular ejection fraction was observed and no cardiac event was documented. Mean NT-proBNP levels did not significantly change after anthracycline administration. However, in 13 (26.5%) patients a marked transient increase of NT-proBNP was obtained during the second-third cycle of ABVD regimen, after low cumulative doxorubicin dose (median 120 mg/sqm-range 75 -150 mg/sqm) and in 2 patients (4%) the values remained higher than baseline up to the end of the chemotherapy. Our data suggest that low to moderate doxorubicin doses can cause a transient increase of NT-proBNP that, even in absence of impairment of the echocardiographic parameters, can be related to altered cardiac load conditions during chemotherapy and may allow early identification of individuals at risk of delayed cardiac damage. Further studies in larger populations with a longer follow-up are warranted to define the role of biochemical markers that can reliably detect early myocardial injury during chemotherapy.

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POSITRON EMISSION TOMOGRAPHY AS A METHOD OF STAGING, EVALUATING RESPONSE AND SELECTING THE TREATMENT STRATEGY IN PATIENTS WITH HODGKIN'S LYMPHOMA: A SINGLE CENTER EXPERIENCE

T. Perrone,[1] F. Gaudio,[1] A. Giordano,[1] P. Curci,[1] A. Pietrapertosa,[1] C. De Risi,[1] N. Merenda,[2] A. Niccoli Asabella,[2] G. Rubini,[2] V. Liso,[1] G. Specchia[1]

[1]Hematology, BARI; [2]Nuclear Medicin Department, BARI, Italy

Background. Metabolic imaging by 18F-fluorodeoxyglucose (F-FDG) positron emission tomography (PET) is a useful tool for the management of patients (pts) with Hodgkin's Lymphoma (HL), both for staging purposes and for evaluating the response to treatment. Because of its accuracy, in the initial staging of HL, PET frequently reveals a higher stage than conventional methods such as CT scans. Furthermore, several studies have shown the prognostic value of PET performed after two, three or four cycles of ABVD chemotherapy, to predict progression free survival and overall survival. *Aims.* The aim of the present study was to investigate the value of PET for the management of pts with HL observed in our institution from July 2005 to July 2007. *Methods.* A total of 40 pts with newly diagnosed HL were staged with: total body CT scan, bone marrow biopsy and whole body PET. In 5 pts (12%) a higher stage was indicated by PET than by conventional staging. All pts received chemotherapy according to the ABVD regimen. After four cycles, restaging was performed including CT and PET scans. During restaging, in 9 pts (22%) there was no correspondence between the CT scan and PET *Results.* in 6 (15%) pts CT scan showed residual masses, while the F-FDG PET study was negative. In addition, in 3 (7%) pts PET revealed F-FDG uptake in residual areas not shown by CT scan. In view of the accuracy and specificity of PET, we considered 27 (67%) pts with negative PET to be in Complete Remission (CR); 10 (25%) pts with positive PET in Partial Remission (PR) and 3 (7%) had progressive disease. Among pts with negative PET, 11 pts (41%) with adverse prognostic factors at diagnosis (stage IIB - IV, bulky disease, IPS 3-5) received further therapy (2 more cycles of ABVD or Radiotherapy). All pts with positive PET (13) continued the treatment: with ABVD or Radiotherapy, and those with progressive disease or with adverse prognostic factors at diagnosis received salvage treatment consisting of: two cycles of IGEV chemotherapy, stem cells collection, BEAM chemotherapy with Autologous stem cells transplantation. *Results.* During a median follow up of 20 months (7-28), only 2 (7%) pts with negative PET after 4 cycles of therapy relapsed; while the others (25) remained in CR. Among pts with positive PET, 3 (23%) had progression, 10 (77%) reached CR and 1 (10%) of them relapsed. Comparing the two subset of pts, with positive PET and negative PET after 4 cycles of therapy, the first group seems to show a worse prognosis. *Conclusions.* In our experience PET scan has proven to be a valuable, important tool for the assessment of initial staging, for therapeutic evaluation and detection of residual disease. Positive PET after 4 cycles of therapy shows a negative predictive value and can identify pts who need a more intensive treatment approach. Therefore PET performed after 4 cycles of ABVD plays an important rule in the selection of more individually tailored therapeutic strategies.

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IS IT HODGKIN LYMPHOMA THE SAME DISEASE IN ALL AGE GROUPS? A STUDY OF MORPHOLOGICAL, CLINICAL AND EBV-ASSOCIATED FEATURES BY AGE-GROUP

M.B. Barros, C.M. Minicelli, D.G. Guiretti, I.Z. Zalcberg, R.H. Hassan

Instituto Nacional do Câncer, RIO DE JANEIRO, Brazil

Background. Hodgkin lymphoma (HL) is a biologically heterogeneous disease, characterized by a bimodal age-specific incidence with identical morphological diagnostic criteria for children and adults. However, several reports show that some biologic differences exist among age-group patients, according to socio-geographic origin. *Aims.* We sought to investigate whether HL diagnosed in Southeastern Brazil exhibited clinical, morphological and virological differences according to age groups. *Methods.* 96 patients diagnosed at INCA, Brazil, between 1998-2005 were included based on availability of formalin-fixed paraffin-embedded tissue and complete clinical data for retrospective analysis. Clinical features [stage, risk group (RG), number of involved anatomic sites (NIAS), Hb levels] were obtained from hospital records. Morphological characteristics [histologic subtypes, number of neoplastic cells (NNC), mitosis and eosinophils] were obtained by histopathological analysis, and EBV-association by EBER-ISH. Patients were classified in 3 age groups: GR1: <10 years; GR2: >10 years and <18 years; GR3: >18 years. *Results.* Median age was 16 years (3 to 81) with an age-group distribution of 18 (18.8%) GR1; 54 (56.3%) GR2 and 24 (25%) GR3. Analysis by age-group showed low mediastinal involvement in GR1 vs. GR2 and GR3 (p=0.005); low disease stages (I/II) in GR1 vs. GR2 and GR3 (p=0.01). A predominance of B-symptoms was observed in GR3 while GR1 showed few patients with this variable and GR2 exhibited an intermediate frequency (p=0.01); a predominance of patients in the high RG was observed in GR2 and GR3 (p=0.03). Normal Hb levels predominate in GR3 patients compared to GR2, which exhibited low Hb levels (p=0.002). Morphological analysis showed a higher frequency of mixed cellularity subtype in GR1 (p=0.001) and nodular sclerosis grade 2 in GR2 (p=0.0001); A high NNC was characteristic of GR2 and GR3 patients (p=0.0001) while an EBV-associated HL was observed more frequently in GR1 and GR3 (p=0.01). By multivariate analysis, B symptoms (p=0.04); nodular sclerosis grade (p=0.03) and NNC (p=0.01) retained significance; the Hb level showing borderline association (p=0.09). Conclusions: Our results confirm that HL is a heterogeneous disease, not only regarding epidemiological features but also at the clinical and morphological level. Children younger than 10 years showed a distinct biologic pattern.

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KSHV TRANSFORMED PRIMARY EFFUSION LYMPHOMA CELLS INDUCE A VEGF DEPENDENT ANGIOGENESIS AND ESTABLISH FUNCTIONAL GAP JUNCTIONS WITH ENDOTHELIAL CELLS

A. Bazarbachi,[1] L. Haddad,[2] H. El Hajj,[2] R. Abou-Merhi,[2] Y. Kfoury,[2] R. Mahieux,[3] M. El-Sabban[2]

[1]American University of Beirut Medical Center, BEIRUT, Lebanon; [2]American University of Beirut, BEIRUT, Lebanon; [3]Institut Pasteur, PARIS, France

Background. Kaposi's sarcoma associated herpesvirus (KSHV) is the causative agent of primary effusion lymphoma (PEL) and of Kaposi sarcoma. PEL is an aggressive proliferation of B cells with poor prognosis. *Aims and Methods.* We evaluated both *in vitro* and *in vivo* the potential role of angiogenic factors secreted by PEL cells i.e. their interaction with endothelial cells and their implication in the invasive behavior of tumoral cells. *Results. In vitro*, PEL cells induced angiogenesis is dependent on vascular endothelial growth factor (VEGF) and VEGF receptors. However, although PEL cells produce VEGF and basic fibroblast growth factor (b-FGF) transcripts, they only secrete VEGF *in vitro*. *In vivo*, very high levels of both VEGF and b-FGF were found in the ascitic fluid of NOD-SCID mice injected with PEL cells. We then show evidence of cell adhesion and gap junction-mediated hetero-cellular communication between PEL cells and endothelial cells. Finally, we show that PEL cells extravasate through the endothelial barrier and that the specific tyrosine kinase inhibitor of VEGF receptors, PTK-787/ZK-222584, the anti-VEGF antibody, bevacizumab, or the gap junction inhibitor 18-alpha glycyrrhetinic acid, partially attenuate PEL cell extravasation. *Conclusions.* Angiogenesis, cell adhesion and communication likely contribute to the development of PEL and represent potential therapeutic targets.

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THE ROLE OF PROTHROMBOTIC GENETIC RISK FACTORS ON DEVELOPMENT OF THROMBOSIS IN PEDIATRIC ONCOLOGY PATIENTS

M. Turker, B. Atabay, I. Yaprak, H. Oniz, I. Coker

Tepecik Teaching and Research Hospital, IZMIR, Turkey

Background. The coagulopathic complications in cancer patients may be life threatening. In less severe cases, the impact of these complications on the patients quality of life, duration and frequency of hospitalization, and on the treatment costs can be substantial. *Aims.* The aim of this study is to evaluate the frequency and the clinical relevance of the genetic risk factors, mainly related to the hemostatic system that are known to influence thrombotic risk in cancer patients. *Methods.* In this prospective study, a total of 101 pediatric cancer patients, 47 patients with solid tumors and 54 with leukemia/lymphoma were included. The patients were treated according to the established pediatric tumor protocols, ALL-BFM, NHL-BFM, and other relevant chemotherapy protocols. After admission, all patients were evaluated for the symptoms and signs of thrombosis. Patients were supported for hydration and mobilization during asparaginase and steroid treatment. In all patients, Factor V G1691A (FVL), Prothrombin G20210A and MTHFR C677T, MTHFR A1298G polymorphisms were evaluated with RT-PCR. Nineteen patients had central venous lines (14 leukemia/lymphoma, 5 solid tumors). Of these patients, only one receives prophylactic anticoagulant therapy. *Results*. Frequency of mutations in the whole group were as follows: Factor V G1691A (9.9% heterozygosity, no homozygotes); Prothrombin G20210A (6.9% heterozygosity, no homozygotes); MTHFR C677T (50.5% heterozygosity, 8.9% homozygosity), MTHFR A1298G (6.9% heterozygosity, 3% homozygosity). Thrombosis developed in six patients (12.7%) with solid tumors (deep venous thrombosis (n:2), hepatic juguler vein thrombosis (n:1), venoocclusive disease (n:1), stroke (n:1), and deep venous thrombosis with pulmonary embolus (n:1)). Among six patients with thrombosis two had FVL heterozygosity, the remaining four patients had compound heterozygosity for MTHFR C677T and MTHFR A1298G, MTHFR C677T heterozygosity and MTHFR C677T homozygosity. When the presence of FVL and Prothrombin G20210A heterozygosity, MTHFR C677T and MTHFR A1298G homozygosity were taken as risk factors, the development of thrombosis in patients with solid tumors was found to be significantly higher (p=0.005, χ^2 test). *Conclusions*. Genetic mutations are likely to be additional risk factors for the development of thrombosis in patients with solid tumors. However, these mutations do not appear to be relevant risk factors for thrombosis in the small number of children with leukemia/lymphoma in our study.

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CEREBRAL VENOUS SINUS THROMBOSIS AS A COMPLICATION OF ASPARAGINASE TREATMENT IN A 15 YEAR-OLD GIRL WITH T CELL NON-HODGKIN LYMPHOMA

T. Ociepa, E. Maloney, T. Urasinski, E. Kamienska, B. Richter

Pomeranian Medical University, SZCZECIN, Poland

Venous thrombosis (VT) including cerebral venous sinus thrombosis (CVST) is a rare condition in pediatric population. It is usually caused by underlying disorders (thrombophilia, malignancy), chemiotherapeutic agents (i.e. steroids, asparaginase) and the use of indwelling catheters. Thanks to modern visual diagnostic techniques VT is diagnosed with increased frequency. The reported incidence of VT in children with malignancy varies from 1% to 36% and still results in high morbidity and mortality rate. We report on a case of 15-year-old girl admitted to our unit due to right supraclavicular lymph node enlargement. Based on the physical, radiological and histological examination the diagnosis of T cell non-Hodgkin lymphoma stage III was made. The treatment according to the EURO-LB 02 protocol was started resulting in regression of initially involved lymphoid regions. On the day 33 of the induction protocol the patient started to complain of headaches, nausea, visual problems, than generalized seizures occurred. The contrast head computed tomography (CT) showed the right transverse and sagittal sinus filled with non-contrasted, hyperdense, heterogenous mass. No brain lesions (including bleeding) were found (Figure 1). Protrombin time and partial protrombin time were in normal ranges. Antitrombin III (AT III), fibrinogen and platelets levels were decreased. D-dimers were elevated. Based upon the head CT, laboratory analysis and the history of asparaginase and steroid treatment the right transverse and sagittal sinus thrombosis was diagnosed.

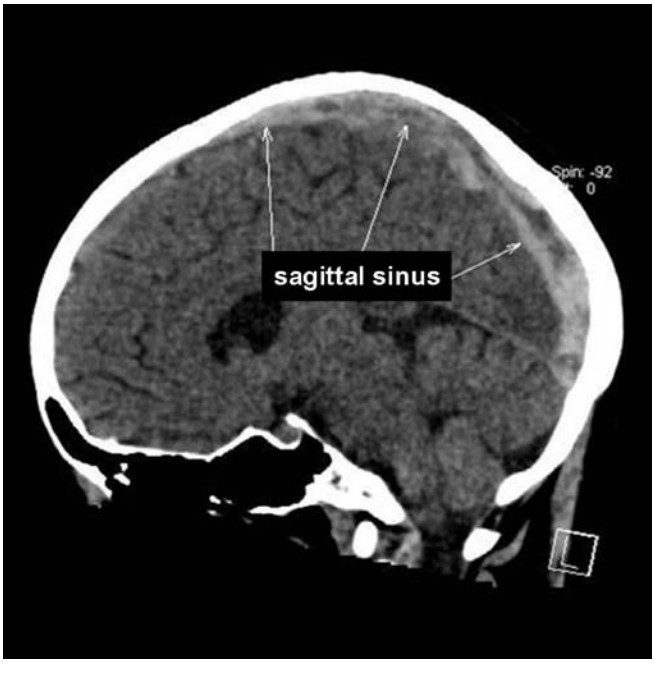

Figure 1.

Thrombolytic therapy was introduced. tPA (Actylise) in continuous infusion in a dose of 0,03 mg/kg/h with concomitant Heparin infusion in a dose of 5 U/kg/h were administered. Due to decreased level of AT III and fibrinogen, fresh frozen plasma and AT III concentrate were transfused. After 24 hours of the treatment the control CT of the brain revealed partial resolution of thrombotic lesions. tPA was increased to the dose of 0,06 mg/kg/h for the next 24 hours followed by 0,03 mg/kg/h. tPA and Heparin were stopped after 96 hours from the onset of infusion and a low molecular weight heparin (LMWH; Fraxiparine) in a single dose 0,1 mL/10 kg was given twice a day. During the thrombolysis elevation of D-dimers was observed and the patient developed mild bleeding from the mucus membranes and subcutaneous bruises in regions of previous vein punctures. On day 8 from the beginning of symptoms angio-MRI was performed showing total resolution of the

left transverse and sagittal sinus thrombosis. Within 10 days from the beginning of initial symptoms patient was well, with no neurological abnormalities on physical examination, and started to continue the chemotherapy schedule. On day 14 from the thrombosis event patient was discharged from the department still on anticoagulation with LMWH. There is no guidelines of anticoagulant therapy in children with CVST. The use of thrombolytic agents still remains controversial and there is no definitive recommendation regarding the therapy. Published data recommend administration of lower doses of tPA in children with deep vein thrombosis compared to those in adults. However there are no recommendations in sinus venous thrombosis. Both local and systemic thrombolytic therapy for the treatment of deep vein and central venous sinus thrombosis have been reported in children. Although the local thrombolysis seems to be the method of choice in CVST treatment, the reported case might indicate that systemic thrombolytic therapy appears to be as effective as safe in children with sinus venous thrombosis.

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VKORC-1 AND CYP 2C9 MUTATIONS AND THEIR IMPACT ON PHARMACODYNAMIC RESPONSE TO ORAL ANTICOAGULATION TREATMENT

N. Tsiara, A. Vartholomatos

University Hospital of Ioannina, IOANNINA, Greece

Background. Anti vitamins K, acenocoumarol (Ac) and warfarin are the most widely used oral anticoagulants for the treatment and prevention of thromboembolic events. They have narrow therapeutic range and wide variation in their anticoagulant effect. The risk for hemorrhagic events is elevated, especially soon after the initiation of treatment. Factors that affect response to orally administered anticoagulants are weight, race, age and gender. Additionally, variants of the CYP2C9 and VKORC-1 genes may affect their metabolism. The aim of our study was to investigate whether polymorphisms of CYP2C9 and VKORC-1 have an impact on the pharmacodynamic response to Ac administration, leading to dose modification resulting to effective and safe anticoagulation. *Material and Methods.* We studied 44 patients on long-term oral anticoagulation treatment with Ac, for atrial fibrillation, deep vein thrombosis or pulmonary embolism. Twenty-four patients were male and 20 female, mean age was 69±14 years. All were treated with Ac to a target INR between 2 to 3. Mean necessary dose was 2.6±1.8 mg Ac. Blood samples were collected from the patients and tested for (-1639G>A), (430 C>T) and (1075G>A), VKORC-1, CYP2C9*2 and CYP2C9*3 gene polymorphisms respectively. *Results.* Sixteen patients were on low dose Ac treatment (<2 mg). Thirteen were heterozygotes for one or more of the above mentioned mutations. Thirteen patients on Ac dose ≥2 mg had wild type genotype and 15 had one or more of the above mentioned mutations. All patients had INR within the therapeutic range. Statistical analysis was performed with the Fisher's exact test. Results were not statistically significant, but showed that patients with wild type of the above mentioned genes have 4 times lower risk for hemorrhage when treated with elevated ≥2 mg Ac doses compared with patients with mutated genes. *Conclusions.* The majority of patients who have a wild type genotype for VKORC-1, CYP2C9 are on higher Ac doses (≥2 mg). Consequently, in patients lacking mutated genes, Ac treatment can be safely initiated at higher doses without increased risk for bleeding. However, we believe that a higher number of patients is needed to confirm that treatment with Ac, in patients with mutated genes, VKORC-1, CYP2C9*2 and CYP2C9*3, should be initiated carefully and in low doses.

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PROSPECTIVE STUDY OF THE CLINICAL AND SEROLOGICAL CHARACTERISTICS AND LONG-TERM EVOLUTION OF THE ANTIPHOSPHOLIPID SYNDROME AT THE AMERICAN UNIVERSITY OF BEIRUT MEDICAL CENTER. THE LEBANESE ANTIPHOSPHOLIPID SYNDROME STUDY GROUP

I.U. Uthman, L.B. Bazzi, A.T. Taher

American University of Beirut Medical Center, BEIRUT, Lebanon

Background. Antiphospholipid syndrome (APS) is a disorder characterized by elevated levels of auto-antibodies directed against the anionic components of phospholipid membranes. It includes a heterogeneous array of consequences, most commonly, arterial and venous thrombosis, as well as fetal loss. Patients with APS suffer from increased risk of thrombosis, leading to complications such as neurological stroke and deep vein thrombosis, among others. *Aims.* The aim of this study is to evaluate prospectively the clinical and serological manifestations and the long-term evolution of the APS patients seen at AUB-MC, and in the future expand this to other centers in the country. We hope to determine the incidence and characteristics of the different APS clinical manifestations at the onset of the disease and during the follow-up. *Methods.* All patients included in the study must fulfill at least one clinical criteria (venous thrombosis, arterial thrombosis, recurrent fetal loss) plus one antiphospholipid antibody (lgG anticardiolipin antibody, IgM anticardiolipin antibody, lupus anticoagulant). Patients with both primary APS and APS associated to systemic lupus erythematosus (SLE) or other autoimmune diseases are enrolled. A standardized questionnaire is used to collect demographic data, including age at onset of disease, time elapsed to protocol, risk factors for thrombosis, coexisting autoimmune disorders, and specific morbidities due to APS. A database was developed to enter and tabulate data from the study. *Results*. Over 200 patients have been reviewed and 110 were entered into the database. Thrombotic: DVT affected 10% and superficial thrombophlebitis 1.8%. Neurological: TIA was seen in 7%, and at least one neurological infarction in 14.5%. Vertigo affected 1.8% and 5.5% reported migraines. Cerebellar ataxia affected 2.7% and cerebral venous thrombosis 1.8%. Epilepsy or seizures were reported by 7%. Cardiac: unstable angina was reported by 2.7% and valve dysfunction by 0.9%. Chronic cardiomyopathy was reported by 0.9% and coronary artery bypass graft by 0.9%. Intestinal: infarcts were reported by 2.7%. Budd Chiari syndrome, along with portal hypertension and nodular regeneration in the liver was reported by 0.9%. Liver infarctions were reported by 1.8%. Pancreatic manifestations were reported by 2.7%; splenic infarcts by 3.6%. Pulmonary: Acute respiratory distress syndrome was reported by 0.9%, and pulmonary embolism and infarction by 3.6%. Osteoarticular: Avascular necrosis of bone was reported in 2.7%. Arthralgias were common, found in 11%; arthritis in 4.5%. Renal vein thrombosis was reported by 1.8% and renal infarction by 0.9%. The most common complications are DVT and TIA, superceded only by stroke. Some patients, of course, suffered from more than one complication, most commonly being arthralgias in combination with some form of thrombosis, such as DVT or neurological stroke. *Summary and Conclusions.* As is apparent by the preliminary data, antiphospholipid syndrome implies a heterogenous array of complications, which include a variety of thromboembolic phenomena. Much work is needed before we can identify the true causes and morbidities associated with the disorder. Furthermore, the wide array of thrombotic events indicates a need for earlier and more effective intervention in treating patients with suspected antiphospholipid syndrome.

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THE SPECTRUM OF INHERITED BLEEDING DISORDERS IN PEDIATRICS

E. El Bostany,[1] N. Omer,[2] S. Al Jaouni,[3] E. El-Ghoroury[4]

[1]National Research Center, CARIO, Egypt; [2]Pediatrics department Cairo University, CARIO, Egypt; [3]King Abdulaziz university, Faculty Of Medicine,Hematology Department., JEDDAH, Saudi Arabi; [4]National Research Center, Clinical Pathology Department, CARIO, Egypt

Background. Inherited bleeding disorders (IBD) are caused by quantitative and qualitative alterations of either platelets or plasma proteins involved in coagulation and fibrinolysis. Hemophilias are the most frequent IBD, however, accumulated data of various studies reported that von Willebrand disease (VWD) is the most common or the second most common cause of IBD, together with an increased incidence of platelet function defects (PFD), mostly due to increased rate of consanguinity in some communities. VWD is inherited disorder of hemostasis due to quantitative (type I and III) or qualitative (type II) defect of von Willebrand factor (VWF). Data on its epidemiology and impact in developing countries are limited. *Aims.* To assess the local prevalence of IBD and establish the clinical and historical variables that are predictive for those bleeding disorders in pediatric age group in our centers. *Methods.* The study involved forty three consecutive children with various bleeding manifestations presented to the clinic in the three centers (Pediatrics Clinic in the National Research Center, Cairo, Pediatrics Hematology Clinic in Cairo University in Egypt and Hematology Clinic in King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia). Fifteen age and sex matched controls. Variables investigated included family history, consanguinity, bleeding and clinical patterns of bleeding manifestations. Hematological profile included platelets count & function, prothrombin time(PT), partial thromboplastin time (PTT), factor VIII antigen (FVIII:Ag) and its activity (FVIII:Ac), factor IX antigen (F IX:Ag) and its activity (F IX:Ac), von Willebrand factor antigen (VWF:Ag) and its activity ((VWF:Ac) assayed with multimeric analysis of VWF. *Results.* The study revealed that 27.9%of studied children matching for criteria of VWD, 25.5% had hemophilia A, 7% had hemophilia B, 16.3% had platelet dysfunction and 23.3% had bleeding manifestations with undiagnosed cause. Multimeric analysis of selected ten VWD cases revealed that two cases had type I, three had type II, four had type III and one case had normal distribution of VWF multimer who suggested to have type IIM. One case from diagnosed type II VWD cases was found to have associated thrombocytopenia which suggested being type IIB VWD. Bruising and epistaxis were the main symptoms in all studied VWD cases while 83.3% of them suffer from bleeding after dental procedures. Consanguinity was documented in 66.7% of VWD, 63.6% of hemophilia A ,33.3% of hemophilia B and 42.9% of platelet dysfunction cases. Positive family history of bruising and bleeding was the most significant predictor for IBD: 83.3% of children with VWD, 72.7% with hemophilia A, 66.6% with hemophilia B and 42.9% with platelet dysfunction disorders the majority were diagnosed as Glanzmann thromboathenia, One had Bernard-Solliar syndrome and the other had platelet gray syndrome. *Conclusions.* VWD and platelet dysfunction disorders should be considered not uncommon causes of inherited bleeding disorders (IBD) in children in these centers. VWD multimeric analysis is useful in the diagnosis of VWD. It is emphasized that routine hematological screening should be mandatory in children with positive family history of bruising and bleeding as a predictor for IBD.

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PERIOPERATIVE MANAGEMENT OF HEMIARTHROPLASTY OF THE HIP WITH RECOMBINANT ACTIVATED FACTOR VII IN A PATIENT WITH ACQUIRED HAEMOPHILIA

V. Thachil, A. Punekar, C. Walker, J. Martlew, C.H. Toh

Royal Liverpool University Hospital, LIVERPOOL, UK

Background. Recombinant activated factor VII (rVIIa) has been used to manage surgery in patients with congenital haemophilia and inhibitors, but its use in elective orthopaedic surgery of patients with acquired haemophilia has not yet been reported. *Aims.* To use bolus infusions of rVIIa in a male with acquired haemophilia who underwent hemiarthroplasty. *Methods.* An 81-year-old man was diagnosed to have acquired haemophilia following a spontaneous right arm bleed. The activated partial thromboplastin time (APTT) was 76 seconds, factor VIII levels were less than 1% and the inhibitor titre was 80 Bethesda units. Investigations into the cause of inhibitor formation revealed the presence of an immunoglobulinG lambda paraprotein. He was treated with cyclophosphamide 100 mg and prednisolone 60 mg daily for inhibitor eradication. With this treatment regimen, the inhibitor levels became undetectable after seven weeks, but the factor VIII levels continued to be low (less than 10%). Nine weeks after the admission, he sustained an intracapsular fracture of the neck of femur. A hemiarthroplasty of the hip was carried out with rVIIa support. Loading dose of rVIIa (90 μg/ kg - 14.4 mg) was administered one hour before the surgery. Maintenance dose of rVIIa (8.4 mg) was given every two hours from postoperative period for the next 48 hours with tranexamic acid (TXA). The frequency of rVIIa was reduced to every 4 hours after day 2, 6 hours after day 4 and 8 hours after day 8 and stopped on day 11. The postoperative period was uneventful with no risk of thrombosis or excess bleeding. *Results.* The most frequent use of rFVIIa in acquired haemophilia is for the treatment of non-surgical bleeding episodes including spontaneous or trauma-associated bleeds. Surgical prophylaxis with rVIIa has been used in one series of 23 patients which included mainly non-orthopaedic operations.[1] Rodriguez-Merchan also reported a case of hemiarthroplasty with rVIIa prophylaxis in a patient with haemophilia A and inhibitors.[2] Surgical prophylaxis with rVIIA in these patients, as in our patient, was by using the standard treatment regimen for rVIIa - 90 μg/ kg of rFVIIa every 2 h for 24-48 h and an increasing dosing interval after the first 2 days. *Summary.* rVIIa is a safe and effective therapeutic measure in managing patients with acquired haemophilia who may require elective orthopaedic surgery.

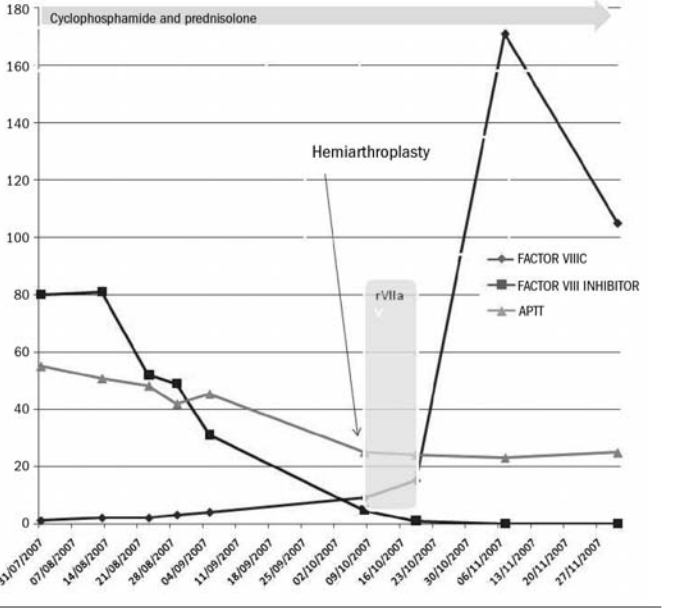

Figure 1.

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THE IMPORTANCE OF BEING HONEST. UPDATING THE INFANT DONOR'S CLINICAL FOLLOW-UP AT TIME OF PROCUREMENT CONTRIBUTES TO THE SAFETY OF UNRELATED CORD BLOOD GRAFT

P. Bergamaschi,[1] C. Parisi,[1] A. Marchesi,[1] M.P. Mercati,[2] G. Viarengo,[3] C. Del Fante,[3] C. Perotti,[1] L. Salvaneschi[1]

[1]Pavia CBB, Transfusion Medical Department, Fondazione IRCCS Policlinico San Matt, PAVIA; [2]Faculty of Medicine, University of Pavia, PAVIA; [3]Pavia CBB, Transfusion Med. Dept., Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy

Large employ of cord blood (CB) for unrelated stem cell transplantation is constantly encouraged by CB banks (CBBs) spreading worldwide. Safety of the graft represents a major goal. With reference to transmit-

table diseases (i.e. hereditary or infectious diseases), CB donors (CBDs) are examined at different times of CB management. First the suitability to donate is assessed at time of enrolment by medical interview of the CBD's mother and father and careful evaluation of all testing performed during pregnancy. Then maternal serum is tested for infectious disease risk at delivery and six months after. Finally depistage of disease transmission is completed by clinical evaluation of the newborn obtained at birth and updated at time of second withdrawal of maternal blood. Despite useful to avoid unsuitable units being entered in the CBB inventory, post-natal check is not mandatory according to FACT-Netcord international standards and may lack for many cryopreserved CBUs potentially available for transplantation. Anyway, the clinical control performed when the neonate is six months old does not definitively prevent potentially unsafe donations from becoming available during a donor's search. In fact CBUs are stored for 15 years and longer maintaining their viability and function. In the meanwhile the newborn grows up and new events unknown during neonatal age may become evident that compromise safety. This is the reason why in our CBB we honestly discourage from using for transplantation units missing the CBD follow-up ascertained at time of CB procurement. Here we review our CBB data aiming to demonstrate the ground of our policy. From 2003 to 2007 we managed 430 unrelated CBD's search requests. Up to then our CBB inventory consisted of 2000 CBUs, 30% of which lacking the neonate's evaluation at six months. One hundred requests reached the final step just missing the formal recruitment by the transplant center. 70 CBUs were finally issued for transplant, all provided with an updated and suitable statement of the baby's health. Mean reasons for release were patient's ineligibility to transplant and availability of alternative stem cell sources. Only in one case the mother refused the second withdrawal of blood; in two cases anti-HBc resulted positive. After review at time of CB procurement, the neonate's clinical follow-up was found pathological in 2 cases (G6PD deficiency and café au lait spots suggestive for von Recklinghausen's disease, respectively), the latter provided with a normal post-natal check at six months. The transplant physician was promptly informed and both CBUs were released. Despite infrequent and smouldering, pathological events may be present in the donor. Their detection contributes to identify the best suitable donor suggesting the importance of doing a honest piece of work at the CBB. In our opinion updating the neonate's clinical follow-up cannot be omitted and should be reasonably pointed out for further implementation of safety. All the more so because in the setting of unrelated CB transplantation even 2 mismatches are accepted, generally more than one unrelated CBD per patient is available and after all ethics imposes honesty as the best policy.

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LONG TERM EFFICACY AND SAFETY OF MIGLUSTAT THERAPY IN TYPE 1 GAUCHER DISEASE. ZAGAL STUDY

P. Giraldo,[1] P. Latre,[2] A. Acedo,[3] D. Alonso,[4] A. Barez,[5] A. Martin,[6] R. Franco,[7] A. Fernandez-Villamor,[8] M. Pocovi,[2] By Spanish Group

[1]Miguel Servet University Hospital, ZARAGOZA; [2]FEETEG, ZARAGOZA; [3]Vega Baja Hospital, ALICANTE; [4]Virgen del Rocío Hospital, SEVILLA; [5]Ntra Sra de Sonsoles Hospital, AVILA; [6]Virgen de la Concha Hospital, ZAMORA; [7]Punta Europa Hospital, CADIZ; [8]San Millan y San Pedro Hospital, LOGROÑO, Spain

Background. and objective: Gaucher disease (GD) is the most common lysosomal storage disease. Miglustat ZAVESCA®, is a synthetic iminosugar which acts as an inhibitor of the enzyme glucosylceramide synthase (SRT). This therapy offers an alternative approach for GD based on the indirect effect reducing the burden of glycolipids delivered to the macrophage system after phagocytosis of formed blood cells. We present the results after more than 36 months on an everyday clinical use of oral therapy in type 1 GD patients. *Design and Methods.* The Spanish group on GD design a structured project named with the acronymus ZAGAL. It includes a set of recommendations in a structured protocol for collecting safety, efficacy and QoL data at 6, 12, 24, 36 months and beyond. The project's aim is to guarantee the safe and proper use of Zavesca in an everyday clinical use. Baseline assessment: complete clinical, analytical and imaging evaluation, detailed neurological exam and superficial electroneurogram in sural and peroneal nerve. Cognitive test and memory impairment screen for dementia assessment were used. A free lactose and low carbohydrate diet (FLLCD) were recommended and applied in first weeks on therapy. A SF-36 survey was evaluated after 24 months on therapy. *Results.* 47 GD patients (females 56.1%). mean age 44.4.y (range: 21-74), SSI 6.8(range: 2-9), spleen removal 9.5%, chitotriosidase activity 3,756 nM/mL.h (range 468-10,553), CCL18/PARC 533 (range 102-1,219). Ten patients were naïve to SRT, mean age: 46.7y. Thirty seven patients were included on SRT once stabilising their disease with Imiglucerase during a mean of 3.8 y (range:2-11), dose 30-60 U/kg, mean age 39.2 y; mainly heterozygous for N370S. In February 2008 15 patients had completed 36 months on SRT, 26 patients 24 months and 10 naïve patients/37 switch 12 months. Response: all patients with anaemia improved haemoglobin concentration (mean 0.85 g/dL). Platelet count improved in patients with lowest values and it was maintained in patients with counts in normal limits. Chitotriosidase activity was maintained in switched patients and slightly decreased in naïve patients. The response was observed at 6 months on therapy and it is maintained after 24 months on therapy. In 7 naïve patients bone marrow MRI improvement was documented. No new symptoms were developed, three patient discontinued SRT due to poor compliance. Gastrointestinal disturbance appeared sporadically in five patients and became normal when they followed the FLLCD. The QOL analysis showed satisfactory results after two years on therapy. *Conclusions*. In our experience type 1 GD patients with mild or moderate disease had a satisfactory clinical, analytical and bone-marrow response to SRT with scarcely adverse events. At six months in naïve patients the response is similar to that observed in clinical trials and in patients treated with Imiglucerase and remained stable at 24 and 36 months, with a satisfactory QOL

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THE COMPARATIVE METHYLATION STATUS OF THE MDR-1 GENE IN PATIENTS WITH ACUTE LEUKEMIAS AND CHRONIC MYELOGENOUS LEUKEMIA

D.V. Marinitch,[1] D. Tsvirko,[1] N. Volkovets,[1] V. Smolnikova,[1] Y. Buryanov,[2] T. Shevtchuk[2]

[1]Byelorussian Hematology Center, MINSK, Belarus, Republic of Belarus; [2]Branch of Institute of Bioorganic Chemistry, Russian Biology Center, PUSTCHINO, Russian Federation

The development of leukemia involves the concurrent disruption of regulation of expression of multiple genes. Epigenetic changes in the promoter region of multidrug-resistance gene (MDR-1) are of such events. The comparative methylation status of the 5'-promoter region of this gene in patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia and chronic myeloid leukemia (CML) patients was investigated. Totally 97 nucleic acid samples (from 12 ALL, 28 AML and 23 CML patients) obtained from mononuclear cells of peripheral blood or bone marrow were involved in the experiment. The DNA and RNA samples of acute leukemia patients were obtained in different stages of the disease (first attack, complete remission and relapse). Twelve CML patients were in chronic phase of the disease, five - in accelerated phase and six - in blast crisis. As a control we used genomic DNA obtained from 24 normal volunteers. We applied methyl-dependent polymerase chain reaction (PCR) method, searching for the intensity of CpG-sites methylation of MDR-1 gene in the 5'-promoter area. The methylsensitive (HpaII) and methylinsensitive (MspI) restriction endonucleases were used for the digestion of the DNA-samples. The products of the PCR were scanned directly in agarose gel using appropriate software. The methylation status was compared with the expression of cell surface P-glycoprotein (Pgp) using immunophenotyping method and RNA-expression of the MDR-1 gene. Overexpression of the MDR1 gene has been known usually to be associated with poor clinical outcome in various hematological malignancies, including acute leukemias. We have not revealed a close correlation between the Pgp and RNA - expression levels and methylation status of the MDR-1 gene in patients with acute leukemias during the period of monitoring. In contrast the intensity of the MDR-1 gene methylation progressively decreased from stage to stage of CML-disease. Our preliminary data shows that the acquisition of multidrug-resistance phenotype is more typical for CML than acute leukemias.

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EFFECTS OF IRON DEFICIENCY ANEMIA ON GROWTH IN CHILDHOOD

M. Ferrara, L. Capozzi, R. Russo

Second University of Naples, NAPLES, Italy

Background. Iron deficiency (ID) is still a major health problem in the world. Impaired intake, blood loss, malabsorbption are the most important causes. Anemia is the most prominent manifestation, but in childhood there are other complications such as impaired growth. *Aims.* We have undertaken a retrospective review from 1999 of the relationship between iron deficiency anemia (IDA) and physical growth and effects

of iron therapy in children. *Methods.* In 65 children aged between 2 and 9 years, divided in 2 groups according to age (2-6 and 7-9 yrs), affected by IDA from various causes, a retrospective study from 1999 to 2007 has been performed to investigate relationships between IDA and physical growth and effects of iron therapy. Hb, MCV, ferritin levels, height calculated as SDS (HSDS) according to sex and age and adjusted for regression to the mean on difference between HSDS and mild parenteral SDS, BMI were evaluated. We defined IDA as Hb<12 g/dL, MCV<74 fl (2-6 yrs), <78 fl (7-9 yrs), ferritin level <15 ng/mL. Causes of IDA were investigated: in group I there was 18 cases of inadequate intake and poor compliance to therapy, 20 cases of cow's milk intolerance and 16 cases of Helicobacter pylori infection (HPI), 4 patients with blood loss due to Meckel's divericulum (MD), in group II 7 cases of inadequate intake, 9 of HPI, 2 of MD. All patients received oral iron supplements until Hb value ≥12 g/dL and ferritin level ≥20 ng/mL. *Results*. At study entry patients from group I, regardless of IDA etiology had Hb levels between 8.1 and 11 g/dL, MCV between 66.2 and 73.1 fl, ferritin values between 4.1 and 10.9 ng/mL, patients from group II showed Hb levels between 7.8 and 11.3 g/dL, MCV between 65.9 and 73.7 fl, ferritin values between 4.8 and 11.6 ng/mL. HSDS was in group I between -1.64 and -0.46, in group II between -1.15 and -0.65. BMI values were between 14.5 and 16.4 Kg/m^2 in group I and one year after recovery patients from group I showed Hb between 12.2 and 14.6 g/dL, MCV between 80.9 and 87.7 fl, HSDS between -0.74 and -0.10, BMI between 14.9 and 16.7 Kg/m^2, patients from group II HB between 12.1 and 14.9 g/dL, MCV between 81.7 and 87.7 fl, ferritin values between 21.4 and 27.1 ng/mL, HSDS values between -0.35 and 0.42, BMI between 14.9 and 16.7 kg/m^2. Statistical analysis by student t-test shows significant differences of haematologic and HSDS parameters at entry and after 1 year. Statistical analysis by ANOVA before and after therapy among patients from each group with IDA from various causes shows non significant differences. *Conclusions*. IDA, determining effects on appetite, metabolism, hormones and immunity, can influence physical growth, particularly heigth. ID, involving enzymatic activity, could reduce energy needed for optimal functions.

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HAEMOGLOBIN F LEVEL IN DIFFERENT HAEMOGLOBIN VARIANTS IN OSOGBO, NIGERIA

E.O. Akanni,[1] B. Oseni,[2] O. Adebisi,[2] E. Fakunle[3]

[1]Ladoke Akintola University of Technology, OSOGBO; [2]Biomedical Science Department, Ladoke Akintola University of Technology, OSOGBO; [3]Haematology & Blood Trans. Department, Ladoke Akintola University of, OSOGBO., Nigeria

Foetal haemoglobin (HbF) level in different haemoglobin variants in Osogbo was estimated in view of the derth of information on HbF concentration and distribution in various Haemoglobin variants in the study area. *Aims.* The aim of this study is to determine the HbF level of normal (AA), sickle cell traits (AS), sickle cell homozygous disease (SS), and mixed heterozygous (SC) and AC subjects; to evaluate the results of the two principal methods of estimating HbF level and sex distribution in the study area. This is also to provide information on under diagnosis of cases of thalassemia in this part of the world owing to limited facilities and resources. *Methods.* 260 samples were analysed comprising of HbSS, HbSC, HbAA, HbAS and HbAC subjects. The parameters determined in this study were HbF level, haemoglobin genotype and haematocrit. The Haemoglobin genotype was determined using the alkaline globin chain electrophoresis (International Committee for Standardization in Haematology (ICSH) 1988); HbF level was estimated using the acid elution method and alkali denaturation method of Kleihauer and Betke respectively as recommended by the ICSH while haematocrit level was determined using microhaematocrit method of ICSH (1980). *Results.* The mean+S.D of foetal haemoglobin level in respective haemoglobin variants studied is: HbSS, 2.09+1.94%; HbSC, 0.85+0.54%, HbAA, 0.69+0.46%; HbAS, 0.52+0.31% and HbAC, 0.57+0.26%. The mean HbF level across the Haemoglobin variants studied is statistically significant (p<0.05). Sex distribution of Hb F level in the population studied shows that 127 males had the mean+S.D HbF level of 1.09+1.36% while 133 females have the mean+S.D HbF level of 1.13+1.26%. This shows that female have a slightly higher mean HbF level than male counterparts. There is also a close association between the alkali denaturation method and acid elution method of estimating HbF as there was no significant difference between the results from the two methods (p>0.05). Conclusions: This study therefore shows that the mean HbF level in HbSS is highest compared with HbSC, HbAA, HbAS, HbAC and the two methods of estimating HbF are equally reliable.

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ANAEMIA IN CHRONIC RENAL DISEASE PATIENTS: EVALUATION OF INFLAMMATORY FACTORS, INCLUDING PRO-HEPCIDIN

Z.W. Grotto, A.F.M. Lima

State University of Campinas, UNICAMP, CAMPINAS, Brazil

Background. anaemia is a frequent and early complication of chronic kidney disease (CKD) that impairs patient's quality of life and has an important impact on the function of several organs. Insufficient production of erythropoietin (EPO) is the major cause of anaemia, but other factors may complicate the diagnosis and management of anaemia. Systemic inflammatory factors play roles in the regulation of erythropoiesis and intracellular iron homeostasis. *Aims.* the objective of this study was to evaluate some pro-inflammatory factors in a group of patients with CKD and the possible action on erythropoiesis and iron metabolism. *Methods.* a total of 114 adult patients with CKD without dialysis treatment were studied. Control group (CG): 21 healthy individuals. Fifty of 114 patients showed normal haemoglobin levels. Sixty-four patients were anaemic: 26 had iron deficiency (IDA) (transferrin saturation - TS - <20% and/or serum ferritin - SF - < 100 µg/L), 32 were classified as renal anaemia (RA) (normal serum iron and TS, and normal or elevated SF) and 6 had other causes of anaemia. C-reactive protein (CRP), interleukin 1β (IL-1 β), IL-6 and serum pro-hepcidin were determined by commercial kits. Kruskal-Wallis test and Spearman coefficient were used in the statistic analysis. *Results.* CKD group with anaemia x CG: PCR, IL-1β and IL-6 showed higher levels in CKD patients (p<0.0001). CKD group without anaemia x CG: PCR and IL-1β were higher in CKD patients (p<0.001), but IL-6 levels were similar. CKD with anaemia x CKD without anaemia: EPO and IL-6 levels were higher in patients with anaemia (p=0.0042 and p=0.0005, respectively). Reticulocyte haemoglobin content (RET-Y) was lower in anaemic patients (p=0.0053). There was no difference in IL-1β, CRP and pro-hepcidin levels. When patients with anaemia were divided in RA and IDA groups the results showed differences only in EPO (p=0.0163) and RET-Y (0.0014) parameters. There was no difference among groups concerning serum pro-hepcidin levels. Nineteen CKD patients without anaemia showed TS<20% and/or SF <100 µg/L, suggesting a functional iron deficiency. sTfR levels were higher in that group than in RA (p=0.0098) and CG (p=0.0023), indicating that even with the absence of anaemia those patients may have an inappropriate amount of iron available for erythropoiesis. *Conclusions.* as CKD is a chronic inflammatory state it was expected high levels of cytokines in serum. Moreover, our results showed that inflammatory factors contribute to anaemia in renal patients. Hepcidin is an iron regulatory hormone whose overexpression is induced by inflammatory factors. Data on hepcidin are relatively scanty and controversial in CKD studies, most of them conducted in patients undergone haemodialysis and receiving i.v. iron, a possible bias in hepcidin measurement. Although none of our patients were receiving those treatments pro-hepcidin levels were not different among CKD groups and even between CKD group and normal population group. The role of hepcidin as possible factor to explain the pathogenesis of disordered iron metabolism in CKD remains to be clarified.

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ERYTHROCYTAPHERESIS IN SICKLE CELL DISEASE: TOLERABILITY AND EFFICACY IN PAEDIATRIC PATIENTS

M. Ryan, H. Conway, R. Geoghegan, H. Eliwan, C. McMahon

Our Lady's Children's Hospital, DUBLIN 12 Ireland

Sickle cell disease is a monogenic haemoglobinopathy whose incidence is increasing in paediatric populations in Western Europe due to inward migrations from endemic areas. Complications seen in paediatric patients include painful crises, splenic sequestration syndromes and serious neurological sequellae resulting from hypoperfusion within the cerebral vasculature resulting in ischaemia. Current treatment options include Hb F modulating agents and chronic blood transfusion. However, long term transfusion may in itself be complicated by serious iron overload and resultant organ damage. Exchange transfusion is a treatment method which, while reducing Hb S levels, may be less likely to lead to iron overload due to a lower net volume of RBC transfused. *Methods.* We present a cohort of 7 paediatric patients who have been treated with exchange transfusion at our institution between October 2006 and the present. Age at commencement of exchange transfusion was 3.3 yrs -10.6yrs. Reasons for commencement of such a program included clinical neurological disease (4), radiological evidence of ischaemia (2) and abnormal TransCranial Doppler examination (1). 5

patients had previously undergone some form of disease modifying therapy, with either chronic transfusion (3), hydroxyurea (1) or both (1). In those on a transfusion program, volumes of blood transfused ranged from 1520 mls - 10,621 mls (mean 6601 mls, median 7131 mls). 4 had received iron chelation therapy. *Results.* The number of exchange transfusions performed ranged from 3 - 25 procedures. In total 63 procedures have been performed. Of these, 40 were successfully completed. Reasons for the non-completion of the remaining 23 included difficulties with vascular access (11), Clot formation within the extracorporeal circuit (5), patient non-cooperation (2), non-availability of staff (2), technical difficulties (2) and patient illness (1). Mean number of donor exposures per procedure was 2.47 (1.6 - 3.92). Ferritin levels at commencement of exchange transfusion ranged 99-2457 (mean 970.2, median 243) and at most recently for each patient 106-1787 (mean 709, median 477). At present, all 7 remain on an exchange transfusion program and none have had further neurological complications. Radiological findings have remained stable or improved in all. *Conclusions.* We conclude that exchange transfusion is an effective treatment modality in paediatric patients which appears to reduce the risk of iron overload in these patients while successfully controlling their disease. However, difficulties in obtaining adequate vascular access may prove problematic, especially in younger patients, and limits the effectiveness of therapy. Erythrocytapheresis is also associated with increased exposure to donated blood products.

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SPONTANEOUS HEMATOLOGICAL REMISSION IN CLL: LESSONS FROM THE ISRAELI CLL REGISTRY

L. Shvidel,[1] M Shtalrid,[1] A. Braester,[2] A. Klepfish,[3] A. Berrebi[1]

[1]Kaplan Medical Center, REHOVOT; [2]Naharia hospital, NAHARIA; [3]Wolfson hospital, HOLON, Israel

Spontaneous regression in CLL is characterized by documented decrease in tumor burden not related to specific treatments. Cases of CLL regression have been occasionally documented. In the data of the Medical Research Council CLL trials, Thomas et al reported a series of 10 patients who showed spontaneous remission out of 2370 (0.42%) registered cases (Br J Haematol, 2002, 116, 341). A possible association of this phenomenon in CLL with infection, smallpox vaccination or development of second malignancy has been previously suggested. The pathogenesis of spontaneous regression may be related to apoptotic pathways including secretion of cytokines or natural steroids or stimulation of cellular immune response by cytotoxic cells. We analyzed the incidence of hematological remission in a large group of patients included in the Registry of the Israeli Study Group on CLL. Out of 1013 patients registered with CLL, 13 patients (1.3%) experienced spontaneous remission during the course of their disease. There were 6 women and 7 men, with median age at diagnosis of 71 years (range, 50-87). All but one were diagnosed in clinical Binet stage A, the remainder had enlarged spleen compatible to stage Binet B. On flow cytometry, the median CD19/CD5 antigen coexpression on circulating CLL lymphocytes was 50%. Serum b2-microglobulin and LDH values were normal; in these early stages no additional prognostic parameters were evaluated. The median follow-up period was 12 years (3-23). The regression was noted after a median of 8 years (range 1-22) of the follow-up. The maximal lymphocyte count during the course of CLL was 15×10^9/L (9-75) and at regression dropped up to 2.3×10^9/L (1.2-4.7). The median CD19/CD5 antigen at regression coexpression was 28% (range, 17-37%). In order to search associated diseases or events that may explain changes in the hematological condition, we reviewed medical reports of the patients. Eight patients suffered from recurrent infections, which required multiple hospitalizations and antibiotic treatments. One another patient developed severe ischemic heart disease and was treated by verapamil. This drug by itself found to induce apoptosis in CLL (Berrebi *et al.,* Leukemia, 1994, 8, 2214). Finally, one patient showed hematological improvement while developed brain tumor. In three patients no concomitant diseases at time of regression were documented. In conclusion, spontaneous remission in CLL is a rare phenomenon developed usually in early stage. Concurrent disorders such as infections and/ or second malignancy may contribute to decrease CLL clone through uncertain apoptotic pathways.

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IMPACT OF BIOLOGICAL VARIABLES ON NEED OF TREATMENT AND PROGNOSIS: STUDY OF 347 PATIENTS IN A SINGLE INSTITUTION

M.J. Garcia-Rodriguez, R. De Paz, M.M. Morado, M.C. Canales, H.N. Hernandez Navarro

Hospital La Paz, MADRID, Spain

Background. Chronic Lymphocitic Leukaemia (CLL) has an extremely variable clinical course. Some patients have a rapid and fatal evolution, and die earlier after diagnosis, whereas others have an indolent disease, and live for 10-20 years without symptoms. Ray and Binet systems have been classically considered the standard clinical staging methods, but have a recognised limitation in indentifying those patients with initial stage (Rai 0-2, Binet A) in whom treatment is needed. *Aims.* The objective of this study was to evaluate the relative prognostic impact of biological variables, such as cytogenetic analyses, CD38 and ZAP70, and their relationship with clinical characteristics on need of treatment and prognosis. *Patients and Methods.* Our study included 347 patients with B-CLL, 167 men and 180 women, with a median age of 70 years (range 35-97 years). All patients were diagnosed in our center during a period of 20 years, on the basis of a clinical examination as well as morphological and immunological criteria and retrospectively analyzed. Chromosomal abnormalities were studied using FISH cytogenetic methodology in 79.3% of patients. Patients with normal kariotype or single abnormality of 13q-, were considered in the group of favourable prognosis. Patients with other abnormalities such as trisomy 12, 17p- and 11q-, were considered as high risk of progression. CD38 expression was analysed by flow cytometry in 90.2% of patients. A cut-off ≥30% was defined to distinguish positive and negative expression. Measuring of ZAP70 expression was studied in 64.8% and we considered a cut-off ≥20% positive. *Results.* Favourable risk group was assigned by FISH in the 78.9% of the patients, CD38 positive expression was detected in the 24.6% and ZAP-70 was positive in the 56%. Organomegaly (51.3%), rapid lymphocyte doubling time (32.5%), autoimmune phenomenon (11.1%) and B symptoms (5.1%) were the causes that motivated to start treatment. When we analyzed the biological characteristics of treated patients we observed that they expressed CD38, ZAP70 and unfavourable kariotype more frequently than total population studied. These differences were more significant than the relationship with Rai and Binet stages. The results are described in Table 1. *Conclusions.* It has been suggested that biological markers, specially lack of CD38 expression, measured at moment of diagnosis, might be used in our patients to predict need to treat and separate those cases of smouldering CLL that do not need to be observed so intensively in which the best option of treatment is *watch and wait.*

Table 1. Frequency of biological markers and need of treatm.

Biological characteristics	Total population (%) N=347	Need of treatment (%) N=347
Rai 0-2	97.9	32.3
Rai 3-4	2.1	100
Binet A	83.6	24.2
Binet B-C	16.4	84.1
FISH favourauble	78.9	37.3
FISH unfavourable	21.1	48.3
CD38 positive	24.6	55.8
CD38 negative	75.4	28.4
ZAP-70 positive	56	49.2
ZAP-70 negative	44	27.3

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THE ASSESSMENT OF ANTILEUCEMIC ACTIVITY OF THALIDOMIDE IN COMBINATION WITH FLUDARABINE IN PATIENTS WITH NEWLY DIAGNOSED AND REFRACTORY OR RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA

M. Kowal, M. Podhorecka, T. Gromek, K. Giannopoulos, A. Dmoszynska

Medical University of Lublin, LUBLIN, Poland

Background. Thalidomide (THAL) is an immunomodulatory agent with pleiotropic activities. It was reported that in group of CLL patients THAL enhanced proapoptotic activity of fludarabine (F) that improves

the clinical response. In the present study we report the findings of the efficacy of THAL+F combined therapy in newly diagnosed and refractory or relapsed CLL patients. *Methods.* Forty patients were enrolled into this study. The median age was 67 years (ranging from 43 to 75). Twenty patients (14 males, 6 females) were newly diagnosed and 20 of them (11 males, 9 females) were refractory or relapsed (12 and 8, respectively). The median number of prior lines of therapy was 3 (ranging from 1 to 6). THAL 100 mg was given orally for the first 7 days of cycle 1 and continued for 6 months, F 25 mg/m^2 intravenously or 40 mg/m^2 orally, was given for 5 days every 28 days for up to 6 cycles, starting on the seventh day of THAL administration. Acetylsalicylic acid was used for preventation of venous thromboembolism. *Results*. The duration of follow-up ranged from 2 to 26 months (median 14 months). Six patients were inevaluable (unable to complete 2 treatment cycles; either for toxicity or progression). Two pretreated patients attained stable disease after 2 cycles but therapy was stopped due to toxicity. Thirty two patients received treatment for at least 3 months and were therefore evaluable for clinical response acc to NCI-WG criteria. The results of the efficacy of THAL+F combined therapy in the group of newly diagnosed and refractory or relapsed CLL patients are presented in the Table 1. The progression free survival among analyzed patients ranged from 3 to 19 months (median 11 months) *Toxicity.* Constipation and fatigue were noted in nearly all patients. A tumor flare reaction developed in 25% of patients during the first week of treatment with THAL. Infections occured in 45% of patients, the severe ones were observed only in 4 pretreated patients. The main hematological toxicites were neutropenia (40%), thrombocytopenia (25%) and AIHA (20%). *Conclusions.* THAL in combination with F seems to be very efficient as a first line therapy. THAL$^+$ F therapy may also be used as a subsequent line in pretreated patients, with tolerable toxicity. These results warrant further investigation of larger group of patients .

Table 1. The efficacy of THAL+F combined therapy in newly diagnosed (A) and refractory or relapsed (B) CLL patients. The data are presented as the number of patients (CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease).

Clinical response	A	B
CR	5	1
PR	13	9
SD	-	4
PD	-	2

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CYTOTOXIC EFFECT OF MGCD-0103, A NEW GENERATION HISTONE DEACETYLASE INHIBITOR, ALONE OR IN COMBINATION WITH A BCL-2 INHIBITOR, IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

V. El Khoury,[1] E. Moussay,[1] N.H.C. Brons,[1] N. Aouali,[1] K. Van Moer,[1] B. Janji,[1] V. Palissot,[1] G. Berchem[2]

[1]CRP-Santé, LUXEMBOURG; [2]Centre Hospitalier de Luxembourg, LUXEMBOURG, Luxembourg

Background. HDAC inhibitors are a promising new approach to the treatment of cancer. MGCD-0103 (gracefully given by Pharmion/ MethylGene Inc.), an orally active molecule belonging to the benzamides class of HDAC inhibitors, is currently undergoing clinical trials for the treatment of solid malignancies as well as hematologic tumors. *Aims.* This study aimed at understanding the mechanism of action of MGCD-0103, alone or in combination with a Bcl-2 inhibitor, in the chronic lymphocytic leukemia (CLL). *Methods.* The antitumor activity of MGCD-0103 was demonstrated, *ex vivo*, in a CCK-8 assay. Apoptosis induction was investigated with annexin V/propidium iodide staining and immunoblots analysis. Caspase activity was evaluated by a fluorometric assay. *Results.* MGCD-0103 induced cell death of PBMC (peripheral blood mononuclear cells) from 13 CLL patients, with a marked difference in sensitivity among patients analyzed. The apoptotic effect of MGCD-0103 was characterized by the presence of Annexin V-positive cells. Although discrepancies were observed among patients, MGCD-0103 activated caspases 2, 3/7, 4, 6, 9 and 10, and to lesser extent caspases 5, 6 and 8. MGCD-0103 induced cleavage of pro-caspase 3 and PARP, as well as pro-caspases 4, 8, 9 and the Bcl-2 family protein Bax. Cleavage of Bax was concomitant with proteolysis of calpain. Apoptosis was enhanced when MGCD-0103 was associated to the Bcl-2 inhibitor, HA14-1. Depending on patients, caspase inhibition through the pan-caspase inhibitor ZVAD-FMK decreased or did not influence MGCD-0103-mediated apoptosis. Pre-treatment of PBMC with ZVAD-FMK reduced markedly MGCD-0103-induced pro-caspases 4/8 and PARP cleavage but not that of pro-caspase3 or Bax. *Conclusions.* These results suggest that MGCD-0103 induces apoptosis by a caspase-dependent and -independent pathway. Calpains, along with caspases, may be involved in MGCD-0103 cytotoxic effect. Inhibiting Bcl-2 protein seems a promising therapeutic strategy for combining with MGCD-0103.

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PRE-TRANSPLANT BONE STATUS EVALUATION IN PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION

I. Yakoub-Agha,[1] C. Wibaux,[2] L. Magro,[1] A. Juilliard,[2] I. Legroux-Gerot,[2] V. Coiteux,[1] L. Terriou,[1] J.P. Jouet,[1] B. Cortet[2]

[1]Maladies du Sang, LILLE; [2]Service de Rhumatologie, LILLE, France

Background. In view of the high observed frequency of bone events following allogeneic haematopoietic stem cell transplantation (allo-SCT), the aim of this prospective study was to evaluate pre-transplant bone status in allo-SCT patients. *Patients and Methods.* In the month before transplantation, bone-loss risk factors were documented for 27 patients. We measured the levels of bone remodelling markers (BRMs: plasma osteocalcin and bone & total alkaline phosphatases for bone formation; CTX and telopeptides for bone resorption), together with plasma creatinine, intact PTH, TSH, testosterone, LH, FSH, 25 OH vitamin D, serum calcium & phosphorus and calciuria. In addition, bone mineral density (BMD) at the lumbar spine, femoral neck and hips were measured using double-energy X-ray absorptiometry (DEXA). Spine X-ray was also made. Bisphosphonate, oral calcium and vitamin D or testosterone were administered according to the results of the evaluation. *Results.* Between June 2006 and March 2007, 13 males and 14 females underwent allo-CST for haematological malignancies. The median age at transplantation was 44 years (range: 22-60). Eighteen had received prior corticosteroid therapy, 10 were smokers and 2 had a history of alcohol abuse. All had received prior chemotherapy, including 2 patients having already undergone autologous SCT. Ten of the 14 women were postmenopausal but none was on hormone replacement therapy. The median BMI was 24 kg/m^2 (range: 19-35). The daily calcium intake was low, with a median value of 950 mg/day (range: 467-1852). While serum calcium, phosphorus and creatinine levels were within the corresponding normal ranges for all patients, 15 individuals displayed vitamin D deficiency and 6 had calciuria <100 mg/day. Two patients suffered from hyperparathyroidism. In terms of BRMs, the patients respectively displayed a normal profile (n=9), high bone resorption activity (n=12) or high bone formation/ resorption activity (n=5). One patient had hyperthyroidism and another presented hypotestosteronaemia. Bone density results were normal in 16 patients and abnormal in 11 (41%), including 8 with osteopaenia and 3 with osteoporosis. Vertebral fractures were observed in 4 patients. Overall, 18 patients (67%) were considered as having a pathological bone status and required treatment with bisphosphonate alone (n=5), vitamin D supplementation alone (n=11) or both (n=2). *Conclusions.* This study revealed that a large proportion of allo-CST patients have pre-existing abnormal bone status and thus demonstrates the importance of pre-transplant bone status evaluation in allo-CST candidates. The implementation of appropriate bone-related treatments may reduce the frequency of post-transplant bone events. This study is now being extended to include a post-transplant bone evaluation.

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EVALUATION OF PEGFILGRASTIM EFFICACY IN PERIPHERAL BLOOD STEM CELL MOBILIZATION OF PATIENTS WITH LYMPHOMA

F. Bijou,[1] N. Milpied,[2] M. Dilhuydy,[2] J.M. Boiron,[1] R. Bouzgarou,[1] M. Sauvezie,[2] R. Tabrizi,[2] B. Dazey,[1] T. Leguay,[2] C. Foucaud,[2] G. Marit,[2] S. Vigouroux,[2] D. Fizet,[1] K. Bouabdallah[2]

[1]Etablissement Français du Sang, BORDEAUX; [2]CHU de Bordeaux, Service Maladies du Sang, PESSAC, France

Twenty four patients (pts) with planned autologous stem cell transplantation for lymphoma diseases (Hodgkin's disease=4; non-Hodgkin's lymphoma=20) received chemotherapy (CT) (Induction CT=3 and salvage regimen= 21) followed by a fixed single dose (6 mg) administration

of Pegfilgrastim (PF) after the last day of CT for peripheral blood stem cell collection (PBSC)(target cell dose of > 2×10^6 $CD34^+$/kg). Median age was 53 yrs (24-68) and median weight was 72, 5 kg (45-98). Among the 24 pts, 7 received more than 2 lines of CT regimens. The injection of PF was well tolerated. Median time interval between day 1(D1) of the cycle of CT mobilization and first leukapheresis session was 14 days (10-18) while the median time interval between injection of PF and first leukapheresis session was 9 days (6-13). Stem cell collection was started when the absolute number of circulating $CD34^+$ cells was >$\times10^6$/L and performed with standard volume leukapheresis. Median $CD34^+$ cells level at D1 of leukapheresis was 35,5/mm^3 (11-320) and interestingly, more than 35% of pts could reach this median level of $CD34^+$ early after PF injection (D6). Notably, 22 pts reached the target cell dose in 2 sessions of leukapheresis or less (10 pts after 1 session, 10 other pts after 2 sessions, 2 pts after 3 and 4 sessions respectively). The median number of leukapheresis sessions was 2(1-4) and the median $CD34^+$ cells harvested was 4×10^6/kg (0,8-26,6). Two pts (DLBCL=1 and FL=1) could not reach the level of $CD34^+$ required to start leukapheresis, and both became secondary refractory to CT. In univariate analysis, PBSC collection of > 4×10^6/kg was highly correlated with pts who started their collection at D9 of PF administration (p=0,01) and with those presenting a $CD34^+$ cells level > 35,5/mm^3 at D1 of leukapheresis (p=0,033). White blood cells level higher than 9 G/L was also predictive of circulating $CD34^+$ cells >35,5/mm^3 (p=0,033). These data suggest that PF may represent an attractive option for PBSC mobilization particularly in pts with lymphoma when optimal compliance of frequent sequential regimens of CT is required. We also emphasize that stem cell mobilization is effective even in pts in second or subsequent salvage CT. Importantly, the circulating $CD34^+$ count should be performed from D6 of PF administration.

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PRIOR TREATMENT WITH NOVEL TYROSINE KINASE INHIBITORS ALLOWS STEM-CELL TRANSPLANTATION (SCT) IN A LESS ADVANCED DISEASE PHASE AND DOES NOT INCREASE SCT TOXICITY IN PATIENTS WITH CML AND PH+ ACUTE LYMPHOBLASTIC LEUKEMIA

A. Shimoni,[1] M. Leiba,[1] M. Schleuning,[2] G. Martineau,[3] M. Renaud,[3] M. Koren-Michowitz,[1] E. Ribakovski,[1] P. Le Coutre,[4] F. Guilhot,[3] A. Nagler[1]

[1]Chaim Sheba Medical Center, TEL-HASHOMER, Israel; [2]Stiftung Deutsche Klinik für Diagnostik, WIESBADEN, Germany; [3]CHU La Miletrie, POITIERS, France; [4]Charite-Universitatsmedizin, BERLIN, Germany

Background. Dasatinib and nilotinib are novel tyrosine-kinase inhibitors (nTKI) with activity in imatinib resistant/ intolerant CML in all disease phases and in Philadelphia positive acute lymphoblastic leukemia (Ph+ALL). Many of these patients will ultimately require allogeneic stem-cell transplantation (SCT). *Aims.* Previous studies have confirmed the safety of imatinib given prior to SCT. This analysis was designed to determine whether prior nTKI therapy adversely affects SCT outcomes. *Methods.* We retrospectively studied SCT outcomes of 21 patients, median age 45 years (range, 16-59 years) with CML [n=19; first chronic phase (CP1)-5, accelerated phase/ CP2-6, blastic crisis-8) or Ph+ALL (n=2, both in relapse) who were treated with nTKI (dasatinib-13, nilotinib-5, both-3) and subsequently had allogeneic SCT. All have previously failed imatinib therapy. *Results.* Following nTKI treatment 9 of the 16 patients with advanced disease achieved a hematologic response (56%) which was also a major cytogenetic response in 4 (25%). Seven patients (44%) maintained their response until SCT. Three of the 5 patients in CP1 achieved a major cytogenetic response. Subsequently, all patients had allogeneic SCT from HLA matched sibling (n=7), matched unrelated (n=13) or haplo-identical donors (n=1). The conditioning regimen was myeloablative (n=14) or reduced intensity (n=7). All patients engrafted in a median of 18 days (range, 11-42) and all achieved full donor chimerism. Transplant-related toxicities were relatively infrequent and there was no death related to organ toxicity. Acute and chronic GVHD occurred in 43% and 55%, respectively. Only one patient had grade III-IV acute GVHD and there was only one death due to chronic GVHD. There was no apparent increase in the incidence of infections. In all, the cumulative incidence of non-relapse mortality (NRM) was 7%, a relatively low incidence considering the high proportion of patients with advanced disease and alternative donors. With a median follow-up of 14 months (range, 1-31 months), 15 patients are alive, 12 disease-free; five died of disease progression, and one of NRM. The estimated 2-year overall and disease-free survival (DFS) were 64% (95CI, 41-87) and 46% (95CI, 22-71), respectively. The most significant prognostic factor was a hematologic response to nTKI or chemotherapy. The estimated 2-year DFS was 56% in patients having any response compared with 0% in patients transplanted in active blastic crisis (p=0.01). The 5 patients with CML in CP1 are all alive and currently disease-free (one after a second allograft). *Conclusions.* Prior nTKI treatment allows SCT in a less advanced disease phase in a large subset of patients and does not increase the incidence of treatment related organ toxicity, engraftment failure or GVHD. nTKI treatment is a safe and effective salvage therapy for patients failing imatinib and prior to SCT. Larger-scale studies are indicated to confirm these preliminary observations.

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COST-EFFECTIVENESS OF EARLY VERSUS LATE FILGRASTIM ADMINISTRATION IN AUTOLOGOUS STEM-CELL TRANSPLANTATION (ASCT)

M.J. Rodriguez Salazar,[1] M.T. Hernandez-Garcia,[2] B.J. Gonzalez-Gonzalez,[2] J.M. Raya,[2] R.F. Rodriguez-Sanchez,[2] G. Gonzalez-Brito,[2] T. Martin,[2] L. Morabito,[2] L. Hernández-Nieto[2]

[1]HOSPITAL UNIVERSITARIO DE CANARIAS, LA LAGUNA (TENERIFE); [2]Hospital Universitario de Canarias, LA LAGUNA (TENERIFE), Spain

Background. In patients undergoing high-dose chemotherapy followed by ASCT, the administration of filgrastim (granulocyte colony-stimulating factor, GCS-F) accelerates the neutrophil engraftment in the post-transplant period. Early engraftment is defined as the time to reach an absolute neutrophil count, ANC, over 0.5×10^9/L and a platelet count > 20×10^9/L. *Aims.* 1) To evaluate the early engraftment in patients undergoing ASCT in relation to the day in which filgrastim administration was started, both in patients receiving immunoselected peripheral blood stem cells (PBSC) and those receiving non-immunoselected product. 2) To analyze whether early administration of filgrastim after transplantation is a cost-effective strategy. *Methods.* We have retrospectively studied 190 ASCT procedures performed in our hospital (102 males and 88 females). Patient´s distribution considering diagnosis was as follows: non-Hodgkin lymphoma (101), multiple myeloma (47), Hodgkin's disease (16), breast cancer (8), acute leukaemia (6), sarcoma (5), chronic lymphocytic leukaemia (4), Waldeström's disease (2), and Poems syndrome (1). All the patients received post-transplant filgrastim 5 µg/kg/day. We differentiate two groups of patients according to the day in which the administration of filgrastim was initiated: Group A, before the day +6 after ASCT (≤ day +5) and Group B at day +6 or later (≥ day +6). Both groups were equivalent in terms of the total amount of infused $CD34^+$ cells: 4.35×10^6/Kg *vs* 3.69×10^6/Kg, respectively (p=0.08). *Results.* We do not find significant differences between the two groups in the time (days) to reach a neutrophil count >0.5×10^9/L (11.05 *vs* 11.23; p=0.225) or a platelet count >20×10^9/L (12.96 *vs* 12.61; p=0.729). However, the number of days in which filgrastim was administered until getting early engraftment was significantly different (8.54 *vs* 7.21; p=0.01). There were not differences in the global days of hospitalization (20.88 vs. 20.49; p=0.20). When we compared the groups of patients according to the nature of the infused product (immunoselected PBSC vs. non-immunoselected PBSC) we found the following *Results.* 1) Selected PBSC: Days to obtain neutrophil engraftment 11,18 (Group A) *vs* 11,05 (Group B) (p=0.31); days to obtain platelet engraftment 12,84 vs.13,09 (p=0.817); days of filgrastim administration 8,86 vs. 6,92 (p=0.002). 2) Non-selected PBSC: Days to obtain neutrophil engraftment 10,93 *vs* 11,42 (p=0.75); days to obtain platelet engraftment 12,64 *vs* 12.84 (p=0.532); days of filgrastim administration 8.26 *vs* 7.24 (p=0.001). *Conclusions.* In our experience, early filgrastim administration in the post-ASCT period (before day +6) does not accelerate the hematopoietic engraftment and also does not short the time of hospitalization, only increasing the number of days in which the drug is administered. We conclude that the early administration of filgrastim in the post-transplant period is not cost-effective.

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GRAFT VERSUS HOST DISEASE INCIDENCE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION ACCORDING TO CONDITIONING REGIMEN AND AGE IN 635 PATIENTS WITH HAEMATOLOGICAL DISORDERS (MALIGNANT AND NO MALIGNANT DISEASES)

M. Benakli, R. Ahmed Nacer, F. Mehdid, R. Belhadj, A. Talbi, M. Baazizi, N. Rahmoune, R.M. Hamladji

Pierre Marie Curie Center, ALGIERS, Algeria

Introduction : graft *vs* host disease (GVHD) is major reason of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT).We report GVHD incidence according to age of patients (pts) and to type of conditioning regimen (myeloablative and reduced intensity conditioning regimen or RIC) in 635 pts with haematological disorders

who underwent allogeneic HSCT. *Patients and methods.* during an 104 months period (from April 1998 to December 2006) 683 pts underwent an allogeneic HSCT. Only 635 pts are appraisable for the study (myeloablative : 458 pts, RIC: 177 pts). Five hundred and fourty two pts are under 40 years old and 93 pts up to 40 years old. Hematological disorders interest various diseases: leukaemia (453), severe aplastic anemia (111), others (71). In the myeloablative conditioning regimen group: median age 28 years (range 4 to 57), sex ratio 1,4 , conditioning regimen with chemotherapy alone, GVHD prophylaxis with ciclosporine and methotrexate (Seattle), for allograft 415 pts received peripheral blood stem cell and 40 pts bone marrow and 3 umbilical blood cord. In the group of RIC regimen: median age 37,5 years (range 18 to 59), sex ratio 0,96, all pts received chemotherapy alone with Fludarabine based conditioning regimen , GVHD prophylaxis with ciclosporine and mycophenolate mofetil (J1 to J40) and all pts grafted with peripheral blood stem cell. e statistic test is used to compare the groups. *Results.* acute GVHD and chronic GVHD are higher in group of RIC regimen with respectively 47,4% *vs* 37,7% (p=0,01) and 67,9% *vs* 47,6% (p=10-8). Inversely no significant difference is observed for aGVHD and cGVHD between the two groups according to age (up to 40 years old and under 40 years old) respectively 43% *vs* 40% (p=0,57) and 49,3% *vs* 31,3% (p=0,74). *Conclusions.* In this study GVHD incidence seems to be related with type of conditioning regimen more than to age of pts.

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FOXP3 EXPRESSION AND ITS CORRELATION WITH GVHD IN ALLO-SCT

B.S. Bellesi, P. Chiusolo, M. Palladino, D.G. De Ritiis, S. Marietti, L. Laurenti, F. Sorà, S. Sica, G. Leone

Hematology Department, Università Cattolica S. Cuore, ROME, Italy

Background. Several studies have demonstrated that $CD4^{+}CD25^{high}$ Treg cells suppress GVHD in animal models. FoxP3 gene encodes a trascription factor that is a key regulator for thymic development and function of naïve Tregs. In humans some authors reported the association between reduced Foxp3 expression and development of GVHD and a low risk of GVHD in patients receiving a graft with high donor FoxP3-positive Tregs. *Aims.* We analized FoxP3 expression and its correlation with GVHD in 6 pts (M/F 4/2, aged 21-55 years) undergoing alloSCT. Pts' characteristics were: 3AML, 1 CML and 1 ALL submitted to a myeloablative PBSCT from siblings in 4 cases and MUD in 1 case. 1 pt affected by MF/SS received RIC-PBSCT from sibling. *Methods.*PB samples were obtained from donors before mobilization and from pts at days +30, +60, +90, +120, +210 after SCT. *Results.* 2 pts developed severe aGVHD and were lost to F-U. 1 pt received DLI from day +90 to day +120 for relapse and developed cGVHD. The pt with non-myeloablative PBSCT developed cGVHD from day +110 until several months post SCT. 5 donors expressed FoxP3 and only 1 pt developed severe aGVHD. Only 1 donor did not express FoxP3 and the pt developed severe aGVHD. Two pts with no GVHD expressed FoxP3 during F-U. The pt who received DLI presented a reduced gene expression at day +90 and +120, with associated cGVHD; at +210 the same pt did not presente cGVHD in association with major expression of FoxP3. The pt undergoing RIC-PBSCT developed cGVHD from day +110 post SCT and presented reduced gene expression at days +90, +120, +210. 1 pt with resistent aGVHD presented reduced FoxP3 expression at days +30, +60,+90 and no expression of FoxP3 in the donor. The other pt with aGVHD did not show FoxP3 at day +30, but he expressed FoxP3 at day +60, after 6 ECP with complete resolution of cutaneous manifestations. In summary 4 pts who did not develop aGVHD expressed FoxP3 at days +30. Two of these pts developed cGVHD in association with reduced FoxP3 expression. Both pts with severe aGVHD presented reduced expression and no expression of FoxP3 at day +30. In conclusion FoxP3 expression decreased in pts with GVHD and its early expression at day +30 in the recipient and in the donor may be associated with low risk of GVHD following alloSCT. *Conclusions*. According to other recent studies FoxP3 expression increased post six ECP in the pt receiving this treatment for aGVHD. The induction of Treg cells may be the mechanism required for a tolerogenic shift of immune system post ECP.

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PERITRANSPLANT SITAGLIPTIN MODIFIES SERUM SDF-1ALPHA LEVELS IN MULTIPLE MYELOMA PATIENTS UNDERGOING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

F. Focosi, L. Mattii, M.R. Metelli, M. Maggi, S. Galimberti, E. Benedetti, F. Papineschi, M. Petrini

University of Pisa, PISA, Italy

Background. Hematopoietic stem cell (HSC) engraftment into recipient bone marrow after intravenous infusion is mainly driven by gradients of the chemokine CXCL12 (also known as stromal cell-derived factor 1). Alternative splicing generates 2 isoforms (alpha and beta) differing by 4 amino acid residues in the carboxyterminus. No drug currently exists to modulate this gradient. CXCL12 is inactivated when the 2 amino-terminus amino acids are cleaved by dipeptidyl peptidase IV (DPP-IV), which also degrades glucagon-like peptide 1. Recently, DPP-IV inhibitors (gliptins) have been clinically approved as oral antidiabetes agents. *Aims.* We tested whether sitagliptin was safe and effective at modifying serum SDF-1alpha levels in a homogeneous cohort of multiple myeloma patients receiving high-dose melphalan and HSC transplantation, and tested whether the drug could improve engraftment and reduce transfusion requirements. *Methods.* Eight consecutive patients (6 males and 2 females; median age 59 years) with multiple myeloma receiving high-dose melphalan (range: 100 to 200 mg/m^2 of body surface area) on day -2 and HSC transplantation (mean dose : 4.1 millions CD34+ HSCs per kg of body weight) on day 0 were treated with oral sitagliptin (Januvia®, Merck & Co., Inc) 100 mg b.i.d. since day -1 until day +2. All patients also received pegfilgrastim on day +1, platelet transfusions when lower than 20000/microliter (median : 1 concentrate) and packed red blood cell units when hemoglobin was lower than 8 g/deciliter (median : 1 concentrate). Peripheral blood samples were collected on day -2, 0, +3, +6 and +10. Serum SDF-1alpha levels were measured with the Human SDF-1alpha Quantikine Colorimetric Sandwich ELISA (R&D System, Minneapolis, MN, USA). *Results.* The drug was well tolerated without any significant adverse effect. In comparison with a historical cohort of first and seconds autologous HSC transplantation (n=31), patients receiving sitagliptin exhibited significatively lower serum SDF-1alpha levels during the days of treatment, returning to baseline levels on day +10 (as depicted in Figure 1). *Discussion.* The small sample size allowed to show only noninferiority on engraftment kinetics and transfusion requirements. Although laboratory tests are still pending to explain why serum SDF-1alpha levels dropped instead or rising (as expected), we are currently investigating changes in SDF-1beta levels. We demonstrated for the first time that DPP-IV inhibitors have the potential to modify serum SDF-1alpha levels. Apart from autologous HSC transplantation these drugs could potentially be added to the armamentarium against allogeneic graft failure. Similarly, they could prove useful in disturbances affecting the cognate receptor CXCR4, such as HIV infection or the WHIM syndrome.

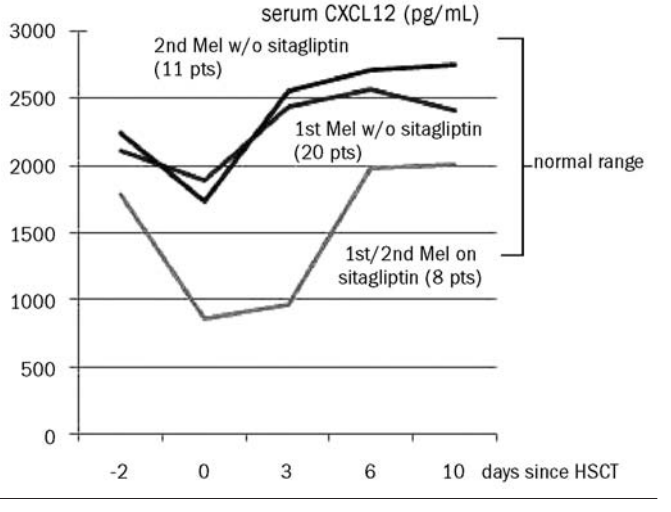

Figure 1.

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EXTRACORPOREAL PHOTOPHERESIS AS FIRST LINE TREATMENT IN HIGH RISK GRAFT-VERSUS-HOST DISEASE

A. Tendas,[1] L. Cupelli,[1] T. Dentamaro,[2] A. Picardi,[3] L. Cudillo,[3] M. Mirabile,[3] L. Scaramucci,[2] P. Niscola,[2] D. Piccioni,[2] A. Spagnoli,[1] A. Bruno,[1] M. Giovannini,[1] G. Adorno,[4] A. Lanti,[4] A. Perrotti,[2] P. de Fabritiis[1]

[1]Sant'Eugenio Hospital, Tor Vergata University, ROME; [2]Hematology, Sant'Eugenio Hospital, ASL Roma C, ROME; [3]Hematology, Policlinico di Tor Vergata, Tor Vergata University, ROME; [4]SIMT, Policlinico di Tor Vergata, Tor Vergata University, ROME, Italy

Background. Chronic graft *vs* host disease (cGVHD) is the major late complication after allogeneic stem cell transplantation. Standard therapy is steroid and Cyclosporine-A (CyA); however, immune suppression (ISS) related infections or unresponsiveness to ISS, are major mortality causes. Extracorporeal photopheresis (ECP) has shown activity in treatment of cGVHD, but its use has been limited to first-line-unresponsive cGVHD. *Aim of the study.* This is a single center pilot study testing feasibility of a programme of photopheresis in association with standard therapy as first line treatment in high risk cGVHD. High risk was defined as the presence of parameters predicting high cGVHD-related mortality. Secondary objectives were response and complications incidence. *Patients.* Among 9 pts fitting enrolling criteria, 2 refused due to logistic problem or low compliance with the procedure, 7 were enrolled. Median age was 40. Donor was HLA identical sibling in 6 cases and MUD in 1. All cases presented with extensive/moderate-severe cGVHD; Akpek score was >0 in 3/7 pts. Treatment plan. Pts started with Prednison (PDN) 1 mg/kg and CyA at cGVHD diagnosis; ECP was started with a frequency of 4 application/month in the first 3 months and 2/month for the subsequent 9 months; PDN and CyA were slowly reduced until suspension, or otherwise modulated. Study duration was 1 year. Pts were ruled out the study in case of ECP suspension; requirement of other ISS drugs in case of GVHD progression unresponsive to standard therapy, or severe infections. Response was evaluated with standard criteria, as progression, partial response (PR), very good PR (vgPR) or complete response (CR). *Results.* Adherence to protocol was: 5/7 pts at 3 months, 4/7 at 6 and 9 months, 3/7 at 12 mm; exit from the study was due to infectious complications (2), ECP suspension due to venous access related thrombosis (1) and clear cGVHD progression (1). In evaluable pts, response (CR+very good PR /≤PR) per trimester was 4/5, 2/4 and 3/4 at I, II and III respectively; at the IV trimester, 1 very good PR, 2 PR and 1 progression were observed. Complications were evaluated in 4 pts and are reported on the table. At 1 year, 2/7 pts died (1 TRM, 1 relapse). *Conclusions.* ECP in association with standard therapy is feasible; complications incidence seems to be similar to those observed in patients not treated with ECP; a larger group of patients is needed to evaluate response in this setting.

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SALVAGE TREATMENT WITH INFLIXIMAB OF RESISTANT GRAFT-VERSUS HOST DISEASE IN ALLOGENEIC TRANSPLANTED PATIENTS

C. Ferra,[1] L. Lopez,[2] B. Xicoy,[2] J.M. Sancho,[2] M. Batlle,[2] E. Sancho,[2] E. Feliu,[2] J.M. Ribera[2]

[1]H. Germans Trias i Pujol, Institut Català d'Oncologia, BADALONA; [2]H. Germans Trias i Pujol, Institut Català d'Oncologia, BADALONA, Spain

Background. Severe acute GVHD and extensive chronic GVHD not responding to steroids, in special the late acute GVHD, have special poor prognosis. Among drugs useful for treatment of steroid-resistant GVHD, infliximab (by its inhibitory effect on TNF-a) has reported to be effective, especially in cases with gastro-intestinal (GI) GVHD. *Aim of the study.* To evaluate the efficacy of infliximab for the treatment of patients with steroid resistant GVHD. *Patients and Methods.* We performed a retrospective analysis in a single centre to evaluate the activity of infliximab in 7 patients with acute or chronic steroid resistant GVHD. *Results.* One-hundred and ten allogeneic stem cell transplants (SCT) were performed in our institution from 2000 to 2007. 58 patients (53%) developed acute GVHD and 41 patients presented chronic GVHD (37% of valuable patients for chronic GVHD). Seven patients received at some time infliximab as salvage treatment for steroid resistant GVHD. Their median age was 28 years (15-60), 4 M/3F. The source of progenitors was mobilized peripheral blood in all patients but in one (unrelated cord blood transplant). Four were matched related SCT and 3 unrelated SCT (the cord blood transplant was not fully matched). The diagnoses were acute leukemia (4), Hodgkin's lymphoma (1), peripheral T-cell NHL (1), and CML in accelerated phase (1). All patients received GVHD prophylaxis wit cyclosporine and methotrexate except in the cord blood SCT (ATG, prednisone and cyclosporine). Table 1. Infectious events were common. All the patients developed CMV infection and two of them CMV disease. Six presented a gram-negative blood stream infection, two of them associated with a gram positive bacteremia and four developed a proved or probable fungal infection. *Conclusions.* Infliximab provides transient response in the treatment of steroid resistant GVHD. However, is associated with a high rate of infections especially when the patients have previously received other immunological therapy. Earlier administration of infliximab should be explored in steroid-refractory GVHD to try to reduce treatment-associated infections.

Supported in part with grants PEF-07 from the Jose Carreras Leukaemia Foundation and RD 06/0020/1014 from RETICS.

Table 1.

	1	2	3	4	5	6	7
GVHD	Acute	Chronic quiescent	Acute	Chronic quiescent	Chronic progresive	Acute	Acute
Severity	Grade IV (II inicial)	Extensive	Grade IV (III inicial)	Extensive	Extensive	Grade IV	Grade IV
Date of onset	+15	+136	+9	+158	+100	+26	+45
Involved organ	Skin, entire GI	Skin, entire GI, lung	Skin, lower GI	Skin, entire GI, liver	Skin, entire GI, lung liver	Skin, entire GI, liver	Skin, lower GI
Previous lines of treatment	2	3	4	3	5	2	2
Previous immunological therapy	No	No	Thymoglobulin	Thymoglobulin	Thymoglobulin	Thymoglobulin	Alemtuzumab
Infiximab 100mg /Kg/d: onset day and total doses	+97 5	+263 3	+46 7	+615 6	+469 3	+42 6	+60 3
Outcome (CR/PR/NR)	CR	NR	PR	PR	Not evaluable	PR	PR
Follow-up (days)	558	361	92	793	494	93	+100
Cause of death	Relapse	GVHD Infection	GVHD Hemorrhage cistitis	GVHD Pneumonia	GVHD Fungal infection	GVHD Fungal infection	-

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HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH HLA-IDENTICAL SIBLINGS IN PATIENTS WITH ACUTE LEUKEMIA - EXPERIENCE FROM AN UNIVERSITY HOSPITAL IN BRAZIL

R.M. Lamego, A.L.B. Costa, M.J.M. Oliveira, N.C.D. Clementino, H. Bittencourt

HC, UFMG, BELO HORIZONTE, Brazil

Background. Acute leukemias are a heterogeneous diseases group with high morbimortality. Allogeneic bone marrow transplantation is the most efficacious therapeutic option for its treatment. The paucity of papers in the Brazilian literature about allogeneic bone marrow transplantation for acute leukemias justifies the studies concerned to know the characteristics and the outcome of these patients. *Aims.* Appraising the results of hematopoietic stem-cell transplantation in transplanted patients with acute leukemia in a reference University Hospital in southwest Brazil and compare them with the available literature data. *Methods.* A retrospective cohort study was performed with all transplanted patients with acute leukemia - myeloid, lymphocytic and biphenotypic - who received an non-manipulated allograft of bone marrow or peripheral blood from a matched sibling in the Stem Cell Transplantation Unit at Hospital das Clinicas - UFMG from July 1995 to December 2005. *Results*. The median age of the 125 included patients was 28.7 years. Eighty-one patients presented acute myeloid leukemia; 38 with acute lymphocytic leukemia; and six patients with biphenotypic leukemia. Thirty-two patients were in first complete remission, while 23 were in second remission and the rest was transplanted in an advanced disease stage (refractory, relapsed, or beyond second remission). The cumulative incidence of neutrophil recovery (D+60), platelet recovery (D+100) and acute graft-*vs*-host disease (D+100) were respectively 90.4%, 74.2% and 47.2%. Twenty-nine patients presented chronic graft-versus-host disease. The overall survival and event free-survival estimates at 10 years were 22.9% and 22.1%, respectively. Regarding patient clinical situation at transplantation, overall survival was 56.3% for patients in first remission, 38% for those in second remission and 3.7% for patients with advanced disease. *Conclusions.* This study shows a worse outcome than reported in literature. It could be due to a higher proportion of transplanted patients with advanced disease. Nevertheless, evolution was similar to other studies taking into consideration patients transplanted in first or second remission.

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KINETIC OF HEMATOLOGICAL TOXICITIES IN PATIENTS (PTS) TREATED WITH RADIOIMMUNOTHERAPY (RIT) AND AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR NON-HODGKIN'S LYMPHOMA (NHL)

V. Pavone,[1] A.R. Messa,[1] C.D.C. Del Casale,[1] A. Rana,[1] A. Mele,[1] G. Greco,[1] R. De Francesco,[1] S. Sibilla,[1] V. Frusciante,[2] V. Frusciante,[2] A. Varraso,[2] F. Dicembrino,[2] P. Tabacco,[2] M. Caputo,[3] A. Ostuni[3]

[1]Panico Hospital, TRICASE (LE); [2]Department of Nuclear Medicine-Casa Sollievo della Sofferenza, SAN GIOVANNI ROTONDO; [3]Department of Transfusion Medicine, TRICASE, Italy

Background. Neutropenia and thrombocytopenia are the most common hematological toxicities in relapsed-refractory NHL pts treated with RIT (Zevalin®). Approximately 50% of treated pts experienced neutropenia grade 3-4 (ANC <1×10^9/L) and 60% of treated pts thrombocytopenia grade 3-4 (PLT <50×10^9/L). The median days for nadir of neutropenia and thrombocytopenia is around +60 after Zevalin® treatment without stem cell rescue. (1-2) *Aims.* Poor information in literature exists about kinetic of late hematological toxicities in pts treated with RIT included in a high dose chemotherapy program (Z-BEAM). *Methods.* We evaluated kinetic of hematological toxicities in 12 high risk NHL pts (Table 1), all pretreated with immunochemotherapy followed by high dose chemotherapy including Zevalin® (Z-BEAM) and ASCT in our Institution from February 06 to November 07. *Results.* At transplant 3/12 pts were in 1st complete remission (CR), 6/12 in partial remission (PR), 2 pts were in relapse (REL) and 1 in progression (PROG). Median CD 34$^+$ cells infused was 4,04×10^6/Kg (range 3,2-21,6). All pts engrafted. Median time to ANC ≥0,500×10^9/L was 11 days, median time to platelets (plt)≥20 ×10^9/L was 13 days. 4/12 pts had delayed neutropenia grade 3-4. Median time of onset was day +94 (range 76-147). ANC ≥1×10^9/L was achieved at day +157 (range 95-175). 1 patient (pt) had neutropenia as part of myelodysplastic syndrome (MDS). 4/12 pts had thrombocytopenia grade 3-4. Median time of onset was day +58 (range 34-291). 2/12 pts died with plt <10000 for BK virus encephalitis at day +34 and hyperosmotic coma at day +55. 1 CMV reactivation, 3 urine infection. 2 blood colture + for bacteria and 1 pneumonia was demonstrated during neutropenia and thrombocytopenia (Table 1). *Conclusions.* In our Institution 12 pts were treated with Z-BEAM + ASCT for NHL from February 06 to November 07. After normal engraft 4 pts had neutropenia and thrombocytopenia grade 3-4. Time of neutropenia onset in this subset of pts seems to be later than in pts treated with Zevalin® alone and therefore Z-BEAM and ASCT is feasible but is necessary play attention for possible late hematological toxicities.

Table 1.

		Date Zevalin	BOM at diagnosis	Status pre SCT	1st day Plt <50 (10^9/L)	1st day Plt >50 (10^9/L)	1st day ANC <1(10^9/L)	1st day ANC >1(10^9/L)	Age	Sex	Histology	Note (During neutropenia o thrombocytopenia)
1	C.R.	27.4.07	-	CR	-	-	0,980 g+104	1,630 g+152	37	f	CCCB	Urine + (E.Coli)
2	P.A.	8.6.07	-	CR	-	-	0,620 g+76	1,010 G+163	68	m	DLBCL	-
3	V.A.	8.9.07	-	PR	-		0,890 g+147	1,010 g+175	58	m	DLBCL	Urine + (Enteroc.faecium + Morganella m.)
4	V.V.	9.11.07	+ (t 11;18)	CR	42 g+74	95 g+94	0,500 g+84	1,910 g+95	57	m	MCL	CMV DNA urine+ gargle+ blood colture CVC+: (Stph.epidermidis)
5	A.C.	7.12.06	+	PR	40 g+291	55 g+300	-	-	57	f	DLBCL	Urine + (Enteroc.faecium) blood colture:+ (Stph.epidermidis) pneumonia
6	F.M.	28.7.06	+	PROG	6 g+34	not achieved	-	-	69	f	DLBCL	Encephalitis exitus g+34
7	N.L.	21.7.06	-	PR	5 g+43	not achieved	-	-	69	f	MCL	Hyperosmotic coma exitus g+55
8	C.M.	29.9.06	-	REL	-	-	0,490 g+143	not achieved	58	f	FOLL	MDS exitus g+209

(1)Witzig JCO 2002; 20 (10) 2453-2463
(2)Witzig JCO 2003; 21 (7) 1263-1270

V.Pavone Haematology Department
Panico Hospital, Tricase (LE) Italy

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NILOTINIB AND DONOR LYMPHOCYTE INFUSION IN THE TREATMENT OF PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH$^+$ ALL) RELAPSING AFTER ALLOGENEIC STEM CELL TRANSPLANTATION AND RESISTANT TO IMATINIB

M. Tiribelli, A. Sperotto, A. Candoni, E. Simeone, S. Buttignol, R. Fanin

Azienda Ospedaliero-Universitaria, UDINE, Italy

Background. Allogeneic stem cell transplantation (SCT) is the treatment of choice in adult Philadelphia-positive acute lymphoblastic leukemia (Ph$^+$ ALL), with long-term survival of 27-65% when SCT is performed in first complete remission (CR). Nonetheless, many patients relapse after SCT, and the prognosis of these cases is dismal. Immunotherapy of post-SCT relapse with donor lymphocyte infusion (DLI) is scarcely active in ALL. Imatinib is active in relapsed / refractory Ph+ ALL, but responses were usually short lived. Nilotinib is a new ATP-competitive BCR-ABL inhibitor, 20 to 50-fold more potent that imatinib. *Aims.* We describe the case of a patient with imatinib-resistant post-transplant relapse of ALL, who attained a complete remission with the combination of nilotinib and DLI. *Methods.* A 55-years-old man with Ph$^+$ ALL and meningeal involvement received induction and consolidation chemotherapy and CNS radiotherapy, obtaining an hematologic CR and a MCyR (95% Ph$^-$). The patient then started imatinib (400 mg/day), with a CCyR after 1 month but disease persistence at molecular level. In January 2006 he underwent a myeloablative (BuCy + ATG) allogeneic SCT from a matched unrelated donor. The patient recovered with a complete cytogenetic and molecular remission. No GvHD developed in the following months. Five and half months after SCT molecular positivity was detected, rapidly followed by testicular relapse of ALL. The patient started imatinib 400 mg/day, but rapidly disease progressed with a bone marrow relapse. Salvage chemotherapy and testicular radiotherapy induced a second hematologic CR, so the patient received a first DLI (0.5×10^7/kg), but on day +28 a third relapse was documented. Treatment with vincristine and dexamethasone attained a partial response with persistent marrow disease and a positive RQ-PCR (4.44 BCR-ABL /102 ABL copies). *Results.* In March 2007 the patient started nilotinib at standard dose (800 mg/day). After three weeks of treatment, bone marrow analysis documented the achievement of a morphologic remission and a 1-log reduction of BCR-ABL fusion transcript (0.38 BCR-ABL / 102 ABL copies). Two weeks later, on day +36 from the start of nilotinib, the patient received a second dose of DLI (1×10^7/kg), that induced a grade I GVHD (skin and liver). On May 16th, at the eight week of nilotinib and three weeks after the second DLI, a molecular negativity on bone marrow sample was documented. The patient continued a combined therapy with nilotinib at standard dose (800 mg/day) and monthly DLI infusion (1-1.5×10^7/kg). Monthly bone marrow analyses always confirmed the complete remission, with a donor chimerism steadily increasing and actually 100% donor, and negative RQ-PCR (Figure 1). *Conclusions.* With the limits of an anecdotal report, our case seems to provide a rationale for the combined use of nilotinib and DLI in the treatment of Ph+ ALL relapse after transplant and failure of imatinib. The use of DLI alone was not effective, but nilotinib may have reduced the bulk of disease to a MRD level at which the immunotherapeutic effect of DLI could be displayed.

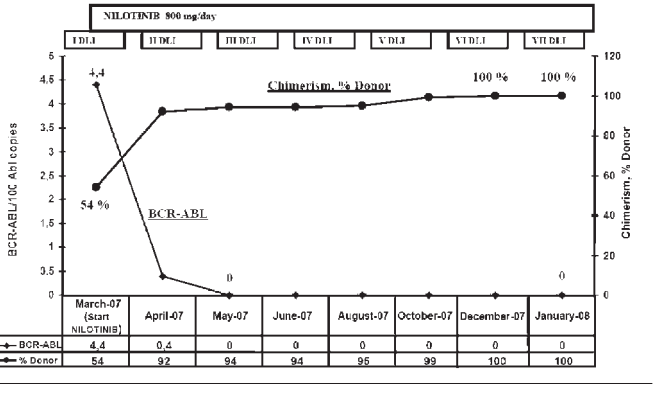

Figure 1.

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NEAR-TETRAPLOID ACUTE T-LYMPHOBLASTIC LEUKEMIAS: EGIL RETROSPECTIVE STUDY

A. Attarbaschi, M-C. Bene, Y. Bertrand, L. Campos, G-L. Castoldi, E. Forestier, R. Garand, E. Homolle, S. Kagialis-Girard, P. Lemez, W-D. Ludwig, E. Matutes, M-P. Pages, W. Pickl, A. Porwit, A. Orfao, J. Stary, W. Strob, P. Lemez

Hospital Jihlava, JIHLAVA, Czech Republic

Aims. To characterize the rarely occurring near-tetraploid T-lymphoblastic acute leukemias (NT-T-ALL) defined by blasts with a karyotype containing 80-104 chromosomes without aberrant near-diploid metaphases. *Methods.* The EGIL collected clinical and laboratory data of 14 patients with NT-T-ALL in a retrospective multicenter study. *Results.* The group comprised 10 childhood and 4 adult T-ALL cases. Childhood NT-T-ALL included 3 girls and 7 boys, 3.6-16.0 (median 9.6) year-old. Blasts in individual patients exhibited various combinations of T-lymphocytic lineage-associated markers, all tested cases were CD34 and HLA-DR negative. The *B-lineage* cytCD79a marker was expressed by blasts of 3 T-ALL cases and the *myeloid-marker* CD117 by one T-ALL. All 10 children reached complete remission (CR) with induction therapy. A 3.6 year-old girl had experienced CNS relapse on the 25th day of CR but reached a 2nd sustained CR now ongoing for 10 years. A 13.6 year-old boy died of septic shock following 3rd consolidation after 6.5 months in CR. A 14 year-old boy had relapsed after 25 months but reached his 2nd CR with chemotherapy. He underwent allogeneic bone marrow transplantation from an HLA-identical donor and has been alive for 43 months since diagnosis. The other 7 children with NT-T-ALL remain in 1st CR for 18-104 months. In contrast to childhood NT-T-ALL, blasts in 2 of the 3 adult cases tested were CD34 positive. Two males (20 and 26 year-old) reached 1st CR ongoing for 17 and 180 months. A 37 year-old female patient relapsed after 7.5 months with breast and skin involvement; since then she has survived for further 7 months with progressing ALL. A 62 year-old woman died of infective complications 40 days after the start of her induction chemotherapy, without signs of ALL. *Conclusions.* The prognosis of NT-T-ALL in children and young adults (below 30 years) seems to be favorable, similar to other T-ALL patients in these age groups. The expression of markers cytCD79a and CD117 can be regarded as lineage-associated but not lineage-specific.

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RESULTS OF EORTC 58951: TREATMENT OF 120 CHILDHOOD ALL (TUNISIAN EXPERIENCE)

L. Aissaoui, Y. Ben Abdennebi, M. Bedoui, R. Jeddi, R. Ben Lakhal, H. Ben Abid, K. Kacem, R. Ben Amor, Z. BelhadjAli, B. Meddeb

Aziza Othmana Hospital, TUNIS, Tunisia

We present the results of two consecutive studies (Protocol EORTC 58951 (I) and EORTC 58951 modified (II)) conducted for 120 children aged between 2-20 years with newly diagnosed acute lymphoblastic leukaemia(ALL) between 2001 and 2005. The mean age was 10 yearsi. The sex-ratio 0.7.The mean leucocyte count was 109×10^9/L. B and T - lineage represent respectively 64% and 34%. Twenty nine per cent of patients were corticoresistant .Complete remission was achieved in 87.5%. The 5year OS was 65.8%. The DFS and the EFS were respectively 59% and 48.6%. When studied separately. The 58 patients enrolled in study (I) were stratified in groups of risk according to criteria of the EORTC protocol without determination of DNA index or Minimal Residual Disease (MRD): 41.3% in AR1(74%), 36% inAR2 and 22.5% inVHR. The 4 year DFS and EFS were respectively 48% and 39%. The relapse occurred in 46% of cases. Eleven patients were eligible for SCT. In view of these worsen results, we introduce others stratification's parameters (bone marrow aspiration on D7/ D19 with determination of MRD by flow cytometry on D19 and on the first month of therapy). Sixty two patients were enrolled in study (II).All patients had an M2 or M3 D7 bone marrow aspiration so all of them receive an induction with HD MTX whatever the corticoresponse. The D19 bone marrow aspiration was realised in55% of patients. Forty four per cent of them had M2 aspect and 56% an M3 one. The MRD D35 was performed in 73% of patients and it was $\geq10^{-2}$ in 31% of cases. So 30% of the patients were enrolled in AVR2 and 70% in VHR. Twenty three patients(42%) were eligible for SCT but done only in 5 cases. The 3 year DFS and EFS were respectively 66% and 58%. The introduction of other parameters to study blastic clearance improve the outcome of our patients. However the results remained insatisfactory. The particular profile of our patients: median age, the high rate of T-ALL, the mean leucocyte count and poor response to treatment (30% MRD>10-2) may explain our results. More intensive post induction intensification should be discussed to improve the outcome of our high-risk acute leukaemia patients.

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NOVEL CHROMOSOMAL ABERRATIONS IN PHILADELPHIA-CHROMOSOME NEGATIVE CELLS OF A PATIENT WITH CHRONIC MYELOGENOUS LEUKEMIA TREATED WITH BCR/ABL KINASE INHIBITORS

A. Athanasiadou, G. Papaioannou, M. Gaitatzi, A. Karpouza, T. Touloumenidou, Z. Lazarou, I. Batsis, C. Vadikolia, C. Lalayanni, N. Stavroyianni, R. Saloum, A. Fassas, A. Anagnostopoulos

G. Papanicolaou Hospital, THESSALONIKI, Greece

The appearance of novel clonal aberrations in Ph-negative metaphases is a rare event in patients with chronic mylogenous leukemia (CML) treated with interferon-alpha. In contrast, the incidence of Ph-negative clones in patients with CML treated with imatinib is significantly higher. Of note, a recent report described the first three CML cases with a new clonal aberration (all trisomy 8) in Ph$^-$ cells after treatment with dasatinib. We assessed the frequency and significance of this phenomenon among 72 CML patients treated with imatinib and three patients treated with dasatinib after imatinib failure. With a median follow-up time of 46 months, 8/75 patients (11%) developed 10 novel chromosomal abnormalities (CAs) in Ph$^-$ cells: seven patients while on treatment with imatinib and one while on treatment with dasatinib subsequent to imatinib failure. The median time from initiation of imatinib to the appearance of CAs was 23 months. The most common CAs were -Y and +8 in 4 and 3 patients, respectively. In two patients with +8, the CAs were transient and disappeared after a median of 9 months. There is only one published report of trisomy 8 in 3/71 patients treated with dasatinib after imatinib failure. Our case developed monosomy 7 in Ph$^-$ cells at 6 months on dasatinib. After a median follow-up time of 26.8 months after the emergence of novel CAs in Ph$^-$ cells, all patients are alive; 7/8 in complete cytogenetic remission and 6/8 in major molecular remission. Importantly, in all cases of the present series with novel CAs in Ph$^-$ negative cells, examination of the BM aspirate and/or biopsy showed no evidence of myelodysplasia (MDS). However, given recent isolated reports of MDS or acute myeloid leukemia in CML patients treated with imatinib who developed novel CAs, a close follow-up of these cases is strongly recommended. That notwithstanding, the true biological significance as well as the mechanism responsible for the emergence of novel CAs in Ph$^-$ cells of CML patients treated with specific ABL kinase inhibitors remain unknown.

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SIMVASTATIN INDUCES PROLIFERATION INHIBITION AND APOPTOSIS IN CML CELLS

B. Oh,[1] T.Y. Kim,[1] H.J. Min,[1] S.H. Song,[1] M. Kim,[1] H.R. Lee,[1] S.H. Kang,[1] Y.S. Lee,[1] S.S. Yoon,[1] D.W. Kim,[2] H.J. Kim,[3] D.S. Lee[1]

[1]Seoul National University College of Medicine, SEOUL; [2]The Catholic University of Korea, SEOUL; [3]Department of Internal Medicine, College of Medicine , Hallym University, CHUNCHEON, South-Korea

Background. Simvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-liminting step for cholesterol synthesis. Simvastatin is known to show antiproliferative effects and are anticipated as a potential drug in the treatment of malignancies including acute myelogenous leukemia (AML) and multiple myeloma (MM). *Aims.* The aim of this study was to determine the effect of simvastain on DNA synthesis, cell cycle progression, and cell proliferation in chronic myelogenous leukemia (CML) cells. *Methods.* To test this approach, cellular proliferation assay based on the quantification of ATP was done on K562, KCL-22, LAMA-84 cell lines and primary CD34$^+$ cells from CML patients. Apoptosis assay by Annexin V staining and Western blot analysis for protein level change were done on CML cell lines. *Results.* We found that simvastatin inhibited cell proliferation and induced G1 arrest. The ability of simvastatin induced G1 arrest and decrease cell growth is thought to be mediated partly through their upregulation of cdk inhibitor p27kip1. Simvastatin also caused apotosis in both caspase dependent and independent pathway. Finally, Simvastatin inhibited cell proliferation in primary CD34$^+$ cells from CML patients. *Summary and Conclusions.* These *in vit-*

ro data indicate that simvastatin may have improved *in vivo* efficacy against CML.

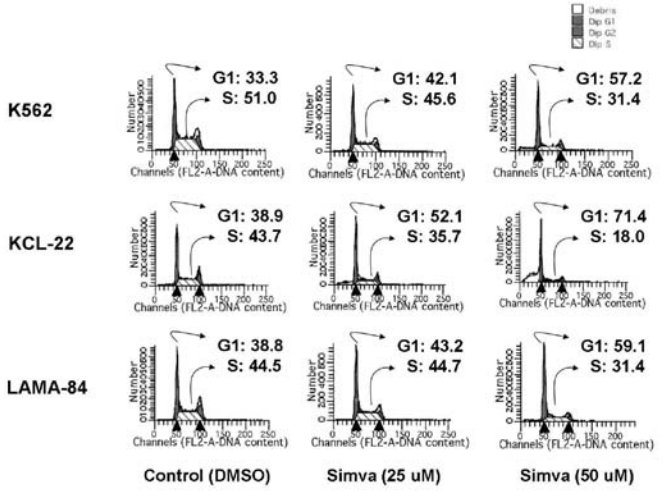

Figure 1.

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ROLE OF HEME OXYGENASE 1 IN CML CELLS RESISTANT TO IMATINIB

F. Di Raimondo,[1] T.B. Tibullo,[2] P. La Cava,[2] C. Giallongo,[2] I. Barbagallo,[3] C. Conticello,[4] F. Stagno,[2] G. Palumbo[2]

[1]University of Catania, CATANIA; [2]Division of Hematology, University of Catania, CATANIA; [3]Biochemistry and Molecular Biology University of Catania, CATANIA; [4]Department of Experimental Oncology, Mediterranean Institute of Oncology, VIAGRANDE (CATANIA), Italy

Background. Chronic myeloid leukemia (CML) is a stem cell disease in which BCR/ABL (tyrosine kinase (TK) activity) promotes the survival of leukemic cells. Imatinib mesylate is now the first-choice treatment for all newly diagnosed CML patients, but the initial striking efficacy of this drug has been overshadowed by the development of clinical resistance. The emergence of resistance to imatinib has prompted researchers to focus on strategies aimed at preventing or overcoming this phenomenon. Heme oxygenase-1 (HO-1) is an inducible rate-limiting enzyme which catalyzes group heme into carbon monoxide, iron and bilirubin. In the recent years, HO-1 expression has been reported as an important protective endogenous mechanism against physical, chemical and biological stress. The cytoprotective role of HO-1 has already been demonstrated for several solid tumors and acute leukemias. In addition, it has been recently showed that HO-1 is constitutively expressed in primary CML cells and that the BCR/ABL oncoprotein promotes expression of HO-1 in leukemic cells. Therefore, HO-1 is considered to play an important role as a survival molecule in CML cells, and an overexpression of HO-1 was found to inhibit apoptosis induced by imatinib. *Methods.* In our laboratory, K562 cells were incubated for 24 hrs with imatinib 1 μM alone, or with an inductor of HO-1 (Hemin 50 μM) or the combination of both. The same experiments were conducted with Dasatinib 2 nM and Nilotinib 100 nM. Cell viability was measured by trypan blue. Gene expression of HO-1 was assessed by Real time PCR (LightCycler, Roche). The results are expressed as mean±S.E.M. and the statistical analysis was performed using student's t test. A value of $p<0.05$ was considered as significant. *Results.* We found that HO-1 gene expression was increased about 3 fold after hemin treatment. The addition of hemin was able to overcome the inhibitory effect of IM (1 μM) on K562 cells ($p<0.002$) and the effect of IM was restored by adding an inhibitor of HO-1 (TIN) to the combination ($p<0.002$). Almost identical results were obtained with dasatinib and nilotinib ($p<0.002$). *Conclusions.* In conclusion, we confirm that HO-1 may represent a mechanism of resistance to IM and we showed that the same mechanism may apply to the other TKI (Dasatinib and Nilotinib). It remain to evaluate the role that HO-1 may have in the clinical setting.

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TELOMERE LENGTH AS A PROGNOSTIC MARKER IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) AT DIAGNOSIS

Y.M. Mortazavi,[1] A.N. Ahmadbeigy lahijani,[2] A. Pourfathollah,[2] M.S. Soleimani,[2] A.O. Omidkhoda,[2] A. Ghavamzadeh,[3] N. Shayan Asl[3]

[1]Zanjan Medical School, ZANJAN; [2]Tarbiat Modares University, Hematology Department, TEHRAN; [3]Shariati, Hematology-Oncology and BMT Reasearch Centre, TEHRAN, Iran

Background. Telomeres are nucleoprotein structures that cap the end of all eukaryotic linear chromosomes, preventing the degradation or fusion of chromosome ends. They are essential for maintaining the integrity and stability of genomes in the mammalian cells. Progressive telomere shortening has a role in genome instability. Progressive telomere length shortening has been reported from a wide range of human cancers including CML. CML has different stages in the process of the disease course and there is a possibility that telomere length shortening may involve in disease transformation and malignancy progress. *Aims.* In this study, we aimed to measure the telomere length changes in peripheral blood leukocytes of patients with CML in chronic and blastic phases and compare the results in each group and with normal controls. *Materials and Methods.* We measured telomere length in 14 patients in chronic phase (CP-CML), and 7 patients in blastic phase (BP-CML). Nine age and sex matched normal individuals were also included in the study as a control group. DNA was extracted from the peripheral blood mononuclear cells and the telomere length was analyzed by non-radioactive Southern blot. *Results.* 71.5% of patients in chronic phase had a shortened telomere compared to age and sex adjusted normal individuals ($p<0.0001$). The mean telomere length in chronic and blastic phase was 6.98 kb and 4.81 kb respectively. Mean telomere length reduction in BP-CML and CP-CML relative to normal controls was 3.31 kb and 5.27 kb respectively. The mean telomere length in BP-CML showed a significant statistical difference compared to CP-CML ($p<0.0001$). These results indicate a significant telomere length variation in different phases of CML. *Conclusions.* The significant statistical difference in mean telomere length of CP-CML and BP-CML relative to age-adjusted normal controls and the apparent difference of TRF in chronic and blastic phases can be useful in the prediction of disease progression and selection of patients at higher risk of disease transformation in CML.

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RESPONSE OF SECONDARY CHRONIC MYELOID LEUKEMIA TO IMATINIB. COOPERATIVE STUDY OF CML REGISTRIES CAMELIA AND INFINITY

J. Voglova,[1] J. Muzik,[2] K. Steinerova,[3] L. Demitrovicova,[4] E. Faber,[3] M. Doubek,[3] H. Klamova,[5] L. Novakova,[6] E. Tothova,[7] Z. Michalovicova,[8] K. Indrak[3]

[1]University Hospital Hradec Kralove, HRADEC KRALOVE, Czech Republic; [2]Institute of Biostatistics and Analyses, BRNO, Czech Republic; [3]University Hospital, PLZEN, Czech Republic; [4]National Cancer Institute, BRATISLAVA, Slovakia; [5]Institute of Hematology and Blood Transfusion, PRAHA, Czech Republic; [6]University Hospital Kralovske Vinohrady, PRAHA, Czech Republic; [7]L. Pasteur University Hospital, KOSICE, Slovakia; [8]University Hospital and Health Centre, BRATISLAVA, Slovakia

Chronic myeloid leukemia as a second malignancy (sCML) is known as a rare late complication of successful cancer therapy. Only a few case reports about the outcome of sCML in imatinib era have been published. Aim of our study was to evaluate the response to imatinib in a larger cohort of sCML patients (pts). *Methods.* Twenty-one pts with sCML (10 males and 11 females, median age, 56 years, range, 35 -69) treated with imatinib have been registered in centers involved in CML registries CAMELIA and INFINITY between January 2000 and December 2007. Characteristics and treatment of primary malignancies, presenting features and the course of sCML were analyzed. *Results.* In 20 patients was sCML diagnosed in chronic phase, in 1 patient sCML presented in accelerated phase. Ph chromosome without additional cytogenetic abnormalities was detected in 20 pts. Additional cytogenetic abnormalities were found out in 1 patient in accelerated phase. Median time from diagnosis of the first malignancy to the diagnosis of sCML was 85 months (range 17-452). Imatinib was used in 12 patients as first-line treatment or after short time pretreatment with hydroxyurea. Nine pts were pretreated with interferon. Median time from sCML diagnosis to start of imatinib was 2 months (range 0-117). Complete hemato-

logical response to imatinib was achieved in all patients in chronic phase of sCML. Complete cytogenetic response was determined in 14 of 20 (70%) assessable pts. Two pts died, 19 pts were alive at the time of evaluation. Median overall survival from diagnosis of sCML was 37 months (range, 6-125), from start of imatinib 31 months (range, 5-64). *Conclusions.* Response of sCML to imatinib seems to be comparable to the response of primary CML. A prospective study of sCML in CAMELIA registry was started with the aim to confirm this finding in a larger cohort of patients.

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AN ATYPICAL JUNCTION OF BCR-ABL GENE AS MOLECULAR MARKER FOR QUANTITATIVE MONITORING OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML)

T. Jurcek, D. Dvorakova, J. Pospisilova, D. Ohlidalova, J. Mayer

University Hospital, BRNO, Czech Republic

Background. A majority of CML patients express one of three most common types of BCR-ABL fusion transcripts b3a2 (e14a2), b2a2 (e13a2) or e1a2. However, a small proportion of patients have rare breakpoints resulting in BCR-ABL transcript with c3a2 (e19a2) junction, or unusual transcripts such as b2a3 (e13a3), b3a3 (e14a3), e2a2 or e8a2. *Aims.* Identification and determination of unusual types BCR-ABL transcripts e19a2 and e14a3 junctions. Design of TaqMan assay for molecular follow-up of minimal residual disease (MRD). Verification of quantitative RT-PCR (RQ-RT-PCR) measurement of expressed BCR-ABL level for routine laboratory monitoring. *Methods.* In the initial screening, single multiplex PCR assay with primers enabling the detection of both most common and rare BCR-ABL fusion transcripts was used. In three patients were obtained PCR amplicons with unmatched length against the expected products. These amplicons were directly sequenced (ABI Prism 310 Genetic Analyzer, Applied Biosystems), and compared with sequences of the ABL gene (NM_007313;GenBank) and the BCR gene (NM_004327; GenBank). Our results showed the presesence of e19a2 transcript in one and e14a3 transcripts in two patients. For long-term follow-up of these patients, primers and TaqMan probe specific for e19a2 and e14a3 transcript, respectively, were designed using Primer ExpressTM software. For absolute quantification, we used cloned plasmid containing target sequences to generate standard curves. Real-time RT-PCR was performed using 7300 Sequence Detection System (Applied Biosystems). In every 25 μL of reaction mixture, there were 300 nM of each primer, 200 nM of probe and a 1x concentration of Master Mix (ABsolute QPCR Mix, ABgene). The amplification protocol consisted of 15 min at 95°C, followed by target amplification via 50 cycles of 15 sec at 95°C and 1 min 60°C. The results were normalized on the number of total-ABL gene copies. *Results.* Our designed RQ-RT-PCR method is highly specific without amplification of another types of BCR-ABL transcripts. Sensitivity of the assay was tested using 10-fold serial dilution of plasmid containing specific target. Maximal reproducible sensitivity was 10 plasmid molecules. As the threshold, value of 0.1 for e19a2, and 0.2 for e14a3 was used. BCR-ABL transcript levels were calculated as BCR-ABL/total-ABL ratios and expressed as percentages. *Conclusions.* We report three cases of CML patients with rare BCR-ABL fusion transcripts. Our findings confirm the heterogeneity of breakpoints in the rearrangement of BCR-ABL gene. Here, we propose a sensitive and reproducible method that could be used for the standard routine laboratory evaluation of MRD in patients bearing atypical rare subtypes of transcripts of BCR-ABL gene.

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HIGH DOSE IMATINIB TO ACHIEVE MOLECULAR RESPONSES IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN LATE CHRONIC PHASE AND COMPLETE CYTOGENETIC RESPONSE: IS IT WORTHWHILE?

F. Stagno,[1] P. Vigneri,[2] M. Massimino,[2] V. Del Fabro,[1] S. Stella,[2] S. Berretta,[1] A. Messina,[2] F. Di Raimondo[1]

[1]Hematology Section, CATANIA; [2]Pathology Section, CATANIA, Italy

Background and Aims. Imatinib Mesylate (IM) currently represents the treatment of choice for patients (pts) diagnosed with Chronic Myeloid Leukemia (CML) inducing high rates of complete cytogenetic response (CCyR) in the majority of pts with chronic phase (CP) CML. Despite these major advances, minimal residual disease is still an issue for the majority of pts that continue to have detectable BCR-ABL transcripts. In fact, IM therapy infrequently induces complete molecular responses with standard dose treatment (400 mg/d). Reported evidence suggests that high dose IM (600-800 mg/d) might be useful in achieving better rates of molecular response (MR), both major (MMR) and complete (CMR). Since obtaining a CCyR together with a MMR or a CMR predicts for a higher rate of progression free survival in CML (Druker *et al.* NEJM 2006), we tried to achieve a molecular response in a cohort of pts with CP-CML who were in CCyR but not in MR. *Patients and Methods.* Five patients (4 males and 1 female; median age 55 yrs; Sokal score 4 low, 1 high risk) received high dose IM. Complete hematologic response, cytogenetic response and molecular response were defined as previously stated (Baccarani *et al.* Blood 2006). Median time from diagnosis was 57 months (range 45-100) with two pts undergoing other treatments before IM therapy (HU, LD-ARA-C, α-IFN). All pts had begun IM at 400 mg with a median time to CCyR of 6 months (range 6-12). *Results.* Prior to high dose IM therapy, median BCR-ABL transcript was 0.166% (on the ISS). Four out of 5 patients (80%) reached a MMR within 3 months. Furthermore, 2 of the 4 patients (50%) also achieved a CMR, although this response was not maintained. None of the pts exhibited dose-limiting toxicities with mild thrombocytopenia, leukopenia and exacerbation of prior edema as the only side effects. *Summary.* In conclusion, these data - although limited to a small number of patients - suggest that high dose IM could be useful to achieve MMR in both early and late CP-CML patients in CCyR.

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A PHASE I/II STUDY OF NILOTINIB IN JAPANESE PATIENTS WITH IMATINIB-RESISTANT OR -INTOLERANT PH+ CHRONIC MYELOGENOUS LEUKEMIA (CML) OR RELAPSED/REFRACTORY PH+ ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

K. Usuki,[1] A. Urabe,[1] A. Tojo,[2] Y. Maeda,[3] Y. Kobayashi,[4] I. Jinnai,[5] K. Ohyashiki,[6] M. Nishimura,[7] T. Kawaguchi,[8] H. Tanaka,[9] K. Miyamura[10], Y. Miyazaki,[11] T. Hughes,[12] S. Branford,[12] S. Okamoto,[13] J. Ishikawa,[14] M. Okada,[15] N. Usui,[16] T. Amagasaki,[17] H. Natori,[17] T. Naoe[18]

[1]NTT Kanto Medical Center, TOKYO, Japan; [2]The Institute of Medical Science,The University of Tokyo, TOKYO, Japan; [3]Kinki University School of Medicine, OSAKA, Japan; [4]National Cancer Center Hospital, TOKYO, Japan; [5]Saitama Medical University International Medical Center, SAITAMA, Japan; [6]Tokyo Medical University Hospital, TOKYO, Japan; [7]Chiba University Hospital, CHIBA, Japan; [8]Kumamoto University Hospital, KUMAMOTO, Japan; [9]Hiroshima University Hospital, HIROSHIMA, Japan; [10]Japanese Red Cross Nagoya First Hospital, NAGOYA, Japan; [11]Nagasaki University Hospital of Medicine and Dentistry, NAGASAKI, Japan; [12]Hanson Institute Centre for Cancer, ADELAIDE, Australia; [13]Keio University Hospital, TOKYO, Japan; [14]Osaka University Hospital, OSAKA, Japan; [15]The Hospital of Hyogo College of Medicine, HYOGO, Japan; [16]Jikei University Hospital, TOKYO, Japan; [17]Novartis Pharma Japan, TOKYO, Japan; [18]Nagoya University Hospital, AICHI, Japan,

Background. Nilotinib, a potent and highly selective BCR-ABL inhibitor, has been approved in both the US and Europe for the treatment of pts with Philadelphia chromosome-positive (Ph^+) CML in chronic phase (CP) and accelerated phase (AP) who are resistant or intolerant to prior therapy, including imatinib. *Methods.* This phase I/II, open-label study was designed to evaluate the efficacy and safety of nilotinib in Japanese pts with imatinib-resistant or -intolerant Philadelphia chromosome-positive (Ph^+) CML CP, AP, blast crisis (BC), or relapsed/refractory Ph^+ ALL patients. In addition, mutational analysis of BCR-ABL was performed. Nilotinib was administered at 400 mg twice daily (BID). *Results.* This analysis includes 34 Japanese pts (16 CP, 7 AP, 4 BC, 7 ALL) who received nilotinib for least 6 months. Of the 6 CP pts without CHR at baseline, 6 (100%) achieved complete hematologic response (CHR) during nilotinib therapy. Overall, MCyR was observed in 15/16 (94%) CP pts; 9/16 (56%) achieved CCyR. The median time to CHR and MCyR was 28 and 84 days, respectively. At the time of data cut-off, the median duration of MCyR was not reached in CP pts. In AP pts, 5/7 (71%) achieved a hematologic response (HR); confirmed HR occurred in 2/5 pts and 1 (14%) achieved CHR. MCyR occurred in 1 (14%) pt, who also achieved CCyR. Confirmed HR occurred in 2/4 (50%) BC pts, and 1 (25%) achieved CHR. MCyR was achieved in 2 (50%) pts, with both pts achieving CCyR. Of the 5 pts with relapsed/refractory Ph+ ALL, HR/CHR was achieved in 1 (20%) pt, 1 (20%) had stable disease and 3 (60%) had disease progression. Of the 2 pts with minimal residual disease, both (100%) had complete response. At baseline, BCR-ABL mutations were observed in 4/16 (25%) of CP pts, 6/7 (86%) AP, 2/4 (50%) BC, and 4/7 (57%) of Ph+ ALL pts; no pts had a

T315I mutation. It was found that, for each disease phase, similar rates of hematologic and cytogenetic responses were observed regardless of the presence/absence of a baseline BCL-ABL mutation and regardless of the mutation type. The overall median duration of exposure to nilotinib was 183 (range 13-456) days with the median dose intensity being 738 mg/day (range 309-799). Treatment with nilotinib is ongoing in 19 (56%) pts; 3 (9%) discontinued due to AE, 8 (24%) due to disease progression and 3 (9%) due to transplantation. The most common study drug related adverse events (AE) of all grades were rash (50%), neutropenia (35%), nausea and headache (32% each). The most common grades 3/4 study-drug related AEs were neutropenia (32%), anemia and leukopenia (18% each). *Conclusions.* The results of this study in Japanese pts confirm that nilotinib induces significant responses in CML-CP and -AP pts with imatinib-resistance or -intolerance. Nilotinib also has clinical activity in CML-BC, a group of CML pts with very advanced disease and limited therapeutic options. Promising activity is also observed in relapsed/refractory Ph^+ ALL pts. Nilotinib therapy was generally well-tolerated. The efficacy and safety of nilotinib in Japanese pts appears to be consistent with pts in the pivotal Phase 2 study.

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OUTCOME OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA WITH T315I BCR-ABL MUTATION

C.A. De Souza,[1] K.B.B. Pagnano,[1] R.A. Silveira,[1] L. Nardinelli,[2] M Mello,[2] C. De Souza,[1] P.H. Dorliac,[2] I. Lorand-metze,[1] V. Funcke,[3] M.A. Zanichelli,[4] N. Clementino,[3] R. Pasquini,[3] I. Bendit[2]

[1]State University of Campinas, CAMPINAS; [2]State University of São Paulo, SÃO PAULO; [3]Federal University of Paraná, CURITIBA; [4]Brigadeiro Hospital, SÃO PAULO, Brazil

The most frequent cause of imatinib resistance is BCR-ABL mutations, usually selected during treatment. Most of them destabilize the inactive conformation necessary for imatinib binding. The T315I mutation, located at the ATP binding pocket is resistant to imatinib and other kinase inhibitors (nilotinib, dasatinib and bosutinib) and is associated with a poor outcome. The aim of this study was to evaluate the frequency of T315I mutation in patients with CML resistant to imatinib and to describe the outcome of these patients. A total of 172 patients with imatinibe resistance were analyzed for mutations following the same protocol. Total RNA was extracted from peripheral blood or bone marrow and amplification of the kinase domain of ABL from BCR/ABL was performed, using a semi-nested RT-PCR, to cover amino acids 244-486. PCR product was submitted to direct automated sequencing and compared with normal sequences of BCR-ABL gene (M14752, GenBank). BCR-ABL mutations was detected in 46 out of 172 (26,7%) and T315I mutation was found in 13 out of 46 patients (28,2%). Patients characteristics: 10 patients were male, 3 female. The median age at diagnosis was 40 years (13,5-72 years). Time between diagnosis and imatinib treatment was 944,3 days (48-2462 days). Patients had been previously treated with Hidroxiurea (5), Hidroxiurea and Interferon (7) and bone marrow transplantation (1). The median duration of imatinib treatment was 685,38 days (273-1873 days). Hematological resistance occurred in seven patients and cytogenetical resistance in six. Mutation was first found in ten patients during imatinib treatment, in one before imatinib treatment and two patients after dasatinib failure. Three patients have more than one mutation analysis, performed during different time-points in treatment: after imatinib resistance, after dasatinib resistance (3) and during a relapse after bone marrow transplantation relapse (1). After imatinib resistance, eight patients were treated with dasatinib and had no hematological response, three were treated with Hydrea and two were submitted to allogeneic bone marrow transplantation. Five patients died of disease progression and one died after bone marrow transplant from GVHD and infection. The median overall survival and EFP from detection of T315I mutation was 793±291days, and 1572±956 days respectively. Overall survival from diagnosis was 38% and 22% from mutation detection. Seven patients are alive, six in chronic phase and one in accelerated phase. Two of them are in complete hematological response, with no cytogenetical response and one in complete cytogenetical response after bone marrow transplantation. The current treatments are: hydrea (4), dasatinib (1), imatinib 800 mg (2). In conclusion, in this study we confirmed the high frequency of T315I mutation in a population of resistant patients and the poor prognosis of these patients. Most of them started imatinib in a late chronic phase and after resistance were treated with dasatinib. However, in two cases treated initially with high dose imatinib the mutation was detected very early, suggesting that there was inhibition of Ph positive sensitive cells and selection of resistant clones.

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MOLECULAR RESPONSE OF CML PATIENTS AFTER INTERRUPTION OF IMATINIB TREATENT

E.Y. Chelysheva, A.G. Turkina, A.V. Misyurin, G.A. Gusarova, E.V. Aksenova, T.I. Kolosheinova, L.Yu. Kolosova, S.R. Goryacheva, E.S. Zakharova, O.Yu. Vinogradova, M.A. Sokolova, N.D. Khoroshko

Hematology Research Centre RAMS, MOSCOW, Russian Federation

Background. At present time imatinib is a standart therapy for chronic myeloid leukemia (CML). For the best effectiveness of therapy the drug is taken constantly. The forced interruptions in treatment have particular reasons. Here we report about the molecular monitoring data of 14 CML patients who had to interrupt imatinib treatment due to the temporary lack of the drug. *Aims.* The aim was to estimate the influence of interruption in imatinib treatment on the hematologic, cytogenetic and molecular response of CML patients. *Patients and methods.* We observed 14 patients (median age 49 years, range 29-69 years) in chronic phase (CP) CML who had complete hematologic response (CHR) and complete cytogenetic response (CCgR) on imatinib therapy. For 10 of them imatinib was a second line therapy after interferon alpha treatment failure, for 4 it was a first line treatment. Median duration of disease was 64 months (28-124 months). Median treatment period before imatinib was 11 months (1-82 months). Median duration of interruption in treatment was 55 days (21-90 days). No additional CML treatment was held during that period. Molecular response (MR) was analyzed by quantitative Real-time PCR. Genes ABL and beta2 microglobulin were used as control genes. Negative PCR results were considered a complete molecular response (CMR), the decreasing of BCR-ABL expression more than for 3 lg as to baseline was considered a major molecular response (MMR). Before the moment of treatment interruption 6 patients had CMR, 5 had MMR, 3 had more than 2 lg decreasing of BCR-ABL transcript. *Results.* After treatment interruption 13 (92.9%)of 14 patients had maintained CHR and CCgR. 1(7.1%) of 14 patients with previous CMR who had been treated with imatinib as a first line therapy for 2 years had a hematologic relapse after 3 months of interruption in therapy. 3 (21.4%) of 14 patients lost CMR, 5 (35.7%) showed BCR-ABL transcript level growth for 0.5-1 lg and 2 of that 5 lost MMR. So for 8 (57.1%) of 14 patients we observed a deterioration of MR. For 6 (42.9%) of 14 patients the situation was more favorable: 3 (21.4%) remained in CMR, 2 (14.3%) in MMR, 1 patient had a slight transcript level depression after the interruption in treatment. After resuming imatinib treatment we continue monitoring all above mentioned patients. *Conclusions*. Our investigation showed a heterogeneity of molecular response in CP CML patients group with CHR and CCgR after 21-90 days interruption of imatinib therapy. We have observed a possibility of CMR, MMR and stable level of minimal residual disease (MRD) without treatment for 42.9% of patients and a deterioration of MR for 57.1%. We also have observed a development of hematologic relapse after interruption of treatment in one case. It is not possible to estimate correctly the role of first and second line imatinib therapy on the kinetics of MRD due to the limited number of cases. The data of molecular monitoring for CML patients with interruptions in treatment should be collected and analyzed furthermore.

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PROGNOSTIC RELEVANCE OF DELETION OF 9Q34 AND THE SUPPRESSOR OF CYTOKINE SIGNALING -1 IN CASES OF CHRONIC MYELOID LEUKEMIA PATIENTS

M. Ghaith,[1] H. Abdou,[1] I. El-Bendary,[1] A. Eid,[1] H. El-Sheikh,[1] I. Farrag,[1] Y. Mohamed[2]

[1]Tanta University, RIYADH, Saudi Arabia; [2]Ain Shams University, CAIRO, Egypt

Background. Chronic Myeloid leukemia (CML) is characterized by formation of the BCR/ABL fusion gene. Although, Imatinib has considerably changed the treatment paradigm and outcome, many patients in underprivileged countries are still being treated with the cytokine Interferon alone and/or in combination with Ara-C. The response to therapy is variable because of development of cytogenetic changes or resistance. In a substantial minority of CML patients, large deletions on the derivative chromosome 9 have recently been suspected as a cause of resistance and were associated with poor prognosis. In addition, Suppressor of cytokine signaling (SOCS) proteins, which are negative regulators of cytokine-induced signaling, are also hypothesized as a cause of resistance and poor prognosis when aberrantly expressed. *Objectives*.

To determine the incidence and role of deletion of 9q34 and SOCS-1 mRNA aberrant expression in CML patients of different phases of their disease and to correlate it to on response to Interferon and prognosis. *Patients and Methods.* 43 CML patients were divided into 3 groups; I: chronic phase (15 patients), II: accelerated phase (10 patients) and III: blastic crisis (18 patients) and IV: 10 apparently healthy individuals of matched age and sex serve as control group. Deletion of 9q34 was determined using Fluorescence *In situ* hybridization (FISH) and SOCS-1 mRNA expression by PCR. Are patients had initial assessment by conventional cytogenetic, FISH analysis and in case of accelerated phase and blastic crisis immunophenotyping of the peripheral blood and/or bone marrow are done. *Results.* FISH Inter-phase studies showed that: all patients were Philadelphia positive, Deletions of 9q 34 were observed in 20.9% of all patients and were present in 13.3% of chronic phase 1 out of 10 (10%) accelerated phase and 6 out of 18 (33.3%) of patients in blast crisis. SOCS expression were 23 (53.4%) patients out of 43 where in group I, 6 patients (40%) were positive and 9 case (60%) were negative. While in group II, 5 patients (50%) were positive and 5 patients (50%) negative and in group III, 12 patients were positive (66.67%) and 6 patients (33.3%) were negative. There was no statistically significant difference between the patients' groups in rate of deletion of 9q34 and SOCS expression rate which may be related to the relatively small number of each group. However, analyzing outcome based on 9q34 deletion and SOCS expression status showed a statistically significant difference in over all survival (OS) and progression free survival (PFS) between those with the deletion (PFS: 29.43+10.68 months, OS: 35.00+10.05 months) and those without (53.28+11.90 months, OS: 58.08+10.80 months) (p<0.001) and those with abnormal SOCS expression (PFS: 45.00.+12.23 months, OS: 42.00+12.73 months) and those without (PFS: 64.00 +6.43. months, OS: 57.14+12.45 months) (p<0.001). *Summary and Conclusions.* Inter-phase FISH for detection of 9q34 deletion and PCR testing for detecting SOCS-1 expression were technically feasible, convenient and reproducible and could detect the relevant abnormalities in a significant number of patients. More importantly, deletion of 9q34 and aberrant expression of SOCS-1 were important poor prognostic factors for CML patients specially those under cytokine therapy. They should be studied prospectively and if confirmed should be incorporated into management decisions particularly for those who may be enrolled on chronic interferon therapy.

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BORTEZOMIB, LIPOSOMAL DOXORUBICIN AND DEXAMETHASONE REGIMEN FOR RELAPSED OR REFRACTORY MULTIPLE MYELOMA AND INTRODUCTION OF SERUM FREE LIGHT CHAINS (SFLC) IN RESPONSE MONITORING: PRELIMINARY DATA

F. Pisani,[1] I. Cordone,[2] M.L. Dessanti,[3] G. Cigliana,[2] B. Frollano,[2] S. Masi,[2] A. Mengarelli,[3] A. Spadea,[3] M. D'Andrea,[3] M.C. Petti[4]

[1]Istituto Nazionale dei Tumori Regina Elena, ROME; [2]SC Patologia Clinica, Istituto Nazionale dei Tumori Regina Elena, ROME; [3]SC Ematologia, Istituto Nazionale dei Tumori Regina Elena, ROME; [4]Sc Ematologia, Istituto Nazionale dei Tumori Regina Elena, ROME, Italy

Background. Bortezomib, liposomal doxorubicin and dexamethasone (BMD) combination could represent a feasible treatment for relapsed/refractory MM. Furthermore the sFLC analysis is providing an efficient way to monitor both disease and response to treatment in MM: reduction to a half of the baseline values of FLC is associated to nCR and increased FLC values could predict a relapse or a non optimal response to treatment. *Aims.* 1) feasibility of BMD in previously treated patients 2) evaluation of k/Ï ratio during and after four BMD cycles as prediction of treatment outcome. *Methods.* We recruited 9 pts, only six (4M; 2F) are currently evaluable having completed the treatment. Bortezomib was given at 1.3 mg/m^2 on days 1,4,8,11, liposomal doxorubicin (Myocet®) 30 mg/ m^2 on day 4 and dexamethasone 20 mg per os days 1-4, 8-11, 15-18, on 28-days cycle with total of 4 courses. The median age at relapse was 52.5 years (range 48-64). All patients had been already treated (range 1-5 lines), including VAD, HDT with ASCT, allogeneic transplant in 1 patient, thalidomide in two and bortezomib in 1 pt. k/λ measurement were carried out at baseline and at the end of each cycle using an immunoassay (Freelite, The Binding Sites, Birmingham, UK). *Results.* After a median follow up of 4 months (range 1-11) 6/6 pts are responders. One pt achieved nCR, 3pts VGPR, 2pts PR. In all 6 pts the proportion of bone marrow aberrant plasma cells, as per flow cytometry, decreased after 4 treatment cycles. In a case with FISH positive for del 13q34 before treatment, we registered a negative FISH analysis and a VGPR at the end of 4th cycle. So far a patient in VGPR was submitted to further ASCT with MEL 100 regimen and subsequently a RIC allo-transplantation from HLA-identical sibling donor and is currently rated as CR. Another 2 pts: one in nCR and one in VGPR, not previously transplanted, received MEL 200 and are both in nCR. One patient in PR is waiting for a further ASCT. A relapsing patient who was previously treated with VAD followed by allogeneic transplant and then irradiated on femora and thus treated with BMD 4 cycles, is actually in VGPR. The last patients who received 5 lines of therapy obtained a PR after BMD but progressed after 2 months. In all patients the absolute values of sFLC and their ratio were correlated with disease, response to treatment and anticipated relapse. All six pts are alive and no severe hematological toxicity or evidence of cardiac toxicity was reported, two patients experienced grade 3 peripheral neurophaty. *Conclusions.* In our experience BMD regimen has shown a feasibility with acceptable toxicity in pts previously heavily treated. BMD provided an optimal disease burden leading almost all patients to HDT and transplant in ideal condition. Although an adequate follow up is needed to drawn any conclusions, BMD appears very effective. Furthermore, sFLC analysis seems to provide a more sensitive mean of monitoring response and identifying residual disease and can be used in assessing stringent complete response.

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RAPID IMPROVEMENT IN RENAL FUNCTION IN PATIENTS WITH MULTIPLE MYELOMA AND RENAL FAILURE TREATED WITH BORTEZOMIB

A. Qayum, A. Aleem, A. Al-Diab, A. Al-Sagheer, F. Niaz, A. Al-Momen

King Khalid University Hospital, RIYADH, Saudi Arabia

Background. Multiple Myeloma (MM) frequently presents with renal dysfunction in addition to other manifestations. Development of renal failure in MM patients carries a poor prognosis. Treatment of multiple myeloma is not curative but development of new agents has improved the outlook for these patients. Bortezomib is a proteasome inhibitor which has shown good activity in MM and carries a favorable safety profile. Previous trials have shown its efficacy in relapsed and refractory MM. It has been shown in small studies that bortezomib is also effective in multiple myeloma patients presenting with renal failure. *Aims.* To report six cases of renal failure secondary to MM treated with bortezomib. *Patients and Methods.* Records of 6 patients with MM and renal failure treated with bortezomib were reviewed. Bortezomib was administered intravenously at a dose of 1.0 or1.3 on day 1, 4, 8, & 11. Each cycle was repeated at 21 days intervals. The dose of bortezomib was increased to 1.3 mg/m^2 in patients who initially received 1.0 mg/m^2, as soon as the serum creatinine reached to near normal levels. *Results.* Three patients were newly diagnosed to have MM while three patients had relapsed or refractory disease. All patients had poor performance status of 3 or 4 on ECOG scale. Mean age of the patients was 71.8±7.2 years. Three patients either had refractory disease (n=1) or relapsed after initial response (n=2). There was a variable degree of renal failure with a mean serum creatinine of 346.8±124 µmol/L (range 193-551 µmol/L). All patients had a creatinine clearance rate of less than 30 mL/min (mean 15.6±4.3 mL/min) at the initiation of bortezomib therapy and three patients required dialysis before first cycle of bortezomib. Five out of six patients showed anti-myeloma response to bortezomib. Reversal of renal failure was observed in all six patients. Serum creatinine normalized in three patients and dropped to less than 50% in other three patients after two cycles of treatment. Mean serum creatinine had dropped to 118.4±30.5 µmol/L at the time of start of 4th cycle of bortezomib. Renal function remained stable in all six patients as indicated by stable lower serum creatinine levels even after completion of the treatment. Adverse effects to bortezomib were mild and manageable. Enhanced toxicity was not seen in patients who received bortezomib at a dose of 1.3 mg/m^2 from the beginning (first cycle). Reversal of renal failure persisted despite incomplete response to MM in 2 cases, and progression of disease in one patient. *Conclusions.* Bortezomib appears to be an effective treatment to restore renal function in patients with MM and renal failure. It seems that bortezomib may have an effect on the kidney in reversal of renal failure independent of its anti-myeloma effect.

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TREATMENT OF LIGHT CHAIN (AL) AMYLOIDOSIS WITH BORTEZOMIB AND DEXAMETASONE

A. Canovas, J.J. Alonso, G. Barreiro

Hospital de Cruces, BILBAO, Spain

Background. Primary systemic amyloidosis is a monoclonal plasma cell

disorder in which diffuse deposit of immunoglobulin light chain causes damage to the involved organs . Treatment is usually based on steroids and standard or high dose of melphalan and autologous stem cell transplantation (ASCT) but patients not suitable for transplantation have an unfavourable outcome. Bortezomib has shown significant activity in multiple myeloma (MM), which is increased if dexametasone is associated, and can be used in patients with renal failure. *Aims.* To assess the effectiveness of bortezomib and dexametasone (BD) in patients with relapsed or refractory AL amyloidosis (AL-A) following at least one prior therapy. *Methods.* Patients with histologically demonstrated amyloidosis, clinical organ dysfunction and relapsed or refractory disease after at least one course of treatment were selected. Bortezomib was administered at a dosage of 1,3 mg/m^2 the days 1, 4, 8 and 11 together with 20 mg of dexametasone each day and the following . Dose modifications were made whenever toxicity developed. Standard criteria of the 10th International Simposium on Amyloid and Amyloidosis (Gertz 2005) were used to assess organ involvement, organ response and hematologic response. *Results.* Three patients with primary systemic amyloidosis and one with IgG lambda MM with AL-A (intestinal involvement and polineuropathy) received treatment. Mean age was 57 years (range 43-74), median performance status (ECOG) was 2 (range 1-3) and mean number of organs involved was 2 (range 1-4), with two symptomatic cardiac disease, one end stage renal failure in dialysis and one gastrointestinal amyloidosis with malabsorption and bleeding. Mean number of previous treatment schedules was 2 (one patient had undergone 2 ASCT). An average of 5 courses were administered (range 2-8). Complete hematologic and partial organ response was ascertained in three patients with involvement of the heart (2), liver (1) and gastrointestinal tract (1), whereas no hematologic nor organ response was achieved in the forth patient, who developed refractory heart failure with early death. CR was sustained in two patients after 12 and 13 months (one of them underwent consolidation ASCT) whereas the patient with MM and AL-A had a relapse of MM at 19 months without clinical amyloidosis. As for toxicity, two cases of grade 2 and 3 polineuropathy, one case of grade 2 mucositis and one case of grade 2 nausea and vomiting have been observed; all of them responded to temporary suspension and/or subsequent treatment dosage reduction. A case of uncomplicated herpes zoster occurred. *Conclusions.* The experience with Bortezomib in AL-A remains limited. In our AL-A patients, BD has been feasible and three of them have achieved haematologic and organ responses with manageable toxicity. Further research is required to optimize the application of BD in this setting. *Off label use.* Bortezomib for the treatment of AL amyloidosis.

1109

BORTEZOMIB PLUS DEXAMETHASONE IN COMBINATION WITH MELPHALAN OR CYCLOPHOSPHAMIDE IN THE TREATMENT OF PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

P. Tsirigotis, K.G Girkas, V. Giannopoulou, S. Chondropoulos, E. Ioannidou, G. Papaxoinis, V. Pappa, D. Vasilatou, K. Dimoula, N. Kapodistrias, E. Papageorgiou, T. Economopoulos, J. Dervenoulas

ATTIKON General University Hospital, ATHENS, Greece

Background. Vortezomib (V) is the first proteasome inhibitor with significant activity in multiple myeloma (MM). The efficacy of V is increased when used in combination with dexamethasone (D). Moreover, preclinical studies using multiple myeloma cell lines showed that V sensitizes myeloma cells to the cytotoxic effects of various antineoplasmatic agents including alkylators. *Aims.* In our study, we retrospectively evaluated the efficacy of V in combination with D plus an alkylator in a cohort of patients with relapsed and/or refractory MM. The primary objectives of our study were to evaluate the response rate, as well as to determine the time to treatment failure (TTF), and overall survival (OS). *Methods.* 21 patients (14 females and 7 males) with relapsed or refractory MM were included in our study. Median age was 58 years (range, 44-80). The median number of previous therapeutic regimens was 2 (range, 1-6). Six patients had previously undergone autologous stem cell transplantation (Auto-SCT). Study design: patients were scheduled to receive 8 cycles of the combination consisted of bortezomib, dexamethasone, and an alkylator (melphalan or cyclophosphamide). Each cycle consisted of 1) bortezomib 1,3 mg/m^2; 2) dexamethasone 40 mg, and 3) melphalan 5 mg/m^2, or cyclophosphamide 200 mg/m^2 administrered on days 1,4,8,11 and followed by a rest period of 11 days before the administration of the next cycle. The preferred alkylator was melphalan because of its well known antimyeloma activity. However, for patients presenting with platelet count of less than 100.000, as well as for patients eligible for future autologous stem cell transplantation melphalan was substituted by cyclophosphamide. Monitoring of response was assessed according to European Group for Blood and Marrow Transplantation (EBMT) criteria. *Statistical analysis.* Time to treatment failure (TTF) was calculated from the date of initiation of treatment to the date of disease progression, unacceptable toxicity or death and overall survival (OS) was determined from the date of initiation of treatment to the date of death. Kaplan-Meyer curves were plotted for TTF and OS. *Results.* The overall response rate was 71%. In summary 7 patients achieved CR, 6 patients achieved PR, while 2 patients achieved MR. Treatment was not completed in 2 patients due to unacceptable toxicity, in 4 due to disease progression, while 3 patients with stable disease underwent Auto-SCT due to preference of the referring physician. Toxicity was manageable except in 2 patients (1 developed grade 3 neurotoxicity, and 1 developed an acute psychotic episode that resolved after discontinuation of treatment). With a median follow-up period of 12 months, the observed median TTF was 9 months, while the estimated median OS was 14 months. The 3 patients who underwent Auto-SCT before the completion of treatment were not included in the statistical analysis for the estimation of TTF (Figure 1). *Conclusions.* The combination regimen used in our study resulted in an overall response rate of 71%, despite the fact that most of our patients had refractory disease and were heavily pretreated. It will be reasonable the combination V-D plus an alkylator agent to be further tested in patients with untreated MM.

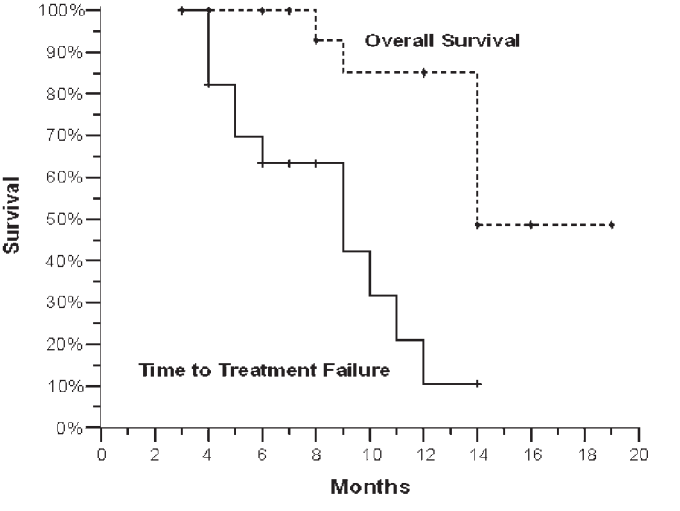

Figure 1. OS and TTF curves.

1110

COMPARISON OF PROGNOSTIC SIGNIFICANCE OF SELECTED BIOLOGICAL PARAMETERS IN MULTIPLE MYELOMA IN THE CASE OF CONVENTIONAL CHEMOTHERAPY OR HIGH DOSE THERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION

V. Scudla,[1] T. Pika,[1] M. Budikova,[2] J. Minarik,[1] J. Bacovsky,[1] K. Langova[3]

[1]Department of Haematology, OLOMOUC; [2]Department of Clinical Biochemistry, OLOMOUC; [3]Department of Medical Biophysics, OLOMOUC, Czech Republic

Background. Multiple myeloma (MM) is an unusually heterogeneous disease with individually different course, response to therapy and prognosis. As the result of significant progress in the treatment of MM with conversion to high dose chemotherapy with autologous stem cell transplantation (HD-T/ASCT) there is an emerging question of the assessment of prognostic significance of the parameters, expressing the intrinsic biological properties of myeloma cells and the microenvironment of the bone marrow. *Aims.* The aim of this study was to compare 12 selected biological parameters in the groups of patients treated with the use of conventional therapy (CT) *vs* HD-T/ASCT. *Methods.* The analysed group consisted of 140 patients evaluated at the time of MM diagnosis before start of therapy. The group was formed by 85 patients treated with CT: MP, VAD or VBMCP regimens *vs* 55 patients treated with HD-T/ASCT. For the assessment of serum levels of examined molecules were used: ELISA, REA, RIA and the technique of quantitative sandwich enzymatic immunoassay. Statistical analysis was carried out using Log rank, Kaplan-Meier and Pearson Chi-Square test (p<0.05). *Results.* In the

group of patients treated with CT, but not in the HD-T/ASCT group, there was a significant difference in the evaluation of prognostic significance of different ranges of serum levels of β2-microglobulin (2.3 mg/L, p=0.011 *vs* 0.43; 3.5 mg/L, p=0.002 *vs* 0.35 and 5.5 mg/L, p=0.001 *vs* 0.13) and thymidinkinase (10 IU, p=0.026 *vs* 0.91; 20 IU, 0.009 *vs* 0.16). A statistically significant difference in the prognosis of patients treated with CT *vs* HD-T/ASCT was found also in the evaluation of abnormal serum levels of following biological markers: IL-6R (p= 0.046), ICTP (p= 0.004), osteoprotegerin (p=0.003), syndecan-1/sCD138 (p= 0.032). In the case of VEGF there was no relation of higher levels to prognosis in the group of patients treated with CT, but there was a relation in the group treated with HD-T/ASCT (p=0.036). In both of the analysed groups, i.e. treated with CT or HD-T/ASCT there was no relationship to prognosis in the case of elevated serum levels of molecules of ICAM-1, VCAM-1, PINP, HGF and Fas. *Conclusions.* The presented study implies that the HD-T/ASCT significantly supresses the prognostic value of all parameters that administered significant prognostic potential with the use of conventional chemotherapy, i.e. β2-microglobulin, thymidinkinase, IL-6R, ICTP, OPG and syndecan-1. These molecules are therefore not suitable for prognostic stratification of patients treated with HD-T/ASCT. The study implies, that the future stratification systems of MM will probably be based on the molecular-biological parameters.

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1111

BORTEZOMIB , PEGYLATED LIPOSOMAL DOXORUBICIN AND DEXAMETHASONE (B-PEGHLD-D) AS THERAPY FOR PATIENTS WITH RELAPSED REFRACTORY MULTIPLE MYELOMA

A. Gozzetti, E. Marchini, A. Fabbri, M. Bocchia, M. Defina, F. Lauria

Ematologia e Trapianti, SIENA, Italy

Background. There are evidences supporting the existence of a synergism between the proteasome inhibitor bortezomib and anthracyclines. In addition, several *in vivo* data show synergic-additive effect of bortezomib and pegylated liposomal doxorubicine (Peg LD). Recently these findings were confirmed by the results of a phase III study. *Patients and therapy*. Based on these findings we are using this combination as salvage therapy in active multiple myeloma (MM). 22 MM patients (F:M 12:10, median age 61 years, range 45-76) with a disease resistant to or relapsing after high or conventional dose chemotherapy have been treated. 19 pts had IgG k/L (10/9); 1 pt had IgA/k, 2 pts had a Bence Jones k/l(1/1). β2-microglobulin was 3.5 (range 1.4-12.1). Treatment was started at a median of 50 months from diagnosis (range 1-120). Patient distribution according to disease status and previous therapy was as follows: 13 patients had an untreated relapse (UR); 5 patients primary refractory (PR) and 4 refractory relapse (RR). A median of 4 lines of chemotherapy were received (range 1-6), with 8 patients with more then 5 lines of CHT. 10 patients were relapsing after autologous stem cell transplantation (ASCT) . Interestingly 5 patients had a disease previously refractory to antracyclines (VAD therapy), and 5 patients had an extramedullary localization of myeloma. Bortezomib 1,3 mg/m^2 was given as a bolus IV injection on days 1,4,8 and 11 every 3 weeks PegLD was given IV at a dose of 30 mg/m^2 on day 4 every 3 weeks, desamethasone was given IV 40 mg on days 1-4 every 3 weeks. *Results.* Patients received a median of 4 cycles (range 2-4). B-PeghLD-D therapy resulted in 18 objective responses for an overall response rate (ORR) of 81% according to EBMT criteria. In particular we observed 8CRs and 2 nCRs (45%); 4 VGPRs and 5 PRs. Seven patients received less then 4 cycles (4NR, 3 for toxicity). Four patients had bortezomib dose reduction to 1mg/m2. Median duration of response was 7 months (range 2-12) and all 9 patients with less then PR relapsed. By contrary 11/13 patients with more then VGPR still maintain a response at median of 8 months (range 4-12). 4/5 VAD refractory obtained a response. 2/2 patients adequately mobilized stem cells for ABMT.Toxicities were mild to moderate in most of the patients and maneageble. Grade 3-4 thrombocytopenia and neutropenia occurred in 7/22 patients (29%), but only 2 developed febrile neutropenia 13 patients (58%) complained grade 1-2 paresthesias, 3 pts (12%) had HSV reactivation, 1 hand-foot syndrome. *Conclusions.* Bortezomib-PegLD-D combination is highly effective in resistant-relapsing MM with an ORR of 81% and 45% CR, nCRs, also in antracyclines refractory patients. Best and more durable responses are seen in patients treated with less then 3 lines of previous chemotherapy. Toxicities are acceptables and maneagebles. Stem cells mobilization is in our small experience feasible.

1112

RECOMBINANT HUMAN ERYTHROPOIETIN IS ASSOCIATED WITH REDUCED OVERALL SURVIVAL IN NEWLY DIAGNOSED PATIENTS WITH MULTIPLE MYELOMA: A SINGLE CENTER EXPERIENCE IN 312 PATIENTS

E. Katodritou,[1] E. Verrou,[2] C. Hadjiaggelidou,[2] V. Gastari,[2] A. Spyrou,[2] P. Konstantinidou,[2] A. Lazaridou,[2] N. Konstantinou,[2] K. Zervas[2]

[1]Theagenion Cancer Center, THESSALONIKI; [2]Department of Hematology, THESSALONIKI, Greece

Background. Anemia is a common clinical problem which affects quality of life and survival in patients with multiple myeloma (MM). The administration of recombinant human erythropoietin (R-HuEPO) induces response of anemia in about 2/3 of MM patients. Several recent studies have addressed the possible negative impact of R-HuEPO on overall survival, particularly in patients with solid tumors. However, the influence of R-HuEPO to overall survival of MM patients has not been sufficiently explored. *Aims.* The aim of this study was to explore the possible impact of R-HuEPO administration on the overall survival of newly diagnosed patients with MM. This is the second reported study, which attempts to clarify this important issue in the research field of MM. *Methods.* Three hundred twelve newly diagnosed symptomatic MM patients, on chemotherapy, 177 males and 135 females, with a median age of 67 years (range 29-90) were evaluated. R-HuEPO was administered when Hemoglobin levels (Hb) were less than 10.5 g/dL and it was titrated and discontinued when Hb levels reached 13 g/dL. The parameters evaluated for predicting survival were: Age, sex, Hb, platelets, bone marrow infiltration, serum creatinine, ISS score, β2-microglobulin (β2M), response to MM treatment and R-HuEPO administration. Patients' characteristics were compared with the Mann Whitney-U test and χ^2 test. Cox regression model was used for multivariate analysis and evaluation of Hazard ratios. *Results.* One hundred sixty-six patients received R-HuEPO and 146 did not. The median duration of R-HuEPO administration was 6 weeks (range 4-10) and the median hemoglobin levels of patients who received R-HuEPO, was 9.2g/dl (range 7.3-10.5). Patients in R-HuEPO group, were older and had a higher ISS score, higher β2M and serum creatinine and lower hemoglobin levels (p<0.01, for all parameters). Univariate analysis resulted that, age, Hb, platelets, serum creatinine, ISS score, β2M and R-HuEPO administration predicted for survival (p<0.05, for all parameters). The multivariate analysis demonstrated that, age, LDH, β2M and R-HuEPO administration were independent predictors for survival (p<0.05 for all parameters), while Hb, platelets, ISS and serum creatinine were non-significant. Hazard ratios for age, LDH, β2M and R-HuEPO administration were: 1.04 (95% CI: 1.007-1.075), 1.001 (95% CI: 1.000-1.001), 1.025 (95% CI: 1.002-1.049) and 2.092 (95% CI: 1.170-3.742), respectively. With a median follow up of 30 months (range 1-231), the median survival of patients in the R-HuEPO group was 36 months (95% CI: 30-42) whereas in the group without R-HuEPO administration it was 67 months (95% CI: 51-83) (p<0.001). *Summary and Conclusions.* These results suggest that, R-HuEPO administration may negatively influence overall survival, in newly diagnosed patients with MM. The observation that, baseline Hb which is considered as a strong predictor of survival in MM, did not predicted for survival in our study group, stressed the importance of the negative R-HuEPO impact on MM patients' survival, demonstrated in the current study. Taking into account our results and the growing evidence, concerning the role of R-HuEPO in cancer growth, we suggest that, R-HuEPO should be used with cautious in cancer-related anemia, including MM, following strictly the international guidelines for cancer and treatment-related anemia.

1113

THE IMPACT OF READY ACCESS TO NOVEL THERAPIES ON THE SURVIVAL OF AN UNSELECTED POPULATION-BASED COHORT OF PATIENTS AGED 60 AND YOUNGER DIAGNOSED WITH MULTIPLE MYELOMA IN REGIONAL HOSPITAL IN IRELAND (2001-2007)

J. Krawczyk,[1] M.B. Kelly,[1] P. Hayden,[2] K. Perera,[1] G.M. Crotty[1]

[1]Tullamore Regional Hospital, TULLAMORE; [2]Galway University Hospital, GALWAY, Ireland

Background. Novel therapies have been shown to contribute to the improved long-term survival now seen in patients with multiple myeloma (MM). The most significant impact of these novel therapies was observed in patients under the age of 60 (Blood, 2008 111: 2521-2526). The national Health Service Executive (HSE) in Ireland provides clinicians with generally unrestricted access to novel therapies such as thalido-

mide, bortezomib and lenalidomide. *Aims.* Our study aim was to examine whether the excellent results reported in the setting of clinical trials were translating into an improvement in overall survival in an unselected population of younger patients with MM. *Methods.* A retrospective audit of the demographics, treatment modalities and outcome for patients with MM diagnosed and treated at Tullamore Regional Hospital (catchment area 300,000) between 2001 and 2007 was performed. Comprehensive data was collected from both the Patient Medical Records and the hospital Laboratory Information System. We analysed data for all patients aged 60 and younger. *Results*. Between 2001 and 2007, 86 patients were diagnosed with MM (53 M, 33 F; M: F ratio 1.60). The mean age of the patients at diagnosis was 68 (68.6 and 67.8 for males and females, respectively). A total of 23(28%) of these patients were 60 years of age or younger at diagnosis (mean age 50.6). This cohort included 14 males (mean age 49) and 8 females (mean age 53). Durie-Salmon staging revealed that 22% of those patients were stage 1, 9% of patients were stage 2 and 68% were stage 3, respectively. Most (15/23 (65%)) of them had received novel agents during their disease course (thalidomide - 9/23 (39%), bortezomib - 4/23 (17%) and 2/23 (9%) both agents). Three (13%) patients received lenalidomide. All 3 had received either thalidomide or bortezomib previously. A total of 16 (70%) of these 23 patients underwent autologous stem cell transplantation. In this unselected younger cohort, the overall survival at 5 years is 58% and the median survival is 62 months. *Conclusions.* In this unselected cohort of young patients with MM treated in a Regional Hospital setting, the utilisation of novel therapies and autologous stem cell transplantation has resulted in a median overall survival of over 5 years, consistent with international reports of improvements in OS for younger patients with MM.

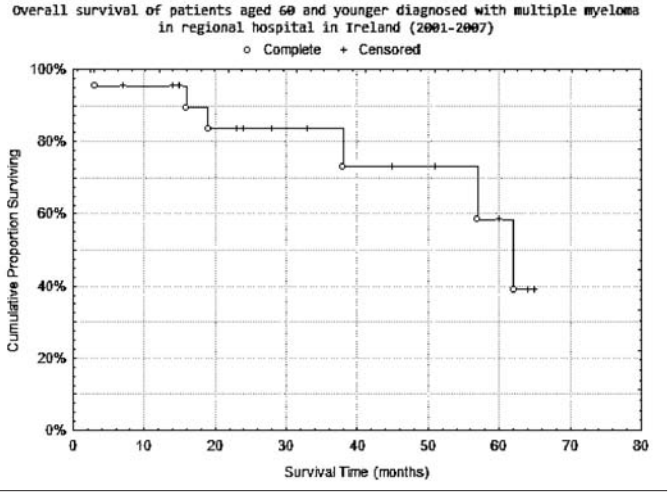

Figure 1. Overall survival in patients aged 60 and younger.

1114

18F-FDG PET/CT CONTRIBUTION IN THE ASSESSMENT OF RESPONSE IN PATIENTS WITH MULTIPLE MYELOMA: PRELIMINARY DATA

E. Lucia,[1] G.L. Cascini,[2] M. Gentile,[1] C. Mazzone,[1] E. Vigna,[1] M.G. Bisconte,[1] C. Gentile,[1] O. Tamburrini,[2] F. Morabito[1]

[1]UO di Ematologia AO di Cosenza, COSENZA; [2]Dipartimento di Diagnostica per Immagini, Università della Magna Graecia, CATANZARO, Italy

Background. New imaging techniques, such as whole-body (18)F-FDG PET/CT, have been introduced to assess the extent and severity of disease in multiple myeloma (MM) patients. Preliminary data suggest that FDG PET allows to detect both medullary and extramedullary disease in MM patients. The aim of our study was to evaluate the contribution of (18)F-FDG PET/CT to the standard criteria in assessing MM patients response to therapy. *Methods.* MM patients who achieved a ≥ PR from July 2007 to September 2007 entered in this study. Response evaluation have been made according to standard EBMT criteria. All patients underwent whole-body (including skull, upper limbs and femora) 18F-FDG PET-CT. Non-physiologic areas of increased 18F-FDG uptake were considered positive for MM bone involvement if the standard uptake volume (SUV) max based on body weight was >2.5 bone lesion was recognized on the corresponding CT images. *Results.* Nine patients were enrolled in our study, 3 males and 6 females. Median age was 71 years (range 49-85). Median number of prior therapy was 2 (range1-4). Five patients were without treatment respectively from 21, 15, 11, 6 and 2 months, while the remaining 4 cases were on maintenance therapy with thalidomide. According to the standard criteria, 5 patients were in complete response (CR) and 4 in partial response (PR). Overall, the whole-body (18)F-FDG PET/CT was positive in 2 cases (all with focal uptakes). All CR patients showed a negative PET/CT with no progression after a median follow-up of 6 months. Conversely, 3 out 4 PR cases progressed, two of them showed a positive (18)F-FDG PET/CT. In particular, one patient showed 4 focal lesions (3 in D7-D8-D9 vertebrae and 1 in the right scapula) while 1 focal lesion in D9 vertebra was demonstrated in the second case. Moreover, the two PR cases with positive (18)F-FDG PET/CT progressed earlier (2 and 4 months), as compared with the only PR case with a negative PET/CT (6 months). *Conclusions.* Our preliminary data failed to add supplementary information to EBMT criteria of response. However, additional cases and a longer follow-up are needed for a better definition of the role of 18 F-FDG PET-CT in predicting time to progression in patients achieving PR. However, the PET-CT predictive value should be assessed in a more sizeable number of cases perhaps integrated with magnetic resonance imaging.

1115

DOSE-ADJUSTED EPOCH PLUS RITUXIMAB (DA-EPOCH-R) REDUCES THE RISK OF CNS RELAPSE IN PATIENTS WITH HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): A SINGLE CENTRE EXPERIENCE

J. García-Suárez, D. De Miguel, Y. Martín, J.J. Gil-Fernández, T. Pascual, H. Magro, M. Lopez-Rubio, C. Burgaleta

Príncipe de Asturias University Hospital, ALCALÁ DE HENARES, Spain

Background. Although the rates of isolated CNS relapse for all diffuse large B-cell lymphoma (DLBCL) patients treated with modern regimens range from 1 to 2.5% subgroups can be identified with risks as high as 20%. High risk scenarios where CNS prophylaxis is most commonly offered include either an increased IPI (or to the factors forming part of it), or disease involving testicle, sinus or breast location.Up to date there is no general agreement on the optimal CNS prophylaxis. Two reports from clinical trials (Feugier *et al.* Ann Oncol 2004,15:129; Boehme *et al.* Ann Oncol 2007,18:149) suggested that administration of systemic etoposide was associated with a lower risk of CNS relapse. *Aims.* The present study aims to evaluate the rate of CNS relapse in patients with high risk-CNS DLBCL treated with an etoposide containing chemotherapy regimen (DA-EPOCH-R: etoposide, vincristine, doxorubicin, bolus cyclophosphamide, prednisone and rituximab). *Methods.* We retrospectively review a cohort of 32 consecutive DLBCL patients (median age 52 years; range 21-74) with high risk-CNS (31 cases had an aaIPI ≥ 2, 8 cases had a raised LDH and > 1 extranodal site involvement, and 1 presented involvement of breast). All patients were treated at our institution with inpatient DA-EPOCH-R (6-8 cycles) between 2002 and 2007. Prophylaxis of CNS disease was not part of the treatment protocol. *Results.* A total of 30 patients out of 32 patients (93%) achieved a CR following treatment. Consolidation radiotherapy was administered to 5 patients with bulky disease, all of them were in CR (18FDG-PET scan negative) after DA-EPOCH-R and were irradiated at the site of prior bulky. The entry-dose level of etoposide was 200 mg/m^2 and the average relative dose intensity was 133%. So far, after a median follow-up of 32 months (range 5-51) only 1 patient (3.1%), with an aa IPI=3 and a combination of an elevated LDH and >1 extranodal site of disease, showed an isolated brain parenchyma relapse 12 months after diagnosis. Three courses of systemic high-dose MTX chemotherapy were given; however, the patient died 4 months later because neurological progression. *Conclusions.* The risk of CNS relapse seen in our series was low (3.1%), although CNS prophylaxis was not administered. Administration of more intensive induction therapy as well as use of etoposide, an agent crossing the blood-brain barrier, could explain the lower number of isolated CNS occurrences with DA-EPOCH-R. Thus, a general prophylaxis for all high risk-CNS patients treated with DA-EPOCH-R is not warranted. More sensitive detection for occult CNS disease might assist in the identification of patients at risk for CNS relapse. These encouraging observations must be confirmed in further randomized trials.

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PRIMARY CUTANEOUS LYMPHOMA: OUR EXPERIENCE IN THE LAST 11 YEARS

M.E. Zerga, S. Cugliari, G. De Stefano, J. Caffaro, A Moreno, R Del Aguila

Roffo Institute, BUENOS AIRES, Argentina

Introduction. The primary cutaneous lymphoma (PCL) are non

Hodgkin´s lymphomas (NHL) with fenotype T or B, whose first manifestation are skin lesions, without evidence of disease in another sites during the first 6 months until the diagnosis. They are the second localization of extranodal NHL (approximately 2% of the whole group of NLH) We analyzed our experience over the past 11 years with patients (p) with PCL *Objetives.* To analyse the distribution of the anatomopathologic subtypes in the overall group, presentation, its relationship with other non-hematologic malignancies, topical and/or systemic treatment, and evolution (rate of response, progression free survival, overall survival, histological transformation) *Patients.* We evaluated 46 ptes, 24 woman and 22 men. Ages between 22 and 90 years (mean: 57.4 years) *Results.* Incidence of subtypes: Cutaneous T-cell lymphomas (CTCL) 34 p (73.9%): mycosis fungoide (MF) 21 p (45.6%), large-cell CD 30^{+} anaplastic 8 p (17.4%), large cell CD 30 + inmunoblastic 1 p (2.17%), peripheral T 4 p (8.7%). Cutaneous B-cell lymphomas 12 p (26.1%): large cell lymhoma 4 p (8.7%), large cell leg type 1 p (2.17%), centrefollicular lymhoma 4 p (8.7%), marginal zone 3 p (6.52%). A patient presented with diagnosis of MF and peripheral T lymhoma. 1) Treatment: topical: 10 p (21.7%), systemic: 5 p (10.8%), both: 31 p (67.4%). 2) Hystological transformation 1 p (2.17%). 3) Non-hematologic malignancies concomitant 4 p (8.69%). 4) Overall survival: of the 33 p with follow up (71.7%), the overall survival a two years is 84.8% (28 p). *Conclusions.* Distribution of subtypes AP, age and survival are similar to the published, but not the incidence by gender. It hightlights the low percentage of transformation to aggressive lymphoma and hight frecuency of ptes with PCL and other non hematological malignancy associated, which might depend on the population of patients in our institution.

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18F-FDG PET SCAN AT EARLY STAGE OF THERAPY WILL PREDICT THE PROGNOSIS OF DIFFUSE LARGE B CELL LYMPHOMA

Y. Shimazu, T.I Ishimori, N. Kenji, Y. Fujiwara, T. Ito, S. Morita, A. Sato, T. Sato, T. Maeda, T. Onishi, C. Mizutani, F. Matsuyama, C. Tsukayama, Y. Watanabe, Y. Ueda

Kurashiki Central Hospital, KURASHIKI, Japan

Aims. To evaluate 18F-FDG PET (PET) scan for determining the effect of therapy and predicting the prognosis of diffuse large B cell lymphoma (DLBCL). *Methods.* Between July 2006 and June 2007, 35 cases were newly diagnosed as having DLBCL at our institute. Among these, 26 cases that had undergone PET scan during one to four cycles of chemotherapy and had completed the treatment course were analyzed. The primary end point was event-free survival (EFS). Survival curves were analyzed by the Kaplan-Meier method and log-rank test. Standardized uptake values (SUV) were used to assess the results of PET scan. Two doctors judged cases as PET-positive or PET-negative by comparing the maximum SUV of the tumor with those of median pool. The maximum tumor size on computed tomography (CT) scan before treatment was compared with that during treatment. Reduction of tumor size by less than 50% was defined as stable disease (SD), by more than 50% was defined as partial response (PR), by more than 75% was specially defined as good partial response (good PR) and undetectable tumor was defined as complete remission (CR). Four patients could not undergo PET scan because of poor performance status. *Results.* Median patient age was 73 years old (range: 42-88). Risk analyses by the international prognostic index (IPI) of these 26 cases were as follows: low in 6 cases, low intermediate in 3 cases, high-intermediate in 7 cases, and high in 10 cases. Median follow-up periods were 380 days. Final clinical outcome was complete remission in 20 cases, relapse in 5 cases, and 3 patients died of disease. PET scan during the early phase of therapy showed that18 cases were PET-negative and 8 cases were PET-positive. Sixteen cases were determined as CR, 4 cases as good PR, 10 cases as PR on analysis of CT alone. Three cases showed discordant results on PET and CT; namely, PET-positive with CR or good PR on CT. Among these, 2 patients achieved complete remission, but 1 patient has died of disease. Five cases also showed discordant results on PET and CT; namely, PET-negative with PR on CT. All these patients achieved complete remission. Two cases were PET-negative but later developed a relapse in the central nervous system. EFS of the PET-negative groups were significantly better than those of PET-positive groups (p=0.0147). The sensitivity and specificity of PET scan during early therapy for predicting long-term remission were determined as 89% and 50%, respectively. On evaluation of 11 cases in which PET scan was performed after 2 cycles of chemotherapy, the sensitivity and specificity of PET scan was raised as 100% and 75%, respectively. These numbers were considered superior to the sensitivity and specificity of CT, 81% and 30%, respectively. *Conclusions.* PET-negative groups showed significantly better EFS compared with the PET-positive group (p=0.0147). Further analyses in a larger series with long-term follow-up are needed to conclude that 18F-FDG PET scan is superior to CT scan for predicting the prognosis of DLBCL.

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LAPAROSCOPIC DIAGNOSTICS OF INTRAABDOMINAL AND RETROPERITONEAL LYMPHADENOPATHIES

F. Indenko,[1] I.Y. Yevstakhevich,[1] F.P. Indenko,[2] Y.L. Yevstakhevich,[1] M.M. Semerak,[1] T.V. Leshchuk,[1] Y.I. Vyhovska,[1] V.E. Loginsky[1]

[1]Research Institute of blood pathology and transfusion medicine, LVIV; [2]Medical University, LVIV, Ukraine

Background. Diagnostics of lymphadenopathies is based on the analysis of hemogram, bone marrow smear, biopsy of peripheral lymphatic nodes, and immunodiagnostics as well as on the data of instrumental methods, including ultrasound assessment and computer tomography. However, there are patients with isolated lesions involving lymph nodes of chest, abdominal cavity and retroperitoneal space with no signs of peripheral lymphadenopathy. Access to such lymph nodes is complicated. Transcutaneous needle biopsy is often not quite informative, difficult to perform, while it is also dangerous in some cases. Introduction of laparoscopic *Methods.*for diagnostics of such lymphadenopathies would yield very beneficial results. *Aims.* An option of video-surgical diagnostic excision biopsy of intra-abdominal and retroperitoneal lymphatic nodes in patients with lymphadenopathy of unknown origin without enlargement of peripheral lymphatic nodes and no abnormalities in hemogram and bone marrow smear. *Methods.* 18 laparoscopic diagnostic lymphadenectomies for isolated enlargement of lymphatic nodes of abdominal cavity and retroperitoneal space were carried out in the Department of Surgery during 2004-2007. There were 11 male and 6 female patients aged from 26-69 years. *Results.* Affected intra-abdominal lymphatic nodes were mostly located in the area of hepato-gastric and hepato-duodenal ligaments as part of the caul along the big stomach curvature. Lymph nodes were resected for examination by removing the surrounding peritoneum, isolating a vascular pedicle (with both clamping and coagulation using a bipolar device), and subsequent cutting off the body of node. Pathologic changes of retroperitoneal lymphatic nodes were predominantly consistent with enlargement of para-aortic nodes and (sometimes) colic ones. These nodes can be best reached for biopsy from under the mesentery of transverse colon. Therefore, an extra trocar for retractor was used to move both the caul and intestine upwards. Affected retroperitoneal lymphatic nodes were much larger than intra-abdominal ones. As large lymph nodes are better vascularized and located along big vessels in the retroperitoneal space, a resection of such nodes was undertaken. Peritoneum above the lymph node was dissected using bipolar scissors, its frontal and lateral sides were isolated by dissection, and the frontal half of the node body was cut off for examination. The shear surface of the remaining part was coagulated. Using trocars, nodes were removed along with resected fragments (up to 15 mm) without damaging their structure. Tissue samples, collected at biopsy, were sufficient for cytological, histological, immunologic and cytogenetic investigation. Diagnostic laparoscopic lymphadenectomy revealed lymphoproliferative disorders in all patients studied. Neither intra/postoperative complications nor conversions were noted. In-hospital stay lasted 2-3 days. *Conclusions.* Therefore, laparoscopic diagnostic excision biopsy of diseased intra-abdominal and retroperitoneal lymphatic nodes is a safe and effective method of diagnostics.

1119

INFECTIOUS COMPLICATIONS ASSOCIATED TO FIRST LINE STANDARD CHEMOTHERAPY IN A COHORT OF 320 UNSELECTED NON-HODGKIN LINFOMA PATIENTS

J.J. Alonso, G. Barreiro, A. Canovas

Hospital de Cruces UPV, BARACALDO (VIZCAYA), Spain

Background. The incidence of chemotherapy related infection infectious events is usually determined in multicentric trials where selection bias can occurs, making thus these results not applicable to non selected patients. *Aims.* To asses the frequency of severe and febrile neutropenia, CSF prescription, infectious events and related variables on the setting of first line chemotherapy of a non selected population of patients with non-Hodgkin lymphomas(NHL). *Methods.* Prospective observational study of all NHL patients in first line standard chemotherapy at a University Hospital Internal Medicine Lymphoma Unit since 1996-January until 2007-June.The patients were followed since chemotherapy beginning until four weeks after last chemotherapy cycle and neutropenia

end. Filgrastim was prescribed according to ASCO guidelines and ciprofloxacin when 4th grade neutropenia was expected. Blood counts were scheduled next to expected nadir and until neutrophils figure was upper than 500/µL.Variables analysed: demographics, prognostic, filgrastim prescription, 4th grade and febrile neutropenia, febrile events, ethiology of infections and mortality. Infectious events requiring antimicrobial therapy were considered and classified as blood-stream infections(BSI), pneumonia, urinary tract infection(UTI), fever of unknown origin(FUO) and others(OI). Statistical *Methods.* descriptive, Ã2, Fisher exact test, Student's T, Kruskall-Wallis and Cox logistic binary regression model. *Results.*320 NHL patients (mean age:60.5 years; 51% male and 49% female sex) were included. The distribution by lymphoma subtype was(n;%):43(13) WF A; 65(20) WF B-C; 24(7.5) mantle lymphoma;148(46) LBCL; 27(8) peripheral T and 13(4) high grade lymphoma. Filgrastim´s primary prescription was made in 10%, secondary in 43% and never in 47% of patients. 544 4th grade neutropenia (mean:1.7/patient;0.32/month) and 89 febrile neutropenia events, 4 deadly(4.5%), were recorded. 4th grade neutropenia was found in 64% of patiens (40-55% in low grade and 70-85% in the others subtypes) and febrile neutropenia in 20%(1.5-14% in low grade and 23-27% in higher grade lymphomas). 239 infections were observed (mean: 0.75/patient; 0.16/month), 50% microbiologically, 14% clinically documented and 36% FUO; 40(17%) blood-stream infections(BSI), 36(15%) pneumonias,32(13%)UTI and 44(19%) other infections.40% of patients suffered of infection. In 55% BSI Gram positive cocci and in 47,5% Gram negative bacilli were isolated.16 patients (5%) died of infection. Statistical associations: Filgrastim prescription with more advanced IPI/FLIPI and aggressive lymphoma (p<0.001); more advanced IPI/FLIPI or aggressive lymphoma and infection (p:0.0002); advanced IPI/FLIPI(p:0.00003) and aggressive lymphoma (p:0.0008) with febrile neutropenia. There was not an uneven distribution of infection type or aetiology except more frequent FUO in advanced IPI/FLIPI lymphoma(p:0.04) or B symptoms at diagnosis. Only B symtoms initiallly was related to infectious mortality(p:0.009). *Conclusions.* We detected a frequency of 4th grade neutropenia that was clearly superior to the reported to the scientific literature. Gram positive and Gram negative ethiology was balanced. 4th grade neutropenia, febrile neutropenia and infection probability were related to more aggressive lymphoma, advanced IPI/FLIPI and B symtoms at diagnosis. Only B symtoms reached statistical significance regarding infectious mortality.

1120

SAFETY AND EFFICACY OF R-CHOP14 IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) REDUCING GRANULOCYTE COLONY-STIMULATING FACTORS (GCS-F)

L. Rigacci,[1] B. Puccini,[2] L. Rigacci,[2] R. Alterini,[2] V. Carrai,[2] F. Bernardi,[2] A. Bosi[2]

[1]*Azienda Ospedaliero Universitaria Careggi, FLORENCE;* [2]*Department of Hematology Azienda Ospedaliero Universitaria Careggi, FLORENCE, Italy*

Bakground. DLBCL represents 40% of all non-Hodgkin lymphomas. CHOP plus rituximab administered every 14 days (R-CHOP14) has been considered the standard of care for young patients (pts) with DLBCL good-prognosis (MInT trial). The RICOVER-60 trial have recently demonstrated that 6 cycles of R-CHOP-14 could be considered the new standard for elderly pts with DLBCL. Dose-dense therapy is feasible with GCS-F support which is recommended for 10 days. *Aims.* this study was designed to evaluate the feasibility and the haematological toxicity of R-CHOP14 supported by G-CSF in pts with DLBCL. We also want to assess the possibility to reduce the number of GCS-F vials without increasing incidence of febrile neutropenia, hospedalizations and delays of therapy. *Methods.* we have included 45 pts with DLBCL and 5 with follicular lymphoma grade IIIb, median age was 62 years (range 34-79), 60% had an high-intermediate or high IPI. CHOP was administered every 14 days, preceded on day 1 by rituximab and followed by 7 (in the first period) or 5 days of G-CSF (filgastrim). Haematological toxicity and feasibility was calculated over 293 cycles administered. We have used 1246 GCS-F vials, 5 vials for cycle and a median of 25 vials (range 10-35) for every pts for complete treatment. *Results.* the programmed therapy was completed in 48 out 50 pts (96%); two pts swichted to a different therapy (CHOP every 21 days). Eleven cycles (3,8%) have been delayed in 9 pts for severe adverse events. Neutropenia grade 3-4 developed in 3% of cycles, febrile episodes in 1% of cycles, thrombocytopenia grade 3 or 4 in 1% of cycles and hospedalization in 1% of pts. Considering the 293 cycles, the median nadir of leucocyte was 3785×10^9/L (range 700-8400), the median nadir of haemoglobin was 11.1 gr/dl (range 5.8-15.5) and the median nadir of platelets was 146000 (range 47000-328000). The large majority of pts reported musculoskeletal pain. The complete remission rate and overall survival were 84% and 82% respectively. *Conclusions.* in our experience, the dose dense chemotherapy (R-CHOP14) with G-CSF support was feasible also in elderly pts. We have observed that the reduction from 10 to 5 GCS-F vials has not determined an increase of neutropenia, febrile episodes, delays and hospedalizations. We can conclude that in a dose-dense therapy the primary prophylactic use of G-CSF could be redcued to five or three vials per cycle. Moreover at the moment there are insufficient data to assess the impact of G-CSF on disease-free and overall survival.

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PROGNOSTICALLY STRATIFIED TREATMENT OF DIFFUSE LARGE B-CELL LYMPHOMA: 13 YEARS OF THE MORAVIAN EXPERIENCE

T. Papajik,[1] L. Raida,[1] V. Prochazka,[1] E. Faber,[1] J. Vondrakova,[1] A. Hlusi,[1] R. Urbanova,[1] Z. Kubova,[1] T. Szotkowski,[1] L. Kucerova,[1] M. Myslivecek,[1] M. Sticha,[2] T. Pavlik,[2] K. Indrak[1]

[1]*University Hospital, OLOMOUC;* [2]*Masaryk University, BRNO, Czech Republic*

Background. Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma, accounting for approximately one third of all new diagnoses. Anthracycline-based combined therapy has been the standard treatment for more than 25 years. The addition of rituximab to chemotherapy has significantly improved outcome in elderly and low-risk young patients (pts.) with DLBCL and some data have suggested that the early administration of rituximab-supplemented intensive chemotherapy may improve prognosis of poor-risk younger patients. *Aims.* To prove the efficacy of a treatment stratified according to prognostic factors for adults with DLBCL in the pre-rituximab and rituximab era. *Methods.* From May 1995 to June 2007, 313 pts. with newly diagnosed DLBCL were registered, staged and prognostically evaluated at the Hemato-oncology Dpt. Olomouc (CZ). Patients younger than 65 years were divided into low-risk, intermediate-risk and high-risk group and treated with standard CHOP-like, intensified PACEBO-like and intensive sequential chemotherapy (± rituximab). Patients older than 65 years were treated with CHOP-like regimen (± rituximab) if they had no major comorbidity, others were treated with only palliative intent. *Results.* 3-year overall survival (OS) were statistically different in pts. younger 45 years, pts. 45-64 years old and pts. older than 64 years (86% *vs* 75% *vs* 46%, p<0.001). Intensified and intensive chemotherapy significantly improved 3-year progression-free survival (PFS) and OS of intermediate-risk and high-risk pts. younger than 65 years (PFS 85% and 72%, p<0.001; OS 77% and 79%, p<0.001). 69 younger pts. with combination of poor-risk factors were treated with high-dose chemotherapy (BEAM) and autologous transplantation in first complete or partial remission. 3-year PFS and OS of these pts. were 77% and 89%. The addition of rituximab to chemotherapy significantly decreased the probability of relapse (10% *vs* 29%, p<0.001) and improved 3-year PFS a OS in all treated pts. (PFS 84% *vs* 63%, p=0.001; OS 84% *vs* 56%, p<0.001). *Conclusions.* Prognostically stratified therapy and the addition of rituximab markedly improved PFS and OS for DLBCL treated at the Hemato-oncology Dpt., Olomouc, CZ.

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ROLE OF FDG-PET IN T-CELL AND NK-CELL LYMPHOMAS

I. Vasova,[1] M. Navratil,[1] D. Salek,[1] K. Bolcak,[2] L. Smardova,[1] J. Mayer,[1] Z. Kral[1]

[1]*Masaryk University Hospital, BRNO;* [2]*Department of Nuclear Medicine, Masaryk Memorial Cancer Institute, BRNO, Czech Republic*

Bakground. Role of PET scan is extensively studied for B-cell neoplasms and recently PET findings were incorporated into the response criteria but there is lack of data for T and NK-cell lymphomas in this filed. *Aims.* We studied the utility of FDG-PET for staging, response assessment during or after first line therapy and it´s prognostic value for the patient´s outcome in T-NHL. *Methods.* We retrospectively reviewed results of FDG-PET in patients with T/NK-cell NHL treated in our department from 2003 to 2007. We treated 67 patients with T/NK-cell NHL except cutaneous lymphomas in this period. 31 patients (ALCL 15, PTCL 7, AILT 6, NK/T-L 2, T-NHL not specified 1) underwent PET scanning and were included into the retrospective study. PET scans were performed at initial diagnosis in13 pts, during or after the first line therapy in 28 pts

and at relapse in 5 pts. *Results.* All pts were PET positive in CT or other clinical investigation positive locations before initial treatment or at the clinicaly evident relapse. Only one negative initial PET scan was in patient with surgically removed solitary subcutanous lesion. So the positive rate of FDG-PET is 100%. Therapeutic response was evaluated with PET during or after the first line therapy in in 28 pts and 23 were PET negative. From these 23 negative patients 8 (29%) relapsed in median of 5,5 (3-48) months after their negative scan. 5 scans were positive after the treatment, 4 of these patients progressed and only 1 was false positive. *Conclusions.* All T/NK-cell NHL in our study were FDG avid, but predictive value of negative PET scan after the treatment is uncertain because one third of negative patients relapsed during further course of their disease.

1123

A RETROSPECTIVE STUDY TO ASSESS THE CLINICAL OUTCOME, BIOLOGICAL PATTERN AND TREATMENT OPTIONS IN PATIENTS WITH SPLENIC MARGINAL ZONE LYMPHOMA - A SHORT TERM ANALYSIS IN ONE CENTER

A.M. Vladareanu,[1] H. Bumbea,[1] A. Nicolescu,[1] M. Onisai,[1] D. Cisleanu,[1] I. Voican,[1] S. Radesi,[1] C. Ciufu,[1] V. Vasilache,[1] C. Marinescu,[1] M. Begu,[1] M. Dervesteanu,[1] O. Jercan,[1] C. Dobrea,[2] S. Neagu,[1] C. Savlovschi,[1] A. Stamatoiu,[1] H. Pantu[1]

[1]Emergency Universitary Hospital Bucharest, BUCHAREST; [2]Victor Babes National Institute of Development and Research, BUCHAREST, Romania

Background. Splenic marginal zone lymphoma (SMZL) is an indolent lymphoproliferation, which affects especially men over 30 years, possibly with associated hepatitis virus infection. Presentation typically includes splenomegaly, frequent bone marrow involvement, lymphocytosis ($CD19^+CD5^-CD10^-CD23^-$). *Aims.* The study will present the response in correlation with different treatment options and the importance of splenectomy in those patients. *Methods.* We performed a retrospective analysis of the 38 patients diagnosed with SMZL in the last 3 years in the Hematology Department. The diagnosis was according to WHO classification. We also performed flowcytometry analysis in cases with leukemic or bone marrow involvement. In lack of a standard therapy, we used several treatment *Methods. Watch and wait*, splenectomy (diagnostic and therapeutic), spleen radiotherapy, chemotherapy, and especially combined *Methods.* Three groups were established: 1) Splenectomy + chemotherapy: 6/38(16%); 2) Chemotherapy + splenectomy: 10/38(26%); 3) Chemotherapy: 22/38(58%) (8 monotherapy, 14 polichemotherapy). The parameters followed after treatment were: disappearance of lymphocytosis, adenopathias, splenomegaly, decreased bone marrow infiltrate, decreased LDH, treatment free period and performance status. *Results.* SMZL represented 5.71% of all NHL diagnosed in our Department. 36/38 patients (95%) were over 30 years old; 47% were men. All patients presented splenomegaly, which was tumoral in 58%; 24/38 (63%) had B symptoms; 34/38 (89%) were stage III-IV Ann-Arbor at diagnosis, 26 patients (68%) had medullar involvement and 22(58%) lymphocytosis; LDH was moderately increased in 20/38 cases(53%). 32 cases(84%) had typical marginal phenotype ($CD19^+CD20^+CD79a^+CD5^-CD10^-CD23^-sIgD^+CD25^-$) and 6 were atypical (with aberrant $CD5^+$). Performance status according to ECOG was good in most of the patients (28/38-74%). Autoimmune phenomena were present in 4 cases (AIHA); 4 patients had monoclonal serum protein. Splenectomy was indicated as first line of treatment in patients with abdominal symptoms and/or cytopenia; there were no major complications after the operation. Splenectomy improved performance status, decreased lymphocytosis, corrected the cytopenia, induced a debulking effect. The patients who underwent splenectomy as the first treatment option followed by chemotherapy had the most favorable evolution and the longest treatment free period (2.6 years). Splenectomy served in four cases as diagnostic procedure (onset with abdominal tumor). Chemotherapy included monotherapy, polichemotherapy, chemo-immunotherapy (chemo followed by maintain treatment with Rituximab or after splenectomy). Monoclonal antibodies (Rituximab) were administered in four cases, either only as polichemotherapy (R-CHOP) or after splenectomy (8 R-CHOP then maintenance treatment only with Rituximab). Spleen radiotherapy was performed in 8 cases (palliative). Patients with chronic hepatitis infection (6 cases) received alfa-interferon ± Ribavirin (in HCV-4 cases) or Lamivudin (in HBV/HBV+HDV-2cases), with good effect on hematological, hepatic and virus parameters. Complete response was obtained in 12/38 patients(32%); partial response-18/38 cases(47%). 8/38 patients(21%) had no response to treatment. *Conclusions.* The study shows the benefit of combined therapy, needed for a certain number of SMZL with a more aggressive evolution, especially those with an atypical phenotype. In selected cases, the best treatment option seems to be splenectomy followed by chemotherapy - complete response was obtained in all those cases.

1124

ATTENUATED CFU-EPC COLONY GENERATION FOLLOWING GRANULOCYTE COLONY STIMULATING FACTOR MAY BE A GRANULOCYTE-DRIVEN PHENOMENON WITH POTENTIAL TO INFLUENCE THE PREFERRED CELL SOURCE FOR VASCULAR REPAIR

J. Crawford, G.R. Barclay, M.L. Turner

SNBTS Cell Therapy Group, EDINBURGH, UK

Background. The endothelium plays a critical role in the maintenance of vascular integrity. Endothelial progenitor cells (EPCs) have been isolated from peripheral blood (PB). Controversy exists as to EPC origin and phenotype with many supporting a bone marrow haemangioblast source. Bone marrow has been proposed as a source of EPCs for clinical use. Haematopoietic stem cell transplantation is the paradigm for stem cell (SC) therapies. BM harvest is now rarely performed as peripheral blood stem cells (PBSC) are collected after mobilisation of SC from BM with cytokines (granulocyte colony stimulating factor (G-CSF)). G-CSF administration leads to neutrophil hyperplasia and protease release. This, together with reduced osteoblast SDF-1 production, disrupts the BM SC retention signal leading to SC mobilisation. Some authors propose that G-CSF-mobilised PBSCs hold potential for endothelial regeneration. Our group has observed depleted CFU-EPC activity in post G-CSF samples and we believe that current assays of endothelial potential may not support the use of unmanipulated mobilised PB mononuclear cells (MNC) in regenerative activities. *Aims*. To establish the phenotype of MNCs plated into culture pre and post G-CSF administration and explore whether there is evidence of granulocyte-induced alteration to colony forming potential. *Methods.* PB samples were obtained pre and post G-CSF from consecutive patients (autologous and allogeneic patients) referred for PBSC mobilisation and collection. MNC obtained from buoyant density separation were cultured into the CFU-EPC (Hill) assay using commercial reagents (Stem Cell Technologies). Whole blood and MNC immunophenotyping was performed. MACS CD66b selection was undertaken in 5 subjects to reduce MNC granulocytic content with the resulting fractions plated into CFU-EPC culture, following the same method as for standard MNCs. *Results.* Both allogeneic and autologous post G-CSF MNCs contained high levels of granulocytes compared to pre G-CSF samples (allogeneic pre G-CSF 2.6%, post G-CSF 32.3%; autologous 6.2% and 34.1% respectively). Erythrocyte contamination was observed in autologous patient samples pre and post G-CSF. This was less of a feature of allogeneic MNCs (Figure 1).

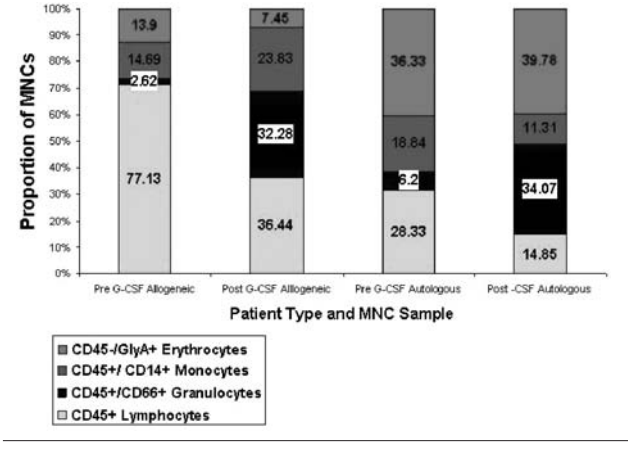


Figure 1. Changes in MNC content Pre and Post G-CSF administration.

Consistent with previous results by our group, CFU-EPCs were severely reduced post G-CSF administration. $CD66b^+$ granulocytes were reduced by a mean of 48.8% post MACS separation. Plating of MACS CD66b-depleted post G-CSF MNCs in CFU-EPC assay produced partial recovery of CFU-EPC activity. *Summary and Conclusions.* Published studies have emphasised the CFU-EPC colony-forming potential of $CD14^+$ monocytes with recent articles also suggesting the importance of lymphocyte subsets to colony formation. We have shown that MNCs obtained from mobilised PB contain significant levels of granulocytes that may inhibit CFU-EPC formation and that this is restored following

granulocyte depletion. Recent studies have queried the usefulness of the CFU-EPC assay as a measure of endothelial-potential and our data raises further questions. Our work suggests that this assay is susceptible to the influence of bystander cells and that CFU-EPC results do not necessarily reflect the presence of target cells.

1125

RADIOISOTOPE SYNOVECTOMY IN SEVERE HEMOPHILIAC PATIENTS WITH CHRONIC SYNOVITIS IN TARGET JOINTS: THE ONE YEAR EXPERIENCE OF CUKUROVA UNIVERSITY, MEDICAL FACULTY HEMOPHILIA TEAM

B. Antmen, I. Sasmaz, Y. Kilinc, C. Ozkan, M. Kibar, E. Kozanoglu, S. Kurdak, K. Bicakci, A. Polat, G. Leblebisatan

Cukurova University, ADANA, Turkey

*Background. and Aims.*The most common sites of bleeding in a person with hemophilia are the joints and muscles of the extremities. Once a joint develops recurrent bleeding episodes (target joint), chronic changes occur. Options for synovectomy include chemical or radioisotopic synoviorthesis and arthroscopic or open surgical synovectomy. Non-surgical synovectomy should be the procedure of choice for treating chronic hemophilic synovitis. Clearly, radioisotopic synovectomy using a pure beta emitter (phosphorus-32 or yttrium-90) is the most effective and least invasive. *Methods.and results.* In this study, we present 26 radioisotope synovectomy (RS) in 18 children, age ranging from 6 to 18 years with a average of 12 years in Çukurova University, Medical Faculty, hemophilia Team, Adana, Turkey. All patients except one patient with won willebrand disease had severe haemophilia A and B. One patient (2 joints) had high responder inhibitor.Of 26 target joints, 17 were knees, 7 were elbows, 2 were ankles. All patients and target joints were evaluated by the hemophilia team which is included hematologists, radiologist, orthopedics, phisical medicine- rehabilitation and nuclear medicine specialists, and sports physiologists.During the procedure, no complication was seen. *Summary*. Radioactive synovectomy seems to be a safe and effective treatment for chronic synovitis causing recurrent joint bleedings

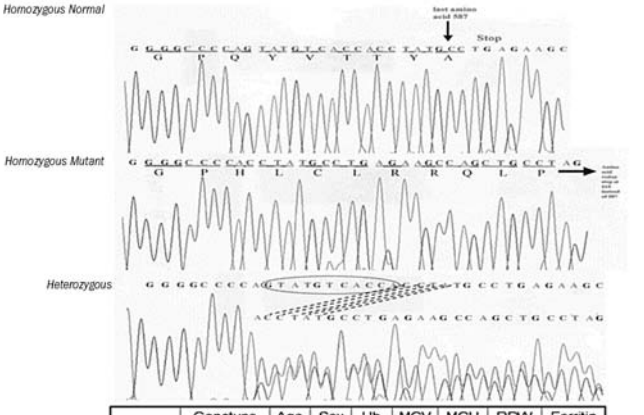
1126

A NOVEL MUTATION IN EXON 11 OF THE ALAS2 GENE(11BP DEL) RESULTS IN X-LINKED SIDEROBLASTIC ANEMIA

A. Al Madhani,[1] S. Alkindi,[2] S. Al Zadjali,[3] A. Pathare[3]

[1]Dept. of Medicine, Sohar Hospital, SOHAR; [2]College of Medicine & Health Science, SQU MUSCAT; [3]Sultan Qaboos University Hospital, MUSCAT, Oman

Background. Mutations in the erythroid-specific 5-aminolevulinate-synthase gene (ALAS2) have been identified in many cases of X-linked sideroblastic anemia (XLSA). It is a clinically heterogenous disorder characterized by ineffective erythropoiesis with hypochromic, microcytic anemia, splenomegaly, elevated tissue and serum iron, and ringed sideroblasts in the bone marrow. Females may also be affected by lyonization of the X chromosome. *Aims.* To investigate the underlying molecular mechanism in a proband referred with hypochromic microcytic anemia with raised serum ferritin. *Methods.* The proband was a 29year old ethnic Omani male, referred for evaluation of hypochromic microcytic anemia with hyperferritinemia. CBC revealed hemoglobin of 8.84 g/dL, hematocrit of 29%, mean corpuscular hemoglobin (MCH) 14.5 pg, a mean corpuscular volume (MCV) of 48 fl. and an RDW of 34.2%. Hemoglobin electrophoresis, liver and renal function studies were normal with raised S. ferritin. The mother of the proband, aged 61 years, and all other 10 siblings were also available for evaluation. The ALAS2 gene was evaluated by direct sequencing using the ABI 3100 Genetic analyzer. All 11 exons, including the exon-intron boundaries were sequenced using 9800 fast thermal cycler (Applied Biosystems) on standard fast PCR program. *Results.* The proband and two other male siblings were documented to have a homozygous 11-bp del in exon 11 resulting in a longer polypeptide of 615 aminoacids (Figure 1). The mother and four other female siblings were heterozygous carriers whereas, the remaining four siblings (1M;3F) had normal germline ALAS2 configuration. Complete blood counts and peripheral smears on the proband and his two siblings who had the 11bp deletion, demonstrating a dimorphic RBC population with marked hypochromic microcytic RBCs and raised serum ferritin (Figure1). Amongst the four normal siblings in the family, surprisingly two had hypochromic microcytic red cell morphology with low S. ferritin suggestive of associated iron deficiency anemia. Summary/*Conclusions.* We have identified a novel mutation[11-bp del] in exon 11 of the ALAS2 gene in the proband and his two brothers born of a consanguineous marriage. The same mutation was also found in heterozygous state in the proband's mother and his four female siblings. The 11-bp del resulted in a much larger protein which was probably dysfunctional, resulting in the clinical phenotype of sideroblastic anemia. The fact that all three affected patients were males; and all the 5 heterozygous subjects, including the mother were females, underscores the X-linked nature of inheritance in this inherited form of XLSA.



	Genotype	Age yrs	Sex	Hb g/dl	MCV fl	MCH pg	RDW %	Ferritin ng/ml
Proband	11-bp del	29	M	8.8	48	14.5	34.2	2716
Sib 1	11-bp del	30	M	7.4	47.4	13.2	34.5	5836
Sib 2	11-bp del	19	M	6.8	49.4	13.5	35.7	563
Mother	Heterozygous	61	F	12.8	84.6	26.4	15.4	ND
Sib 3	Heterozygous	33	F	12.2	78	24.7	16.6	ND
Sib 4	Heterozygous	17	F	10.1	71.2	21	16.7	ND
Sib 5	Heterozygous	15	F	9.6	66	19.6	18.4	ND
Sib 6	Heterozygous	12	F	11.7	77.5	23.9	13.4	ND
Sib 7	Normal	27	M	15.5	79.8	25.7	12.6	ND
Sib 8	Normal	22	F	12.7	75.3	23	15.7	ND
Sib 9	Normal	21	F	5.25	50.5	12.8	22.5	4
Sib 10	Normal	21	F	9.4	58.6	16.5	21.6	6

Figure 1. Lab. data & electropherogram on proband & family

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MIDAZOLAM IN CONJUNCTION WITH LOCAL ANAESTHESIA IS SUPERIOR TO ENTONOX IN PROVIDING PAIN RELIEF DURING A BONE MARROW ASPIRATE AND TREPHINE BIOPSY

G. Chakupurakal, K. Holder, E. Nikolousis, S. Pitchaipillai, L. Bratby, J. De la Rue, J. Delgado, D.W. Milligan

Heart of England Hospital, BIRMINGHAM, UK

We conducted a randomised, controlled trial comparing titrated midazolam 5-10 mg iv and inhaled entonox in addition to local anaesthesia to identify which agent provides superior pain relief. 49 patients were recruited of which 46 were evaluable. 24 and 22 patients were recruited into the entonox and midazolam arms respectively. Patient experiences as well as staff observations were recorded with questionnaires immediately after the procedure and 24 hours later. 45% and 59% of the patients in the midazolam arm could recollect the procedure after 15min and 24hrs respectively compared to 96% and 88% in the entonox arm. Midazolam provided a more comfortable experience ($p<0.01$) and improved pain relief ($p=0.01$) compared to entonox immediately after the procedure, further improved when recalled 24 hours later. Nausea, dizziness and hallucinations were observed with both treatments, but hallucinations were significantly more frequent in the entonox arm. Clinically relevant respiratory depression (O_2 saturation <90%) occurred in 19% of patients in the midazolam arm and sedation was reversed with flumazenil. We conclude that midazolam in conjunction with local anaesthesia provides rapid and reversible sedation as well as effective pain relief during bone marrow biopsy and is superior to entonox however care must be taken to monitor respiratory function.

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DETERMINATION OF IMATINIB IN PLASMA BY HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS

E. Faber, D. Friedecky, K. Micova, T. Adam, K. Indrak

University Hospital, OLOMOUC, Czech Republic

Background. Imatinib mesylate (IM) is an inhibitor of tyrosine kinase Abl, Bcr-Abl and c-kit used for targeted treatment in haematology and oncology. Recent studies described association of through IM plasma levels with cytogenetic and molecular response in patients with chronic myeloid leukaemia. There is evidence that inclusion of plasma drug levels measurement may be important for successful management of resistance to IM. *Aims.* The aim of our work was to develop capillary electrophoretic method for determination of IM in plasma. *Methods.* Samples were deproteinated with methanol, dried under the stream of nitrogen, reconstituted in water and directly analysed. Analyses were performed in 50 mmol/L citrate buffer adjusted with γ-amino-n-butyric acid to pH 3.6. Electric field of 556 V/cm was applied on fused silica capillary (length of 27 cm, inner diameter of 75 um) thermostated at temperature of 25°C. *Results.* Limit of detection of the method is 10 nmol/L (S/N=3). The method is linear in the range of 1 - 100 μmol/L. The recovery of IM was 86.7 - 95.5%, imprecision value (CV, n=10) were 1.59% and 1.69% (within-day) and 4.25% and 4.68% (between-day), respectively. Analysis of IM (and its desmethylated metabolite) is completed within 4 minutes. Plasma IM concentrations were measured in 150 samples from treated patients with chronic myeloid leukaemia. No interferences were observed in all samples analyzed. In a group of patients treated with 400 mg per day for least month (samples collected 18 - 28 h post dose) IM level range from 0.46 to 5.61 μmol/L. Normal distribution of data with no outliers was obtained by descriptive statistics. Calculated mean of 2.58 μmol/L with SD of 1.62 shows broad distribution of IM plasma levels in patients on standard dose highlighting importance of IM plasma monitoring. *Conclusions.* The developed method is fast and sensitive tool for precise measurement of IM levels in plasma.

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EFFECTIVE TREATMENT OF RESISTANT FORM OF AIHA BY RITUXIMAB

N. Tsvetaeva, O. Nikulina, E. Shurkhina, M. Dmitrieva, H.E. Varlamova, E. Gretsov, I. Vorobjev, N. Khoroshko

Hematology Research Center, MOSCOW, Russian Federation

AIHA is a group of autoagressive deseases caused by distractive action of autoantibodes on red cells. AIHA relapsed in 20-30% cases. New messages about treatment of resistant form of AIHA have been appeared in literature. Rituximab is a chimeric, human IgG1 monoclonal antibody specific for the CD20 antigen, expressed on the surface of B lymphocytes. *Aims.* To estimate results of the treatment of AIHA relapse form by Rituximab. *Results.* Ten patients (8-w, 2-m, 25-66 y.o) were enrolled to our study with relapsed form of AIHA. Previously all patients had been resistant to another form of treatment (prednisolone, azathioprine, cyclophosphamide and splenectomy). All patients had low level of Hb(from 33 up to 66 g/L), reticulocytes and bilirubin count was increased. Direct antiglobulin test was positive (1:8-1:64). Acute hemolysis was interrupted by infusion of methylprednisolone in dose 500 mg, 3 days. Rituximab was administered intravenously at a dose of 375 mg/m^2. The dose of Rituximab was controlled by depletion of B lymphocytes in blood. If the count of B lymphocytes was below 1% - the patients had 2 infusions of the drug. If the count of B lymphocytes was more than 1% it was 4 infusions. After the treatment a complete remission was achieved in 5 patients (50%). Hemoglobin, reticulocytes, bilirubin levels with additional tests (creatine, deformability of erythrocytes) riched the normal values. Additional tests could be a help for conrolling complete remission continuing from 6 to 17 month. The partial remission noticed in 5 patients (50%). B lymphocytes became undetectable after treatment and returned to normal values by 5 - 6 months without hemolysis. Immunoglobulin level was normal after the treatment. After remission 3 patients (33%) had relapse of AIHA (increased creatine level and deformability of erythrocytes) in 2, 8, 17 months after the first course of Rituximab. After the second course of therapy they achieved a second remission. All 10 patients hadn' t had any supporting therapy. *Conclusions.* Rituximab is an effective medicine in the treatment of relapse of AIHA as all patients observed responding to the treatment applied with complete remission in 50% cases.

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MUTATION ANALYSIS OF NOLA1 GENE AND MOLECULAR DIAGNOSIS IN APLASTIC ANEMIA PATIENTS

S.P. Pigullo,[1] G.S. Santamaria,[1] E.P. Pavesi,[2] J. Svahn,[1] M. Risso,[1] M. Van Lint,[3] A. Bacigalupo,[3] D. Longoni,[4] M. PIllon,[5] A.P. Iori,[6] U. Ramenghi,[7] P. Saracco,[7] O. Burlando,[1] I. Dianzani,[2] M. Lanciotti,[1] C. Dufour[1]

[1]G.Gaslini Institute, GENOVA; [2]A.Avogadro University, NOVARA; [3]San Martino Hospital, GENOVA; [4]Milano Bicocca University, MONZA; [5]Padua University, PADOVA; [6]La Sapienza University, ROMA; [7]Regina Margherita Hospital, TORINO, Italy

Background. Telomeres are nucleoprotein structures that protect chromosomes ends. The ribonucleoprotein complex is responsible for *de novo* synthesis and maintenance of telomere, mutations in complex genes lead to premature telomere shortening and are responsible for different forms of dyskeratosis congenital: X-linked (DKC1), autosomal dominant (TERC) and autosomal recessive (NOP10, TERT). Heterozygous mutations in TERC gene were previously found by our group also in patients with apparently acquired aplastic anemia (AA). The aim of this work is to analyze the possible involvement of the telomerase complex gene NOLA1, in a population of Italian AA patients. *Methods.* DNA of 108 AA patients (74 pediatrics and 34 adults) and 170 normal controls (94 pediatrics and 76 adults) was amplified by PCR analyzed by DHPLC. For each abnormal elution profile PCR products were directly sequenced using ABI prism 3100 genetic analyzer. *Results*.No mutations in NOLA1 gene (GenBank NM_018983) were found in AA patients. DHPLC analysis revealed the presence in patients and controls of the following polymorphisms: Exon1: 5' UTR -148 -/T (refSNP ID rs11433030). Exon 2: c.376 T>C synonimus (refSNP ID: rs2276326). Exon 3: IVS3 - 46C>T, not reported in GenBank. Exon 7: 3'UTR + 1228C>G (refSNP ID: rs10365). All these polymorphisms had similar frequency in patients and controls. Moreover, in exon 2, we identified, in two patients and one control (frequency respectively of 1.8%% and 0.6%)and the new c.390A>T variation, not reported in GenBank, that and leads to p.H28L. amminoacidic change. Telomere length analysis shoved that subjects carrying this change have a telomere length comparable to that of age matched healthy controls thus suggesting that this variation has no effect on telomerase complex activity. *Conclusions*. We did not found any clear disruptive mutation in NOLA1 gene. The non conservative variation identified in our sample has no effect on telomere length. All these data suggest that polymorphisms or mutations of NOLA1 gene are unlikely to play a major role in the pathogenesis of AA in our patients at least via telomere shortening. In addition they suggests that, differently from other telomerase complex gene (TERC, TERT) NOLA1 mutation study should not be part of the diagnostic work up of AA.

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A RETROSPECTIVE ANALYSIS OF LONG-TERM SURVIVAL IN ACQUIRED APLASTIC ANEMIA PATIENTS TREATED WITH ALLOGENEIC BONE MARROW TRANSPLANTATION OR IMMUNOSUPPRESSIVE THERAPY IN A SINGLE INSTITUTION

Lj. Tukic, D. Stamatovic, O. Tarabar, M. Elez, Z. Tatomirovic, B. Balint, M. Malesevic, S. Marjanovic

Military Medical Academy, BELGRADE, Serbia

Background. The treatment of acquired aplastic anemia (AA) is based on allogeneic bone marrow transplantation (BMT) and immunosuppressive therapy (IST). The aim of this study was to assess the outcome of children and adults with AA treated in the last twenty years in our institution. *Patients and Methods.* between 2/1986 and 2/2008 45 patients (pts) with newly diagnosed AA were treated either with allogeneic BMT (15 pts) or with IST (30 pts). The median interval from diagnosis to therapy was 2 months (range 1-9 months) in IST group and 3 months in BMT group (range 1-16). There was no statistical difference between the two treatment groups in sex, severity of AA, disease duration and previous trasfusion amount. They differ only in age (pts in BMT group were younger). Seventeen allogeneic BMT were performed in 15 pts. All donors were HLA-identical sibling (1 donor was identical twin). Source of stem cells were bone marrow in 14 (two with second transplants) and peripheral stem cells in 3 BMTs. Conditioning regimens were based on cyclophosphamide (CY) with antithymocyte (ATG) in 12 and Flud with CY and ATG in 3 BMTs. Seventeen pts received combined IST with ATG or ALG (antilymphocyte globuline), cyclosporine A and steroids and 13 pts ATG with steroids (from which 4 were splenectomized). *Results.* engraftment was documented in 14 pts with allogene-

ic BMT (1 pts died without engraftment). One patient developed acute GvHD grade 3-4 and died on 48 days, the other pneumonitis interstitialis (CMV+) and died on 60 days and the third rejected allograft after 7 months and died 8 days following the second BMT. Up February 2008, 10 of 14 pts (71.4%) are alive with sustained engraftment. Median survival in BMT group was 25 months (range 6-224). Concerning IST group-25 of 30 patients (83.3%) achieved a response (7 had two or tree cycles IST). Four pts (13.3%) from IST group died, major causes of death were infection and hemorrhage. Five pts (20%) relapsed after IST (range 1 to 3 years). Overall survival in the IST group is 66.6% (20/30 pts) after 56.5 months (range 1-265) of median follow up. In two pts from IST group (8%) MDS/AML was diagnosed after 87 months (range 72-102), at 5 pts avascular necrosis of hip and at 1 patient Ca prostate gland. There was no statistical difference between the two treatment group in rate and overall survival. *Conclusions.* The long-term survival rates of our AA receiving BMT or IST were excellent compared with the other data. Problems still remains, such as incomplete responses, clonal evolution and relapse of the original disease.

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EFFICACY OF EPOETIN ZETA IN PATIENTS WITH CHEMOTHERAPY-INDUCED ANEMIA AND HEMATOLOGICAL MALIGNANCIES: INTERIM RESULTS

V. Tzekova,[1] R. Koytchev[2]

[1]Queen Joanna University Hospital, SOFIA, Bulgaria; [2]CCDRD AG, NEUENHAGEN, Germany

Background. Anemia is a common complication of chemotherapy. Treatment with erythropoiesis-stimulating agents may improve quality of life and reduce transfusion requirements in patients with chemotherapy-induced anemia (CIA). Epoetin zeta is a biosimilar epoetin alfa licensed for subcutaneous use in this group of patients. *Aims.* To determine the efficacy of epoetin zeta in patients with CIA and hematological malignancies. *Methods.* This multicentre, open-label, Phase III trial in patients with solid tumour(s), malignant lymphoma or multiple myeloma studied the effects of subcutaneous epoetin zeta in patients with CIA. All patients gave written, informed consent prior to study enrolment, and were receiving chemotherapy and at risk of blood transfusion (pre-existing anemia, defined as hemoglobin [Hb] <10 g/dL at screening). Subcutaneous epoetin zeta was administered 1-3 times weekly for 12 weeks. Patients received an initial dose of 150 IU/kg 3 times per week or 450 IU/kg once weekly. If a treatment response (defined as an increase in Hb ≥1 g/dL or in reticulocyte count ≥40,000 cells/μL) was achieved within 4 weeks, the dose was maintained at 450 IU/kg per week. If this target was not met, the dose was increased to 900 IU/kg per week. If, following another 4 weeks of therapy, the target Hb increase was achieved, the dose remained at 900 IU/kg per week; however, if the Hb increase was <1 g/dL, response was considered unlikely and treatment discontinued. *Results*. A total of 216 patients were enrolled, of whom 71 had hematological malignancies (all malignant lymphoma). These patients received a mean weekly epoetin zeta dose of 501.6 IU/kg at week 1. Patients with hematological malignancies achieved a treatment response within 2 weeks in 59.2% of cases, with 87.3% achieving a response within 6 weeks. Fifty-eight (81.7%) patients achieved a response in the first 4 weeks of treatment and thus did not require any dose increase. The mean increase in Hb level ranged from 1.1 g/dL at week 4 to 2.9 g/dL at week 12 (p<0.0001 at all time points). *Conclusions*. Epoetin zeta is effective in increasing Hb levels in patients with hematological malignancies and CIA. The majority of patients achieved a treatment response without requiring a dose increase.

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BLUNTED ERYTHROPOIESIS IN ANEMIA DURING PREGNANCY IS OBVIOUSLY RELATED TO INCREASED PRODUCTION OF INFLAMMATORY CYTOKINES

V.G. Demikhov, E.F. Morshchakova, A.D. Pavlov, E.V. Demikhova, E.V. Klimovskaya

Federal Research Center Ped Hematol, RYAZAN, Russian Federation

Background. Iron deficiency is the main cause of an anemia in pregnancy. But we suppose that anemia in pregnancy pathogenesis is more complex, than ineffective erythropoiesis, caused by iron or folate deficiency alone. According to last our investigations inadequately low production of EPO for the degree of the anemia is the one of number mechanisms of anemia in pregnancy pathogenesis. Nevertheless causes of blunt erythropoiesis in anemic pregnant women are unclear. In our opinion blunted erythropoiesis in anemia during pregnancy is obviously related on the one hand to increased production of inflammatory cytokines and on the other hand to hyperestrogenemia. *Aims.* To evaluate serum levels of some inflammatory cytokines in anemic pregnants, healthy pregnants and in healthy non-pregnant women. *Methods.* A total 60 pregnant women were tested. All pregnant women were divided into 3 groups on the basis of iron status and Hb level: group1 - iron deficiency anemia (IDA) - n=19, group 2 - anemia with normal iron status and inadequately low production of EPO for the degree of the anemia - n=17 and group 3 - pregnant females with normal Hb levels - n=24. Control group consisted of 13 healthy non-pregnant women. We determined serum levels of IL-6, IL-8, INF-γ immunoenzymometrically by using commercial ELISA kits. *Results.* The significant elevated serum levels of IL-8 and INF-γ observed at all pregnant women groups *vs* control (Table 1). Serum INF-γ concentration in IDA pregnant women (group1) was significant higher than in group 2: 240.2±80.37 ng/L and 84.2±30.59 ng/L respectively (p<0.05). Increased IL-6 serum level was in group 3 only: 78.4±35.1 ng/L *vs* 3.1±3.13 ng/L in control (p<0.05). Serum levels of IL-8 in groups 1 and 2 were over then in group 3 and control: 85.7±43.18 ng/L, 81.2±34.59 ng/L and 27.4±5.99 ng/L, 10.2±0.98 ng/L respectively. *Conclusions.* Blunted erythropoiesis in anemia during pregnancy is obviously related to increased production of inflammatory cytokines. We suppose some causes of inflammatory cytokines increased production in anemic pregnant women. First, infections during pregnancy may lead to elevated serum levels of inflammatory cytokines. Second, it is known that reduced O_2 stimulates placental production of inflammatory cytokines. That is why hypoxia may lead to decreased production of EPO in the group of anemic pregnant women with IDA. Disruption of the delicate balance of cytokines by bacteria or other factors increases production of inflammatory cytokines at the maternal-fetal interface and leads to blunt erythropoiesis and anemia.

Table 1. Serum levels of inflammatory cytokines in different groups pregnant females.

Groups		Hb g/L	IL-6 ng/L	IL-8 ng/L	INF-γ ng/L	EPO IU/L
PREGNANT FEMALES	Iron deficiency anemia (group 1) n=19	95,9± 1,71	36,1± 16,80	85,7± 43,18	240,2± 80,37	35,4± 10,44
	Anemia with normal iron status (group 2) n=17	97,4± 1,05	55,6± 38,26	81,2± 34,59	84,2± 30,59	9,6± 1,99
	Normal Hb level (group 3) n=24	119,4± 1,56	78,4± 35,06	27,4± 5,99	70,3± 32,15	-
Healthy non-pregnant females (control) n=13		126,7± 1,62	3,1± 2,01	10,2± 0,98	6,9± 2,88	-

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EPOETIN BETA 30.000 I.U. ONCE WEEKLY IS EFECTIVE AND WELL TOLERATED IN ANEMIC PATIENTS WITH NON-MYELOID HEMATOLOGICAL MALIGNANCIES RECEIVING CHEMOTHERAPY. A SINGLE CENTRE EXPERIENCE

H. Ionita, I. Ionita, L. Cheveresan, A. Isac, M. Ionita, D. Calamar, D. Oros, I. Musta, N. Basa

University of Medicine and Pharmacy Victor Babes, TIMISOARA, Romania

Background. Anemia is a common feature in patients with cancer resulting from the disease it self and the effects of myelosupressive treatment. In the European Cancer Anemia Survey [ECAS] with included cancer patients at various stages of treatment from 24 European countries, 73% of 2956 patients with lymphoid malignancies were found to have anemia (hemoglobin [Hb]<12 g/dL) at some point during the 6 month evaluation period (Ludwig H. *et al.* Blood 2002). Anemia has a detrimental effect on patient quality of life[QoL] including debilitating fatique, dyspnaea, cardiovascular complication, weakness and depression. Anemia is also a negativ pronostic factor for survival in patients with lymphoid malignacies. Epoetin beta (NeoRecormon) has been shown to be effective in increasing Hb levels, reducing transfusion requirements and improving QoL when administered three times weekly (t.i.w) subcutaneously (S.C). However the option to administer epoetin beta once weekly (QW) would provide a more convenient treatment schedule for patients. *Aims.* To evaluate the efficacy and safety of epoetin beta 300000IU QW in patient with lymphoproliferative malignancies. *Methods.* This was an single-arm study from a single center experience about patients treated from january 2003- june 2007. Adult patients with non-myeloid hematological malignacies and anemia (Hb levels 8-11 g/dL), a WHO performance status 0-2% and a life expectancy >6 month who scheduled receive chemotherapy were enrolled. Patients received epoetin beta 300000 IU sc QW over 16 weeks. Follow-up visits scheduled after each chemoterapy cycle.The end point included change in Hb level during epoetin beta therapy.The clinical response was defined as an increase in Hb concentration of ≥2 g/dL during the treatment phase without transfusion requirement after the initial 4 weeks of tretment. Hb response was defined according to patients baseline Hb level. *Results.* A total of 143 patients were included in the intention-to-treat population. Patients with anemia (Hb ≥11 g/dL) associated with multiple myloma 52 pts(36.36%), non Hodgkin limphoma 58 pts (40,56%) and chronic lymphocytic leukaemia 33 pts (23,08%) were eligible for the study. Mean age was 63 years, M/F ratio was 78/65, mean serum Hb (SD) g/dL=9,2(1,1), mean hematocrit (SD)%=28,2(4,7), mean transferrin saturation (%)=38. The median duration of the treatment was 14 weeks, Hb response was observed in 65% of patients during the study. The median time to response was 53 (range 25-120) days. Hb response was seen with all types of chemotherapy used in the studied lymphoproliferative malignancies. The mean Hb level at study end point was 12.0(SD 2,2 g/dL). Epoetin beta treatment was well tolerated. Five percent of patients presented tromboembolic events, other few adverse advents were related to the study medication but without major significance. *Conclusions.* In patients with non-myeloid hematological malignancies, Epoetin beta 30000UI s.c.QW effectively and rapidly increased Hb to target level, reduced the need for blood transfusions with their associated risks and was well tolerated.

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THALASSEMIA INTERMEDIA REGISTRY

A. Taher,[1] A.H. El Chafic,[1] M. Karimi,[2] A. El-Beshlawy,[3] K. Belhoul,[4] S. Daar,[5] P. Pattoneri,[6] R. Fasulo,[6] M. Salah,[4] M. Cappellini[6]

[1]AUB-MC, BEIRUT, Lebanon; [2]Nemazee Hospital, SHIRAZ, Iran; [3]Cairo university, CAIRO, Egypt; [4]Dubai Thalassemia Centre, DUBAI, United Arab Emirates; [5]Sultan Qaboos University, MUSCAT, Oman; [6]Universita di Milano, MILAN, Italy

Background. Thalassemia intermedia (TI) is a heterogeneous genetic disease caused by mutations in the β-globin gene, which results in a unique and broad clinical spectrum. *Aims*. A multicenter registry was conducted on a huge number of thalassemia intermedia patients to describe the prevalence of certain complications, treatment strategies, and genotypes in five different countries including Lebanon, Italy, Iran, Egypt, Dubai, and Oman. *Methods.* A questionnaire form about the patients was sent to each of the five different countries. Patients' charts were reviewed; forms were filled out and then sent to the American University of Beirut-Medical Center where the data was collected, entered and stratified using Microsoft Office Excel' 2007 and SPSS' for Windows 15.0 software programs. *Results.* A total of 583 thalassemia intermedia patients entered the registry. Mean age was highest for patients from the Italian center (40.03±11.34) and lowest for patients from Egypt who were of pediatric age (12.58±5.72). Hemoglobin was similar in all countries and levels ranged between 8 g/dL and 9 g/dL. Ferritin levels were highest in the Lebanese TI population with a mean of 1068.35±802.82 ng/ml and lowest in Egypt with a mean of 648.57±443.9 ng/mL. Splenectomy and cholecystectomy were performed mostly in Lebanon and Italy and least in Egypt. Hypothyroidism and osteoporosis had the highest prevalence in Italy which is 11.8% and 57.4% respectively. Thrombotic events such as deep vein thrombosis, portal vein thrombosis, or pulmonary embolism occurred most frequently in the Lebanese TI group with a prevalence of 24% slightly higher than that of Italy where the prevalence is 22.2%. Eighty nine percent of the Iranian TI patients had never been transfused; a percentage that is remarkably higher than that of the other countries which was 20 to 30 percent at most. Hydroxyurea is being widely utilized in Iran and Egypt (83.5% and 52.9% of the respective TI population), to some extent in Dubai, and rarely in Lebanon, Italy, and Oman. Chelation therapy is administered to most TI patients in Italy and Oman but only to around 20% of patients in Lebanon and Egypt. The most frequent point mutation of the TI patients was found to be IVS- II- 1 (G --> A) in Iran, codon 39 in the Italy, IVS-I-6 in Lebanon and Egypt, and finally IVS-I-5 in Dubai and Oman. *Summary and Conclusions.*The high prevalence of thrombosis in the Lebanese and Italian group might be a reflection of the high degree of splenectomy and/or iron overload (high ferritin), as mentioned extensively in the literature. The older age of the Italian group was apparently the major risk factor for the development of gallstones and endocrine dysfunction. Hydroxyurea is the primary cause for the decrease in transfusion rate in the Iranian group and it might also have a protective role against thrombosis in TI like that established in essential thrombocythemia. The impact of the genetic *Background.* on the development of complications and on the response to chelation and hydroxyurea is still to be discovered.

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A NEW CASE OF NON CLASSIFIABLE CONGENITAL DYSERYTHROPOIETIC ANAEMIA WITH IRON OVERLOAD

J. Gay,[1] A. Charpentier,[2] P. Gosset,[1] M. Fournier,[3] C. Beaumont,[4] M. Zandecki,[3] N. Cambier,[1] E. Bourgeois,[1] C. Rose[1]

[1]Hopital St. Vincent de Paul, LILLE; [2]Hopital St. Philibert, LILLE; [3]Centre Hospitalier et Universitaire, LILLE; [4]Centre de Recherche Biologique Bichat Beaujon, PARIS, France

Background. A caucasian man with moderate mental retardation, has presented a stable and moderate non regenerative chronical anemia (>10 g/dL) since childhood. There was not any transfusion requirement. He had no familial antecedent. At 33, as he presented signs of asthenia, haemochromatosis was suspected. *Aims.* We report a new case of non classifiable congenital dyserythropoietic anaemia similar to a type IV and revealed by an important iron overload. *Methods.* We explored this haemochromatosis and this chronic anemia by blood test, liver biopsy, bone marrow aspiration and electron microscopy. *Results.* Serum ferritin and siderophilin saturation coefficient were: 3480 ng/L and 99% respectively. He has presented a stable splenomegaly since childhood and a moderate anemia at 10,6 g/dL normocytic, with a low reticulocyte count without any other abnormality at the full blood count. Serum indirect bilirubin was:18 mg/mL. LDH et haptoglobin levels were normal. The complete etiologic investigation of haemolytic anemia was negative. Genetics tests for HFE and ferroportin mutations were negative. A liver biopsy showed a moderate fibrosis and a liver iron concentration of 559 µmol/g dw . Bone marrow aspiration revealed isolated major anomalies of the erythroid lignaege: massive dyserythropoiesis with pictures of caryorrhexis and abnormal erythroblastic mitosis. Neither ringed sideroblast, nor anomalies in the distribution of intraerythroblastic iron were found. Neither bone marrow aspiration nor electron microscopy could detect arguments in favour of congenital dyserythropoietic anaemia types: I (neither internuclear chromatin bridges, nor megaloblastic), II (insufficient number of binucleate erythroblasts), or III (absence of multinucleate giant erythroblast). *Summary and Conclusions*. Our case belongs to a new unclassifiable congenital dyserythropoietic anaemia, sharing more similarities with a type IV given the pictures of karyorrhexis. The interesting feature of this case is the contrast between major cytologic abnormalities affecting the erythroid mitosis only, and its very small impact on the total production of haemoglobin. However, there is a high hepatic iron overload without erythroid iron distribution abnormalities (mitochondries, siderosoms) or abnormalities in iron metabolism pro-

teins. This case could be used as a model for a specific research aiming at finding the mechanism responsible for stimulating intestinal hyperabsorbtion of iron which occur in all dyserythropoietic anaemia.

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POLYCYTHEMIA CAUSED BY A NOVEL MUTATION IN THE BETA GLOBIN GENE WITH HIGH AFFINITY FOR OXYGEN

J. Rey,[1] A. Murati,[1] S. Delpierre,[2] V. Ivanov,[1] D. Coso,[1] J.A. Gastaut,[1] R. Bouabdallah[1]

[1]Paoli-Calmettes, MARSEILLE; [2]Sainte Marguerite Hospital, MARSEILLE, France

Background. Management of polycythemia has been dramatically changed with the discovery of Jak2 mutation. However, after exclusion of polycythemia vera, exploration of secondary polycythemias remain a difficult challenge. The differentiation of secondary polycythemia as high affinity hemoglobin from polycythemia vera is crucial to avoid therapy as chemotherapy. *Aims.* We report the case of a man with polycythemia caused by a novel mutation in the beta globin gene with high affinity for oxygen (Hb Fort Dodge). *Methods.* A 57 year old man was referred to our center for suspicion of polycythemia vera. He was asymptomatic but his blood count shows polycythemia with 6 500 000 erythrocytes, 18,5 g/dL hemoglobin and 58% hematocrite. Leucocytes and platelets were normal. Serum erythropoietin level was normal. Jak2 screening was negative. The P50 was found to be low at 20 mmHg and 2,3 DPG level was normal. Routine hemoglobin electrophoresis and HPLC detect abnormal hemoglobin but cannot identify the mutant. Globin chain analysis by HPLC revealed an unidentified beta globin variant. Beta globin gene sequencing revealed a mutation (TGT-TAT) of codon 93. *Results.* The variant described here has been already found in a 75-year-old caucasian woman (Hb Fort Dodge). However, the patient was clinically normal and hematological parameters were normal. High affinity for oxygen was not demonstrated in this patient. The difference in this propriety of this same hemoglobin variant in theses two patients is under investigation. *Conclusions.* Estimation of P50 remain an essential step in the evaluation of polycythemia patients in the era of Jak2 screening.

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PARTIAL SPLENECTOMY IN PEDIATRIC HEMATOLOGY/ONCOLOGY: A SINGLE-INSTITUTION EXPERIENCE

Y. Pastore,[1] M. Beck-Popovic,[2] M. Diezi,[2] N. von der Weid,[2] N. Sekarski,[3] O. Reinberg[4]

[1]H.O.P - CHUV, LAUSANNE; [2]Unit of Pediatric Hematology Oncology (H.O.P), CHUV, LAUSANNE; [3]CHUV- Unit of pediatric Cardiology, LAUSANNE; [4]CHUV- Pediatric Surgery, LAUSANNE, Switzerland

Background. Splenectomy can improve chronic hemolytic anemias, such as pyruvate kinase deficiency (PK) and hereditary spherocytosis (HS). However, total splenectomy (T.S) results in partial immune defect and increases risk of overwhelming post-splenectomy infection (OPSI) especially in young children. Over the past two decades, partial splenectomy (P.S) has emerged as an alternative to T.S and has been successfully used in HS It can be performed at younger age, and does not appear to be associated with OPSI. Reports on P.S in pediatric population however are still sparse, and possible benefit of P.S in other types of hemolytic anemia is unclear. *Aims.* To evaluate our local experience with P.S in a pediatric population. *Methods.* Retrospective chart review of P.S in pediatric patients between 1995-2005. Need to perform secondary T.S was used as an outcome measure. Patients requiring T.S within 1 yr of P.S (early T.S group) were compared with those having a T.S performed at a later time or not (late or no T.S group). T-test was used for statistical analysis. *Results.* 7 patients, 2 PK and 5 HS, had a P.S performed at a mean age of 4.4yrs (27months-9yrs). Two of 5 HS patients also had congenital heart disease, one of which with partial protein C and S deficiency. Surgical procedure was laparoscopy in all but one, the latter having a surgical contraindication to laparoscopy. All patients were followed by systematic ultrasound twice a year and spleen size was measured. There were no surgical or infectious complications. In 1/2 PK patients, no decrease of transfusion requirements was observed and a T.S was performed within 12 months; the second patient showed transient improvement but eventually had T.S 2 1/2 yrs later. In 3/5 HS patients, T.S was performed within 12 months because of persistent hemolytic anemia. Two patients had clear improvement: one with complex cyanotic heart disease needed no transfusions for 4 yrs, but hemolytic anemia worsened during the 5th year of follow-up, and eventually had T.S. The 5th HS patient is still doing well almost 5yrs post P.S. Overall, 6/7 patients required T.S of which 4 were done within one year of P.S. All three patients younger than 3 belonged to the *early T.S* group. While all patients had spleen reduction by 50 to 70% by surgery, spleen size in the *early T.S* group returned more rapidly to pre-surgery size than those of *late or no T.S* group (spleen size within 1st year of P.S: 92% compared to 54% respectively; p=0.001). *Conclusions.* P.S is a safe procedure. It can permit a gain of several months with reduced PRBC requirement, but rapid spleen regrowth seems to predict the need for T.S. While P.S benefited the most in H.S patients, its role in PK and in patients operated at a very young age remains questionable. Indication and benefit of P.S. need to be determined in a larger prospective study.

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NORMAL PATTERN OF RED BLOOD CELLS ANALYZED BY EKTACYTOMETRY IN HEALTHY DONORS

S. Goede, J. Deuel, J. Fehr

University Hospital Zürich, ZÜRICH, Switzerland

Background. Ektacytometry has been developed in the seventies to describe the red cell deformability in function of extracellular osmolarity. This technology allows a record of red cell deformability (DI) with continued increase of extracellular osmolarity as an uninterrupted curve. This curve is highly reproducible and represents a detailed characterisation of red blood cell properties. Standard values to describe normal curves are lacking. A reliable description of pathologic states such as hereditary spherocytosis needs a reference to standardised normal curves. The optimal description of these curves includes the points of minimal deformability at low osmolarity (Omin), of maximal deformability (Omax), of half the maximal deformability at high osmolarity (O') and the integral under the curve between Omin and O'. *Aims.* We aimed to determine normal values of Omin, Omax, O' and the integral between Omin and O' (integral). *Methods.* We analyzed fresh whole blood of 19 healthy volunteers (11 females) with a completely normal erythrogramm (ADVIA 120) and performed ektacytometry with an ektacytometer by Technicon/Bayer. All data have been recorded and illustrated by means of an analog-digital converter (NI USB-6008). *Results.* In 13 cases we performed ektacytometry in double. These curves show a high reproducibility with an average intra-individual variability of only 1.6, 3.0 and 2.4 mOsmol/kg for Omin, Omax and O'. Variations with regard to the maximal DI and the integral are minimal (0.0006, 1.33). Compared to these results the inter-individual variability of all 19 volunteers is clearly higher with ranges of 156-171, 334-362 and 454-473 mOsmol for Omin, Omax and O'. The ranges of the maximal DI and the integral are also higher (0.62-0.679, 125.8-150.9). All curves provided with standard deviations for Omin, Omax and O' are summarised in Figure 1. Comparing these results we found a positive correlation between the mean cellular volume (MCV) and the osmolarity at O' (correlation coefficient 0.64). *Conculusions.* Analyzing fresh whole blood by ektacytometry shows a higher inter- then intra-individual variability. This variability is mainly explained by the individual variability of MCV and MCHC. This has to be considered in the description and interpretation of pathological curves.

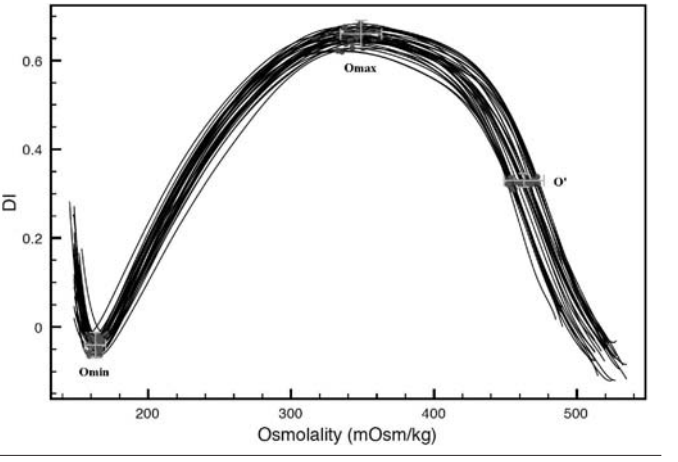

Figure 1.

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REFLECTIONS OF T2 MRI IN 19 THALASSEMIA INTERMEDIA PATIENTS

F. El Rassi,[1] A. Taher,[1] A. Inati,[2] S. Koussa,[3] M.D. Cappellini[4]

[1]American University of Beirut Medical Center, BEIRUT, Lebanon; [2]Rafic Hariri University Hopsital, BEIRUT, Lebanon; [3]Chronic Care ; [4]University of Milano, MILANO, Italy

Background. Unlike patients with thalassemia major (TM), patients with thalassemia intermedia (TI) do not require regular blood transfusion therapy but are still susceptible to iron overload due to increased intestinal iron uptake triggered by ineffective erythropoiesis. Effective monitoring of iron burden is therefore an important element of patient management. Different modalities have been used to assess the iron status in the different organs of the body. The newest modality includes the use of MRI evaluation. R2 MRI has been successfully used to determine the liver iron concentration and has been approved worldwide. Similarly, T2 MRI of the heart has been employed for determining the cardiac iron concentration by using functional correlates. The prevalence of left ventricular dysfunction secondary to iron overload has progressively increased as T2 level dropped below 20 ms. The implication of decreased cardiac T2 value has been correlated with increased iron content of the heart. *Aims.* To assess the cardiac iron status in TI patients and draw conclusions concerning the best chelation regimen. *Methods.* This was a cross-sectional study of randomly selected TI patients treated at a chronic care center in Hazmieh, Lebanon. Patient charts were reviewed and a medical history, including previous blood transfusion therapy, was compiled. Blood samples were taken for NTBI, SF assessment and LIC was determined by R2 MRI. In addition, a subpopulation of 19 TI patients who were found to be iron overloaded were selected to undergo T2 MRI evaluation of the cardiac iron status. *Results.* Data from 19 patients were included in this analysis (11 male, 8 female) Mean SF values were 1317 ng/mL (range 460‡3157) and mean LIC levels were 15.0 mg Fe/g dry weight [dw] (range 3.4‡32.1). T2* cardiac MRI was done accordingly and the mean T2 level was 47.3 ms (range 35-66.9) and this was above the cutoff of 20 ms for implicating iron overload of the heart. The 19 selected patients were infrequently transfused and off any iron chelation regimen. Their selection was done according to the prior assessment of the iron load by SF and LIC. All the results of the cardiac MRI have shown very little uptake of iron in the heart of these iron overloaded thalassemia intermedia patients. *Conclusions.* Although the number of patients in this sub study is small, valuable conclusions can be underlined. T2* cardiac MRI is an important modality for assessing the iron status of the heart. This is extremely crucial in selecting the best chelation regimen. The studied TI patients have shown that although they have elevated SF and LIC, their cardiac status is not as infiltrated by iron. This draws us to conclude that unlike thalassemia major patients, thalassemia intermeida patients tend to develop iron overload of the liver and not the heart. Accordingly, special consideration should be addressed for the best iron chelator regimen in thalassemia intermedia.

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PREVALENCE OF ABNORMAL OR CONDITIONAL TCDS IN LONDON AND PRACTICALITIES OF SETTING UP A TCD SERVICE

S. Trompeter,[1] E. Aimiuwu,[2] F.J. Kirkham,[3] A. Robins[2]

[1]Great Ormond Street Hospital for Children NHS Trust, LONDON; [2]Whittington Hospital NHS Trust, LONDON; [3]Institute of Child Health, UCL, LONDON, UK

Background and Aims. In sickle cell anaemia (SCA), internal carotid/middle cerebral artery velocities of >200 cm/sec (abnormal) and 170-199 cm/sec (conditional) on transcranial Doppler Ultrasound (TCD) predict stroke risks of 40% and 7% over the next 40 months respectively. The prevalence of abnormal and conditional TCD was around 8-10% in the 1990s but there is little recent data. There are a variety of different screening programs in London using imaging (South) and non-imaging (North) TCD. Some hospitals run regular Saturday services to improve attendance and decrease time spent off school. Others feel compliance is best achieved by having a second room in a normal clinic so patients have only one appointment to keep. Some use ultrasonographers, others radiologists and some haematologists. In view of the recently published UK Standards we undertook to screen the paediatric sickle cell population at the Whittington Hospital NHS Trust in North London, a haemoglobinopathy centre. The purposes of the sessions were to i) screen the population with TCD and compare the current prevalence with previous data ii) ascertain the important factors in setting up such a service and iii) determine whether birth factors increased risk for abnormal TCD. *Methods.* Patients were selected according to the following criteria: age >2 <20 and HbSS. HBSC and HbSBthal patients were screened if there were concerns regarding their neurology or if they had a sibling with HbSS attending. All patients were contacted by telephone. All appointments were during normal clinic times and 3 of the four sessions were during school times. Most patients did not have a simultaneous follow-up appointment with the clinician. All patients and parents were given written and verbal information in either French or English and gave verbal consent. Using a non-imaging 2Hz probe, the patients were screened by a specialist registrar in haematology (ST) under the supervision of an experienced consultant paediatric neurologist (FJK). All data was recorded manually and then transcribed onto the Trust's blood results system. *Results.* TCD screening took an average of 15 minutes for a healthy patient and 30 minutes for a patient with known neurological problems. A further one hour per 10 patients was required to load the data onto the computer system. Of 67 patients screened (100% of those given appointments; 63 SCA), 5 of whom had already had a stroke, 7 (10%; 1 stroke) had abnormal TCD, 10 (14%, 1 stroke) were conditional and 2 (3%; 1 stroke) had low velocities. The 4 patients with haemoglobin SC disease had normal TCD. There was no effect of gestational age, birth weight or mode of delivery. *Summary and Conclusions.* The prevalences of abnormal and conditional TCD appear similar to previously reported data. Compliance was excellent. Those with previous infarction take longer to screen. Time is required to upload the results to the hospital computer system but the clinicians in charge of the patients voiced great satisfaction with the easy retrieval of results. A follow-up questionnaire will be looking at patient's satisfaction and the screening program for the rest of the cohort is planned.

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LATIN AMERICAN REGISTRY OF PATIENTS (PTS) WITH TRANSFUSIONAL HEMOSIDEROSIS (TH): THE RELATH STUDY

R.C. Rodolfo,[1] C.L. Lobo,[2] Z.P. Plumacher,[3] O.P. Perez,[4] M.M. Moreno,[5] F.S. Sanchez,[6] G.D. Drelichman[7]

[1]Hemocentro do Hospital Santa Casa, SAO PAULO, Brazil; [2]Hemorio, SAO PAULO, Brazil; [3]Instituto Hematológico de Occidente, MARACAIBO, Venezuela; [4]Banco Municipal de Sangre, CARACAS, Venezuela; [5]Hospital Edgardo Rebagliati Martins, LIMA, Peru; [6]Hospital Civil Guadalajara Dr. Juan I Menchaca, GUADALAJARA, Mexico; [7]Hospital Municipal de Pediatría, BUENOS AIRES, Argentina

Background. Miscegenation of populations of Mediterranean and African ancestry has occurred for several centuries in Latin America (LA), thus facilitating the spread of hemoglobin variants. The prevalence of thalassemias, sickle-cell disease (SCD) and other hemoglobinopathies, as well as of myelodysplastic syndromes (MDS) and other diseases associated with TH is largely unknown in LA. Likewise, no multinational studies are available regarding the prevalence of TH or patterns of use of iron-chelation therapy in LA. *Aims.* To collect retrospective data on pts with TH in the following countries: Argentina, Brazil, Colombia, Mexico, Panama, Peru, and Venezuela. *Methods.* Cases are accrued by large-volume, tertiary-care hematology centers located in large cities through a CRF designed for the study. Treatment and pt evaluation are left to investigators' discretion. Eligible pts have age >2 yr, consultation in the participating institutions at least once since 01/04, any disorder requiring chronic red-blood-cell (RBC) transfusion, receipt of >9 RBC units, at least one value of serum ferritin >1000 mcg/L, and/or a liver iron content (LIC) >2 mg/g dry weight (pts. with leukemia are excluded). Target accrual is approximately 1,000 pts. *Results.* Between Sep/06 and Jan/08, 859 pts have been accrued, 850 of whom are evaluable. The mean age was 29.2±20.1 (range, 2 to 93), and 53.2% of pts were female. Ethnic distribution was African (37.4%) and Caucasian ancestry (31.4%), Hispanic (26.4%), and others (4.8%). The most frequent diagnoses were SCD (48.9%), beta-thalassemia major (15.4%), aplastic anemia (9.5%), and MDS (7.6%, 41.5% of which had refractory anemia). RBC transfusion was > 9 in 100% and >19 in 87.5% of pts (mean age was lower in the latter group, p=0.002), and mean ferritin was 2627±1964 mcg/L. LIC determination was not available or not done in 89.5% of cases; when it was done, it was elevated in 39.7%. The level of hemoglobin at which transfusion was indicated was 7 to 10 g/dL in 67.7%, and 6 g/dL or less in 23.0% (N/A in 9.2%). The mean number of transfusions received was 12.2±9.2/yr (range, 1 to 80). Iron overload was assessed using ferritin (90.0%), transferring saturation (22.1%), and echocardiogram (21.3%). TH-related complications were reported in 82.1% of cases (62.5% of pts had hepatic complications, 26.8% endocrine, 17.9% cardiac). Iron-chelation therapy was given to 45.1% of pts, more frequently on the basis of

ferritin (84.5%), number of transfusions (29.9%), and complication from iron overload (4.4%). Deferoxamine (88.9%) and deferasirox (13.3%) were the most frequently used chelators. In most cases, treatment was still ongoing, but reasons for discontinuation were poor compliance (5.1%), drug no longer available (3.4%), and pt refusal (2.1%). *Summary and Conclusions.* This interim report from the ongoing RELATH study shows that a registry is feasible and may provide valuable information regarding TH and patterns of use of iron-chelation therapy in LA. In addition, the study suggests so far that most LA patients undergoing chronic transfusion develop TH, whose complications could be prevented by more effective use of iron chelation.

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HEIGHT AND WEIGHT IN A UK POPULATION OF CHILDREN WITH SICKLE CELL DISEASE

J. Kirkham,[1] J. Baird,[2] P. Telfer,[3] J. Khatura,[1] T.-P. Sahota,[1] O. Wilkey,[4] A. Robins,[5] M.A. Morgan,[2] J.P.M. Evans,[1] J. Davies[2]

[1]UCL Institute of Child Health, LONDON; [2]Southampton University Hospitals NHS Trust, SOUTHAMPTON; [3]Royal London hospital, LONDON; [4]North Middlesex hospital, LONDON; [5]Whittington hospital NHS Trust, LONDON, UK

Background. Immigration haematology is becoming increasingly important in Europe and Sickle cell disease (SCD) is now the commonest genetic condition in the UK. Prospective studies from the USA[1] and Jamaica[2] suggest impaired growth in childhood, particularly in males. Growth impairment is likely to be multi-factorial. There is a paucity of growth data in affected European children. *Aims.* To evaluate growth data in UK based children with homozygous sickle cell disease (SS), haemoglobin SC disease (SC) and haemoglobin S-β-thalassaemia *Methods.* 402 children (7% haemoglobin SC, 3% haemoglobin S-β-thalassaemia, and the remainder haemoglobin SS) were followed prospectively from birth (n=258, 66%) or time of diagnosis when suspected clinically. Standard auxological techniques were applied in clinics at least yearly for a median of 7.1 years. We analysed standard deviation scores (SDS) for weight, height and BMI (body mass index) compared to standard UK 1990 reference data.

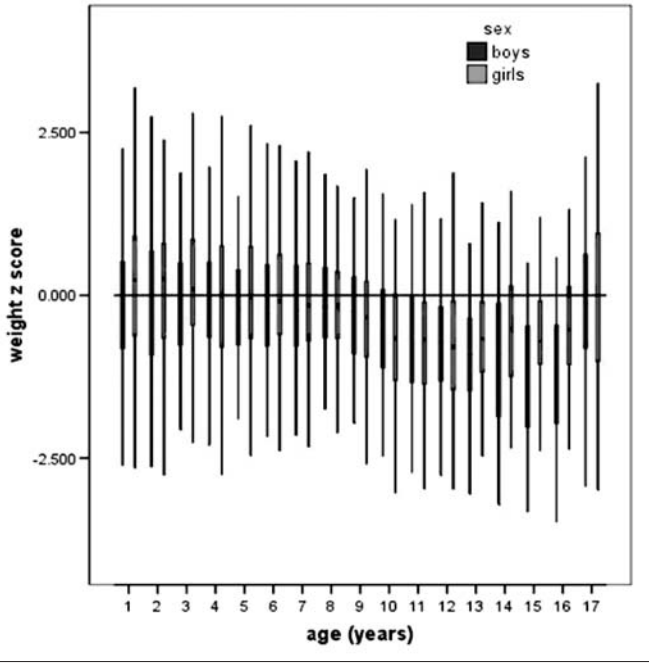

Figure 1. SDS scores for weight, height and BMI in SCA.

Results. Figure 1 shows SDS scores for weight, height and BMI for boys and girls at annual intervals. Although weight and height are close to the mean for normal UK children at age 1, for children of both sexes with homozygous SS, there was a significant reduction in height and weight SDS with increasing age ($p<0.001$). This effect was more pronounced in boys than girls. However, children with haemoglobin SC disease appeared to demonstrate increased height and weight compared to UK reference data. *Discussion.* These data suggest that there is poor growth in children with homozygous sickle cell anaemia. When using UK standard reference data, growth impairment in SCD may be underestimated as UK African-Caribbean children (particularly girls) tend to be taller and heavier during childhood compared to Caucasians[3] and this pattern also seems to occur in haemoglobin SC disease. Although, as has been demonstrated previously, delayed puberty probably plays a role in the reduction in height and weight observed in children with homozygous SCA, growth failure in early childhood may be associated with progressive deterioration in lung function. More data are needed so that appropriate nutritional intervenions can be considered.

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IGF-I, IGFBP-3 AND THE RISK OF MYELODYSPLASTIC SYNDROME

M. Dalamaga,[1] A. Lekka,[2] K. Karmaniolas,[2] J. Chamberland,[3] A. Hsi,[4] M. Triantafilli,[2] A. Dionyssiou-Asteriou,[5] C. Mantzoros[6]

[1]Athens University Medical School, ATHENS, Greece; [2]NIMTS General Hospital, ATHENS, Greece; [3]Beth Israel Deaconess Medical center, BOSTON, USA; [4]Beth Israel Medical center, BOSTON, USA; [5]Attikon General University Hospital,, ATHENS, Greece; [6]Beth Israel Deaconess Medical Center, BOSTON, USA

Background and Aims. Recent evidence suggests that obesity may be implicated in the etiology of myelogenous leukemia and myelodysplastic syndrome (MDS). We thus attempted to explore whether the insulin-like growth factor (IGF) system, a hormonal system linked with several obesity and insulin resistance associated malignancies including leukemia, may be associated with an increased risk for developing MDS. We have designed a case-control study to investigate the role of insulin-like growth factor-I (IGF-I) and its binding protein (IGFBP-3), in the etiopathogenesis of MDS after adjusting for a potential confounding effect of body mass index (BMI), leptin, resistin and adiponectin. We also explored associations between IGF-I and IGFBP-3 and established MDS prognostic factors. *Methods.* Blood samples were collected from 101 cases with incident, histologically confirmed primary MDS, and 101 hospital controls, admitted for non-neoplastic and non-infectious conditions, matched on gender, age and year/month of diagnosis (±1 month) between 2004 and 2007. Serum IGF-I and IGFBP-3 concentrations were measured using a commercially available immunoradiometric assay kit (DSL, Webster, TX). Moreover, serum leptin, resistin and adiponectin were determined. Statistical analysis of the data was performed with SAS 9.1 for Windows XP. *Results.* Cases presented significantly higher height and weight than control subjects, while differences of BMI were only of borderline significance. Cases had significantly lower serum levels of adiponectin, resistin and HMW than controls. IGF-I was found to be significantly higher in cases than in controls in univariate analysis ($p=0.018$). IGFBP3 levels were significantly different in MDS type and IPSS stratification schemes both before and after adjusting for age, gender and BMI. In the unadjusted model of IGF-I, subjects in the highest quartile demonstrated a trend towards lower risk of MDS than subjects in the lowest quartile, but these associations were not particularly strong (OR = 2.30, 95% CI 1.06-5.00). Adjusting for covariates rendered these associations non-significant. Finally, no significant associations between MDS and IGFBP3 were detected. *Conclusions.* In conclusion, although IGF-I levels were significantly higher in MDS patients than controls in univariate analysis, there was no significant association between IGF-I or IGFBP-3 and the likelihood of MDS after adjustment with the other anthropometric and hormonal variables, indicating the importance of IGF-I bioavailability and the role of a potential stem cell hypersensitivity to IGF-I system. These observations need to be replicated and warrant further investigation.

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COMPLETE RESPONSE OF A PATIENT WITH RAEB-II TO SEQUENTIAL THERAPY WITH TIPIFARNIB, 5-AZACYTIDINE AND DECITABINE. A CASE REPORT

I. Möller,[1] A. Kündgen,[1] S. Blum,[2] N. Gattermann,[1] R. Haas,[1] K. Habersang,[1] U. Germing[1]

[1]Heinrich-Heine-University Düsseldorf, DÜSSELDORF, Germany; [2]Centre Hospitalier Universitaire Vaudois, LAUSANNE, Switzerland

Patients with Myelodysplastic Syndromes (MDS) and elevated medullary blast count have a median survival of about 1-2 years. There is no curative approach besides induction chemotherapy and allogeneic stemcell transplantation. The vast majority of the patients can not undergo these procedures due to increased age and/or comorbidities. We here report on a 70 year old male, who was diagnosed as RAEB II in January 2002 and is still alive in good condition. The patient was diagnosed as RAEB II in January 2002 and was transferred to our department in June 2002. At that time he had a normal karyotype and was transfusion independent, but showed deteriorating platelet counts. The IPSS risk category was intermediate-II. A therapy with thalidomide was started, but had to be cancelled one month later due to side effects. In October 2002, the patient still showed a RAEB II and hence we included him into a study with the farnesyl transferase inhibitor (FTI) tipifarnib. After two cycles of treatment, he already achieved a complete remission (bone-marrow (BM) blast count of 3%) with incomplete recovery of cell counts (CRi) (maximum response: WBC 2.300/µL, Hb 12.3 g/dL, platelets 238.000/µL). He obtained a total of 29 cycles of tipifarnib until the therapy was stopped due to polyneuropathy. At time of discontinuation, bone marrow examination revealed progression to RAEB I (BM blast count: 7%). The disease status remained stable for about one year without further treatment. Then, a progression into RAEB II occurred with a blast count of 12% and progressive thrombocytopenia in October 2005 and consequently a therapy with azacytidine was started in the context of a multicenter study. Azacytidine induced a 2nd CRi (BM blast count: 3%; WBC 1.300/µL, Hb 13.1 g/dL, platelets 98.000/µL) after 11 cycles in November 2006. This treatment was stopped in May 2007 after 15 cycles due to progressive disease (RAEB-I: 9% BM blast count). After discontinuation of the drug rapid transformation into AML occurred already in June 2007 (BM blast count: 30-50%). At that time valproic acid was started without response. Two months later he showed progressive thrombocytopenia. We then decided to try decitabine, since although this is another, structurally related, demethylating agent, both drugs have different pharmacological properties and initial data exists, that some patients might benefit from such an approach. Through this treatment he achieved a 3rd CRi (BM blast count 3%; WBC 2.000/µL, Hb 12.4 g/dL, platelets 63.000/µL) after 4 cycles. This treatment is ongoing and our patient still presents in good clinical condition. This case clearly demonstrates that some MDS patients achieve long term benefit from investigational compounds. The enrollment into clinical studies and other unconventional approaches should be taken into account whenever possible. The switch to decitabine from azacytidine should also be regarded as a treatment option.

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MYELODYSPLASTIC SYNDROME IN CHINESE PATIENTS

V. Li, Y.S. Liang, R. Chu, H.K. Sum, F.H.Y. Chan, T.K.H. Lau, B.C.S. Kho, H.S.Y. Liu, J.C.W. Chan

Pamela Youde Nethersole Eastern Hospital, HONG KONG SAR, China

Background. There were limited data on MDS in Chinese patients. *Aims.* To study the demographic and haematologic features, treatment outcome and the prognostic factors of Chinese MDS patients diagnosed in a hospital in Hong Kong. *Methods.* Data of 149 adult Chinese with MDS based on bone marrow examinations from 1994 to 2007 were reviewed. Cases under FAB classification were reclassified according to the WHO criteria by a haematopathologist after reviewing the marrow morphology. Seven RAEB-T patients were excluded. Survival were analysed and compared with Kaplan Meier estimate and Logrank test respectively. *Results.* Demographic and haematologic features: Of the 142 patients, the M:F ratio was 1.19, median age 73 years (range 20-104). The median presenting Hb was 8.6 g/dL (range 3.4-15.4), platelet count 84 $\times 10^9$/L (6.0-646.0), WCC 3.7$\times 10^9$/L (1.1-43.6). The WHO subtypes included RA 23.2%, RA-RS 13.4%, RCMD 12.7%, RCMD-RS 1.4%, RAEB-1 15.5%, RAEB-2 14.1%, MDS-U 19.7%, and 5q- syndrome 0%. Fifteen had karyotyping done - 6 poor-risk karyotypes, 2 intermediate-risk and 7 good-risk. Two patients had concomitant SLE. Treatment and outcome: All patients received supportive care. Three underwent allogeneic HSCT. Two received azacitidine. Each patient had on average 4.8 days of hospitalization, 2.0 units of blood and 4.6 units of platelet concentrate transfused per month. With a mean follow-up of 34.6 months (range 0.43-146.6), 64% died, 9.2% transformed to AML after a mean of 11.96 months. Sixty-eight patients died of MDS-related complications- 72.1% sepsis, 16.2% bleeding, 11.8% AML; 14 deaths were unrelated to MDS; and 9 had unclear COD. The median OS was 24.8 months. All 3 HSCT recipients were stable at 78, 99, and 144 months. One RCMD-RS patient received azacitidine with good response and was alive at 5 months. One RAEB-2 patient on azacitidine had transformed to AML after the 4th course and remained alive 13 months after the diagnosis of MDS. The median OS in RA/RARS, RCMD/RCMD-RS, RAEB-1, RAEB-2 were 86.13, 85.10, 10.17, and 5.87months respectively. Prognostic factors: Significant differences in OS were noted between patients <60 and ≥60 years of age (p=0.018); marrow blasts <5% and ≥5%(p<0.001); 1 and 3 lineages of cytopenia(p=0.001); WHO subtypes of RA/RARS and RAEB-1(p<0.001), RCMD/RCMD-RS and RAEB-1(p<0.001). There is no significant difference in OS between RA/RARS and RCMD/RCMD-RS(p=0.451), and between RAEB-1 and RAEB-2(p=0.470). Transfusion of ≥6units of packed-cell per year is associated with significantly worse OS than those <6(p<0.001), similar finding in those requiring platelet concentrate transfusion of ≥12units per year when compared with those <12(p<0.001). *Summary.* We illustrated the WHO classification has prognostic importance in Chinese patients with MDS, RA/RARS identified a subset of low risk patients with relatively longer OS. Adverse prognostic factors include other WHO subtypes, age above 60 years, pancytopenia, BM blast ≥5%, and transfusion dependency. Younger patients with poor risk factors should consider marrow transplantation as a means of definitive treatment.

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TUMOR NECROSIS FACTOR ALFA AND VASCULAR ENDOTHELIAL GROWTH FACTOR IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

V. Maslyak,[1] L. Lukavetskyy,[1] O. Danysh,[1] N. Tomashevska,[2] K. Kotlyarchuk,[1] S. Prymak[2]

[1]SI Institute of Blood Pathology and Transfusion Medicine UAMS, LVIV; [2]Danylo Halytsky Lviv National Medical University, LVIV, Ukraine

Background. Although MDS according to FAB classification is quite diverse group of diseases, their pathogenesis has common features like chromosomal aberrations, increase of angiogenesis, impairment of apoptosis. Tumor necrosis factor alfa (TNF-α) is considered one of the most important proapoptotic cytokines in this disease; sTNF-RI and sTNF-RII receptors may be not less significant. In MDS significant role belongs also to angiogenesis, the increased level of which is detected in patients with progressive subtypes of MDS and at disease transformation. *Aims and Methods.* Levels of TNF-α, sTNF-RI and sTNF-RII receptors and VEGF were studied in 21 patients with different FAB subtypes of MDS. Patients underwent general clinical, cytogenetic tests and investigation of serum levels of cytokines by immuno-enzyme method using standard *BIOSOURCE* kits: human TNF-α, sTNF-Rß, sTNF-Rßß, human VEGF. *Results and discussion.* In 8 patients with stable course of RA median TNF-α level was 11.81±4.21 pg/mL. Levels of the soluble TNF-α receptors were several times higher than in healthy individuals; sTNF-RII level increase was statistically significant. VEGF level was within the parameters of healthy individuals. In RARS group (4 patients) the stable disease was detected in 2 cases, another 2 patients developed transformation to acute leukemia (AL) with lethal outcome. Median level of serum TNF-α was of no significant difference from RA patients, serum sTNF-RI concentration was 5 times higher than in control group and 2 times higher than in RA. Significant increase in sTNF-RII concentration was found in both patients with transformation to AL (52.76 ng/mL and 38.29 ng/mL respectively), whereas in patients with stable disease this parameter almost did not differ from RA. VEGF level ranged from 20.74 to 45.61 pg/mL in two patients with transformation to AL and in two patients with stable disease it was increased to 750 and 810 pg/mL. In 4 patients with RAEB TNF-α level equaled 9.71±2.42 pg/mL and was of no significant difference form the corresponding parameters of RARS and RA patients. Levels of soluble receptors were 3-4 times higher than control indices; however no difference from RA and RARS patients was detected. Level of VEGF was increased 2-fold comparing with patients with RA. In patients with CMML TNF-α level was 2 times higher than in other patients (29.49±10.79 pg/mL). The levels of soluble receptors were

the highest as well, especially sTNF-RII. VEGF level was several times higher than in other patients (median level - 171.36±48.86 pg/mL). *Conclusions.* Levels of TNF-α, sTNF-RI and sTNF-RII receptors, VEGF depend on MDS subtype, existence or absence of leukemic progression. TNF-α probably plays dual role in MDS: on one hand it is one of the causes of RA, on the other - it is a restricting factor for leukemic clone. It was found, that MDS progression to AL was associated with decrease of TNF-α level, sTNF-RII level increase and gradual decrease of VEGF level, which may indicate an inhibition of apoptosis and angiogenesis by intensively proliferating leukemic clone. Cytokine profile of CMML on our opinion is closer to myeloprolifertive diseases than MDS.

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T-CELL RECEPTOR REPERTOIRE IN MYELODYSPLASTIC SYNDROMES (MDS)

T. Momot, C. Dobbelstein, J. König, O. Schulz, J.-E. Pautsch, A. Ganser, E.M. Weissinger

Medical School Hannover, HANNOVER, Germany

Introduction. The Myelodysplastic syndrome (MDS) is a heterogenios group of malignant disorders of the hematopoietic stem cell, characterized by dysplastic bone marrow and ineffective hematopoiesis resulting in cytopenias. The involvement of T-cells has been shown for low risk MDS, but the mechanisms of action are not yet fully understood. To investigate a T-cell involvement in the pathogenesis of MDS we analysed the T-cell receptor repertoire in T-cells of bone marrow (BM) and peripheral blood samples (PBL) of MDS patients with high- and low-risk MDS (n=71). *Methods.*We used the multiplex PCR-based technique determining the size heterogeneity of the CDR3 region and compared the results those obtained from age-matched controls (n=12). *Results*. TCR Vβ skewing of T-cell repertoire is more frequent in the bone marrow samples of MDS patients than in the whole blood samples. Most frequently skewing of TCR Vβ fragments occurs in Vβ 1 (28%), Vβ 3 (20%) and Vβ 5.3 (25%). CD8 T-cells harbour the most pronounced deviations from a Gaussian distribution of TCR fragment length compared to CD4 T-cells. MDS subtype RA shows more skewing or skewing of different Vβ families than the MDS subtype RAEB. Five of the patients showed normalization of the spectratyping pattern. *Discussion.* We conclude that TCR V-beta skewing is frequent in MDS especially in CD8 T-cells. MDS subtypes RA and RAEB have a different skewing pattern in particular Vβ subfamilies. Normalization of at least one initially skewed V-beta profile after immunosuppressive therapy occurred in 5 MDS patients, suggesting that T-cells have a pathogenetic role in defective hematopoiesis of MDS.

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DIFFERENTIAL ANALYSIS OF EARLY AND LATE APOPTOSIS IN PERIPHERAL BLOOD AND BONE MARROW OF PATIENTS WITH RADIATION-INDUCED MYELODYSPLASIA

A. Bazyka, I. Ilyenko

Research Center for Radiation Medicine, KYIV, Ukraine

Background. Low-dose radiation exposure changing cell function and membrane lipid and protein spectra could influence apoptosis induction that plays important role in myelodysplasia and its transformation into radiation-induced leukemia. Aim of this study was to investigate spontaneous apoptosis at its early and late stages and influence of apoptosis inductor verapamil in PB and BM of patients with radiation-induced myelodysplasia (MDS). *Patients and Methods.* Spontaneus and verapamil induced apoptosis was studied by Annexin-V (AnV) flow cytometry assay in bone marrow and peripheral blood leukocytes of 49 myelodysplasia patients (mean age: 55,7 years). In 14 patients MDS was initiated at the late period after low-dose irradiation during clean-up works at Chernobyl accident (mean dose 239.0+35.6 mSv); 15 were exposed at the radiation-contaminated territories (24.3+6.2 mSv). Control group included 20 healthy donors (mean age 51,0). Apoptosis induction was performed with calcium-channel blocker verapamil in a concentration of 100 μmol/L in 5 h cell culture. *Results.* Percentage of AnV+PI- and AnV+PI+ lymphocytes and granulocytes was significantly higher in BM (p<0,01). Analysis of granulocyte counts showed a moderate increase in RAEB and RAEB-T (14,1±1,81) and highest figures in RA (31,3±1,81). Response of PB cells to apoptosis induction by verapamil has demonstrated in RA a marked increase of AnV+PI- (19,9±2,97, p<0,01) and AnV+PI+ (6,4±0,98, p<0,01) lymphocyte numbers; for granulocytes the elevation was shorn for early cells entering apoptosis (33,5±2,57, p<0,01), and AnV+PI+ cell percentage wasn't changed.. In RAEB and RAEB-T the changes of PB AnV+PI- and AnV+PI+ lymphocyte subsets were similar (p<0,01) while none of the changes were shown for granulocyte counts. In BM verapamil induced and increase of AnV+PI- granulocyte counts (p<0,01), no changes were detected in AnV+PI+ cells. For analysis of radiation exposure influence apoptosis parameters in MDS in radiation-exposed were compared with data in non-exposed MDS patients, healthy cleanup workers at a late period after radiation exposure (comparison group) and control group. Comparison group showed no changes of spontaneous apoptosis (both AnV+PI- and AnV+PI+ cells) and more intensive response to verapamil (p<0,01). In patients with MDS after radiation exposure the percentage of lymphocytes in apoptosis was significantly higher (15,9±1,58, p<0,01) the in control (7,5±2,44), similar changes were shown for verapamil induced apoptosis (p<0,01). *Conclusions.* This study shows predominant changes of early spontaneous lymphocyte and granulocyte apoptosis in peripheral blood. At the late stages there is a decrease of granulocyte sensitivity to apoptosis inductors in peripheral blood. Possible explanation includes block of apoptosis pathways and risk of leukemic transformation that was highest in radiation-induced MDS.

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CLINICO-HEMATOLOGICAL CHARACTERISTICS AND TREATMENT OF 120 NEW MDS PATIENTS. SINGLE CENTER EXPERIENCE DURING 5 YEARS (2000-2005)

I. Ionita,[1] H. Ionita,[1] A. Isac,[1] M. Iordache,[1] R. Pacurar,[2] L. Cheveresan,[1] M. Ionita,[1] M. Delamarian,[2] C. Ionita[1]

[1]University of Medicine and Pharmacy Victor Babes, TIMISOARA; [2]County Hospital, Hematology Departament, TIMISOARA, Romania

Background. Myelodisplastic syndroms (MDS) are clonal hematopoietic stem-cell disoders characterised by ineffective dysplastic hematopoiesis involving one or more cell lineages and characterised by peripheral blood cytopenias and high risk of progression to acute myeloid leukemia (AML). *Aims.* We gathered data on patient characteristics and treatment of 120 MDS patients seen in our hematology center during five years period (2000-2005). *Methods.* We studied all new cases diagnosed and treated since january 2000 in our clinic. All patients were studied on admission with full blood count , bone marrow aspirate smears (BMA) and some were studied with cytogenetics and bone marrow biopsy specimens (BMB).We evaluated morfological features for all MDS patients including the availability of May Grunwald-Giemsa well stained BMA smears and BMB specimens. BMA were examined for dyserythropoesis (DE), dysgranulopoesis (DG) and dysmegakariopoesis (DM) as defined by WHO criteria. For all MDS patients we analised the percentage of blasts and ringed sideroblasts. *Results.* From the 120 patients 91,66% (110pts) had primary MDS and 8,33% (10pts) were diagnosed as treatment related MDS. The distribution among MDS types was 7 - RA, 3 - RARS, 36 - RCMD, 14 - RCMD-RS, 13 - RAEB I, 15 - RAEB II,12 - CMML, 3 patients with 5 - sindrome and 17 patients with RAEB-t. A karyotype analysis was avaible in 72,5% of patients (87pts) and 65% were with a normale kariotype. According to the International Pronostic Scoring System (IPSS) 35% of the patients belonged to the low risk, 30% to the Intermediate-1, 20% to the Intermediate-2 and 15% to the high-risk group.There were 68 males and 52 females. From the 120 patients that we studied 98 patients (81,66%) were treated in our hematology departament; 25% of those treated in our clinic (30pts) were treated in day care hospital and 75% were admitted to the hospital. Reason for hospitalisation were high-risk group patients, disease progression, disease complications like infections, hemorrhages and bad general conditions. From all treated patients, in 41 cases patients were admitted for intensive chemotherapy and any kind of treatment that requires inpatient care. None of the patients received a Stem Cell Transplantation. Eighty five percent of patients received at least one unit of packed red cells and 65% received at least one unit of platlets. The median number of hospitalizations per patient was 2 (1-11). Thirty five patients (29,16%) died during first year of evolution, 41 patients (34,16%) showed progression to AML. *Conclusions.* With regard to MDS subtype distribution, patients seen in our hematological center did not differ much from the MDS population as a whole. Our patient needed hospitalisation inpatient care either for management of MDS related complications or intensive treatment of the underlying bone marrow disease.

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UNDER-EXPRESSION OF NR4A1 AND NR4A3 IN ACUTE LEUKAEMIAS: GENERALIZED ROLES AND SEQUENTIAL EVENTS

S.J. Wu, J.L. Tang, H.F. Tien

National Taiwan University Hospital, TAIPEI, Taiwan

Background. The functions of nuclear receptors Nr4A1 and Nr4A3 remained undefined. Abrogation of both genes in mice was reported to result in the development of acute myeloblastic leukaemia (AML). However, this phenomenon has not been extensively studied in human. *Aims.* To explore the expression pattern of both genes in human acute leukaemias for hypothesis generating about the mechanisms of leukaemogenesis by silencing these two genes. *Methods.* The relative expression levels of both genes were studied by quantitative real-time PCR with unmanipulated bone marrow (BM) cells. The samples were from patients of AML (n=85), acute lymphoblastic leukemia (ALL, n=53) and myelodysplastic syndrome (MDS, n=17), and from normal BM donors (n=14). Paired BM samples at leukemia diagnosis and at complete remission were tested in 18 patients. The expression levels were expressed as the values relative to the median of donors. *Results.* Compared with normal BM donors, the expression levels of Nr4A1 and Nr4A3 were significantly lower in both AML (p<0.001 and p=0.004) and ALL patients (p<0.001 and p=0.002, Figure 1A), indicating that suppression of these two genes may also be related to the leukemogenesis of ALL. The expression levels of both genes did elevate when leukemia remitted (p=0.025 and 0.002, Figure 1B). However, the expression level of Nr4A1 in leukemia patients at disease remission remained subnormal compared with that of normal controls (p=0.053), while the level of Nr4A3 expression returned to a level compatible to that of donors (p=0.597). This observation raised a hypothesis that underexpression of Nr4A1 may be an earlier event than the change of Nr4A3 in AML development and may be associated with a *pre-leukemic status* of blood cells. The expression levels of these two genes in patients with MDS, which is supposed to be a pre-leukemia disorder, were also compatible with this hypothesis that Nr4A1 (p=0.002) but not Nr4A3 (p=0.399) was underexpressed. *Summary and Conclusions.* Underexpression of Nr4A1 and Nr4A3 plays a more generalized role in the pathogenesis of haematological malignancies. The chronology of the molecular changes of these two genes in leukemogenesis needs to be confirmed by further studies.

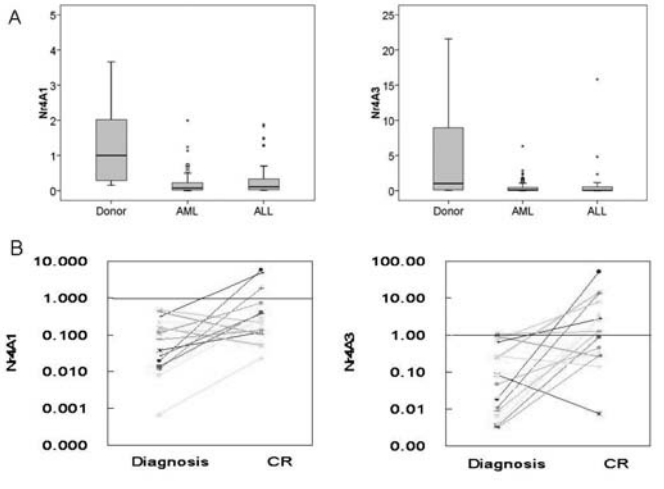

Figure 1. SDS scores for weight, height and BMI in SCA.

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ARSENIC TRIOXIDE INHIBITS ATRA-INDUCED PROSTAGLANDIN E2 AND CYCLOOXYGENASE-1 IN NB4 CELLS, A MODEL OF ACUTE PROMYELOCYTIC LEUKEMIA

A. Bazarbachi,[1] A. Habib,[2] E. Hamade,[2] R. Mahfouz,[2] M. Nasrallah,[2] H. De Thé[3]

[1]American University of Beirut Medical Center, BEIRUT; [2]American University of Beirut, BEIRUT; [3]Hopital Saint Louis, PARIS, France

Background. In acute promyelocytic leukaemia (APL), all-trans retinoic acid (ATRA) triggers cell differentiation while arsenic trioxide (As_2O_3) generates partial differentiation and apoptosis. Animal and human studies suggest that newly diagnosed APL patients can be cured using As_2O_3 combined with ATRA. Cyclooxygenases are involved in prostaglandins and thromboxane synthesis. We have recently demonstrated that ATRA induces COX-1 expression and prostaglandin synthesis in NB4 cells and in blasts from patients with APL. *Aims and Methods.* In the present study we investigated the effect of ATRA and As_2O_3 co-treatment on COX-1 expression and prostaglandin formation and tested the effect of the COX-1/COX-2 non-selective inhibitor indomethacin on cell differentiation. *Results.* Arsenic treatment of NB4 cells resulted in a partial but significant reduction of ATRA-dependent induction of COX-1 expression and activity. Pretreatment of NB4 cells with indomethacin significantly impaired ATRA/ As_2O_3 induced differentiation, as assessed by cell morphology, NBT test, or CD11c expression. PGE2 reversed the negative effect of indomethacin on differentiation of ATRA/As_2O_3 treated NB4 cells. *Conclusions.* In conclusion, COX-1 contributes to ATRA-dependent maturation of NB4 cells and is affected by As_2O_3. These results also suggest that NSAIDS should be avoided in APL patients treated with the combination of ATRA and As_2O_3.

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AUER RODS AND DIFFERENTIATION IN ACUTE PROMYELOCYTIC LEUKEMIA

B. Cassinat,[1] F. Zassadowski,[1] L. Ades,[2] S. Chevret,[1] I. Guillemot,[1] P. Fenaux,[2] C. Chomienne,[1]

[1]AP-HP, Hopital Saint-Louis, PARIS; [2]AP-HP, hopital Avicenne, BOBIGNY, France

Background. Auer rods (AR) are supposed to derive from the crystallisation of myeloperoxydase (MPO) granules and are the hallmark of acute myeloid leukemia, but they can also be observed in Myelodysplastic Syndromes in which they have been associated with a more aggressive disease. *Aims.* We report herein about an unexpected correlation between the presence of AR in APL blast cells at diagnosis and the ability of All-Trans Retinoic Acid (ATRA) to induce these cells to differentiate. *Methods.* Indeed, we have been routinely cultivating fresh APL cells taken at diagnosis to evaluate their sensitivity to the differentiation induction by ATRA. As reported previously (Cassinat *et al.*, Blood 2001), although the% of differentiated cells after 6 days of culture is very high in almost all cases, the results are much more heterogeneous after only 3 days of culture (ranging between 0% and 100% of differentiated cells) reflecting a level of patient heterogeneity in sensitivity to ATRA. *Results.* We then observed on 128 consecutive patients an inverse correlation between the% of cells harbouring AR and the% of differentiated cells at day 3 as measured by the NBT test. Indeed, when analysed as continuous variables the more the cells presented with AR at diagnosis, the less effective ATRA treatment was (p=0.015). The correlation was also found when patients were separated as less than 1% (n=48), between 1 and 15% (n=55) and more than 15% of AR (n=25), as median% of differentiated cells at day 3 were 42%, 25% and 16% respectively (p=0.0016). Interestingly, we had data on clinical outcome for 62 patients included in APL 93 and 2000 trials, and it appeared that patients with high levels of Auer rods tended to have higher risk of relapse (see table). Accordingly, median% of Auer rods positive cells was higher in the group of patients (n=12) that relapsed compared to patients that did not relapse (n=50) (8.5% *vs* 4.5%) in accordance with our previous observation that *in vitro* differentiation could be correlated to patient prognosis (Cassinat *et al.*, Blood 2001). *Conclusions.* All together these data show a novel correlation between biological characteristics of APL blasts and ATRA responsiveness.

Table 1.

	Auer Rods: <5% (n= 32)	Auer Rods: 5 to 10% (n= 15)	Auer Rods: > 10% (n= 15)
Number of relapses (%)	4 (12.5%)	3 (20%)	5 (33%)

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PROGNOSTIC IMPACT OF PHOSPHOINOSITIDE 3-KINASE/AKT ACTIVATION BY SER 473 PHOSPHORYLATION IN ACUTE MYELOGENOUS LEUKEMIA PATIENTS

V. Martin-Palanco, J. Serrano-Lopez, J. Serrano, J. Sanchez-Garcia, R. Rojas, S. Tabares, J. Casaño, J. Roman-Gomez, C. Herrera, A. Torres-Gomez

Hospital Reina Sofia, CORDOBA, Spain

SUMMARY AND *Aims.* Proliferation, differentiation and apoptosis of normal and leukemic hematopoietic cells are regulated by external signals from microenviroment stimuli. These signals activate a variety of intracelullar signaling pathways including RAS/Raf/MEK/ERK, JAK/STAT and PI3/Akt. The latter is activated by phosphorylation of Thr308 and Ser473 by PDK1 and PDK2 respectively. Phosphorylated Akt (pAkt) in turn activates Bad, NF-kB, GSK/Beta-catenina, mTOR, caspases and waf1, promoting cellular proliferation and apoptosis resistance. Constitutive activation of PI3/akt pathway has been reported in acute myelogenous leukemia (AML) but the prognostic importance of this finding is still a matter of controversy. *Methods.* A total of 40 patients diagnosed with AML (28 *de novo* and 12 secondary) were included in this study. Median age was 62 years ranging from 12 to 85 . Median leukocyte count was 9.3×10^9/L (range: 0.8-285). FAB subtypes were: M0=4; M1=11; M2=9; M3=6; M4=4; M5=6; with the following cytogenetic findings: t(15;17) (6), t(8;21) (2) , Complex cariotype (4) , del7 (1) and normal (16). Mononuclear marrow cells were obtained by Ficoll/Histopaque gradient centrifugation. Cell lysates were harvested with Q-Proteome cell compartment (Qiagen) and protein concentration assayed using Protein Assay Kit (Bio-Rad). Protein samples (50 μg/sample) were separated on sodium dodecyl sulphate poliacrylamide gel electrophoresis (Criterion XT Bis-Tris gels 12%, Bio-Rad). Proteins were transferred to nitrocellulose membrane (Pall Life Science) and block with 5% non-fat dry milk. Primary antibodies including total Akt, anti-phospho Ser473 Akt and β-actin (Cell Signalling Technology) were incubated overnight at 4° in blocking buffer. This was followed by incubation with horseradish peroxidase conjugated secondary antibody. Proteins were visualized by enhanced chemiluminescence (ECL-Plus Western blotting detection system) in Chemigenius-2 and quantified using Gene-Tools software. *Results.* In 20 patients constitutive activation of PI3/Akt by p-Ser743 was detected in marrow samples at diagnosis ($PI3K^+$) with high level expression in 8 cases and low levels in 12. No statistical differences were observed comparing $PI3K^+$ and $PI3K^-$ patients regarding age, sex, WBC, Cytogenetic risk or Nucleophosmin or FLT3-ITD mutations. Poor risk FAB subtypes disclosed more frequently $PI3K^-$ whereas good risk FAB subtypes showed $PI3K^+$ (p=0.07). Complete remission rates were similar comparing both groups but 75% of $PI3K^-$ patients and 16% of $PI3K^+$ received more than one cycle to achieve (CR) (p=0.019). Furthermore, relapse rates were 6% and 41% for $PI3K^+$ and $PI3K^-$ patients respectively (p=0.03) and overall survival was also higher for $PI3K^+$ patients (p=0.05). *Conclusions.* Consecutive activation of PI3k/Akt signaling pathway is a frequent event in AML patients. This finding is not related to other clinical or biological features. Our series including 40 consecutive patients, suggests that the detection of p-Ser473 could represent a favorable prognostic factor in AML patients

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SURVIVIN EXPRESSION IN ELDERLY AML PATIENTS BEFORE AND AFTER CHEMOTHERAPY, IN BONE MARROW AND PERIPHERAL BLOOD CELLS

A. Tsiga, E. Ioannidou, A. Avgitidou, E. Vlachaki, S. Dimoudis, P.H. Klonizakis, M. Diamantidis, E. Mandala, S. Haralambidou, I. Klonizakis

Aristoteles University of Thessaloniki, THESSALONIKI, Greece

Background. Apoptosis and cell cycle regulation are two intimately linked processes, acting to preserve homeostasis in tissues. Survivin is a member of the inhibitor of apoptosis protein (IAP) family and is characterized as a bi-functional protein, implicated in both regulation of cell division and suppression of apoptosis. Unlike other IAPs, survivin is expressed primarily in fetal but not in adult tissues and its expression is aberrantly enhanced in most cancers. Survivin takes part in cancer progression and resistance to therapy in diverse tumour types and haematologic malignancies. The AIM of this study is to evaluate the mRNA and protein expression of survivin in elderly AML patients, where several biologic features differ from younger individuals and determine its prognostic impact for disease progression and clinical outcome. For that reason we examined all patients before and after chemotherapy and used both BM and PB cells. *Methods.* Total RNA was isolated from BM and PB cells of 18 elderly AML patients (mean age 76.3±11.7 years, 12 men/ 6 women) before and after chemotherapy. All patients had excess of peripheral and BM blasts at diagnosis, while one month after chemotherapy they had <5% BM blast cells. Real-Time Semi-Quantitative RT-PCR assay was performed in order to detect survivin mRNA levels in both BM and PB. Abl was used as a reference gene. The regulation of survivin was estimated as an expression ratio. Data was analyzed using the REST-XL©-Version2 software. ELISA assay was performed for the evaluation of protein concentration in the serum of each patient. Statistical analysis was performed using the SPSS11.5 program. *Results*. In this study, we have detected survivin mRNA expression in both PB and BM cells, in all AML patients. As far as BM is concerned, patients after chemotherapy had 3.12-times (p>0.05) higher survivin mRNA levels compared to patients before chemotherapy. As regards PB, patients after chemotherapy had 2.27-times (p<0.05) lower mRNA levels, compared to those before chemotherapy. Furthermore, mRNA levels in BM samples were 4.45-fold (p<0.05) higher in patients before chemotherapy and 23.31-fold (p<0.05) higher in patients after chemotherapy, compared to PB samples. The mean concentration of survivin protein in the serum of patients before chemotherapy was 92.45 pgr/mL, while in patients after chemotherapy it was 0.25 pgr/mL. Survivin was undetectable in the control group. *Conclusions.* In our study both PB mRNA levels and protein levels of survivin were down-regulated after chemotherapy. This finding could be related to the reduction of PB blast cells. On the other hand, BM mRNA levels were up-regulated after chemotherapy, when BM blast cells were eliminated. This triggering data suggests that survivin could be expressed from cells other than blasts in higher levels, in order to maintain the homeostasis in BM. Therefore, we could assume that survivin plays a regulatory role when apoptosis is increased with chemotherapeutic drugs. Although our study proposes survivin as a prognostic marker in the AML process, further studies are necessary to determine whether it can also be used as a minimal residual disease (MRD) indicator or a promising cancer therapeutic target.

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THE EXPRESSION OF LEPTIN RECEPTOR ISOFORMS AND THE GLN223ARG POLYMORPHISM OF THE LEPTIN RECEPTOR GENE IN ACUTE MYELOID LEUKEMIA

F. Atalar,[1] O. Ozgen,[2] U. Ozbek[3]

[1]Istanbul University DETAE, ISTANBUL; [2]Istanbul University,Institute of Experimental Medical Research (DETAE), ISTANBUL; [3]Istanbul University,Institute of Experimental Medical Research (DETAE, ISTANBUL, Turkey

Deregulated expression of a variety of growth factors and/or their receptors has been implicated in the pathogenesis of certain leukemias therefore we aimed to characterize the potential differences in the expression pattern of the two major leptin receptor transcript variants in Acute Myeloid Leukemia (AML), we have also investigated whether the genetic variations in LEPR have implications for susceptibility to and prognosis in AML. We used RT-PCR to determine the expression patterns of the two leptin receptor transcripts; the long leptin receptor OBRl and the short leptin receptor OBRs splice variants in 50 AML patients (37 pediatric and 13 adult AML patients) and 71 healthy controls. We also studied the frequency of distribution of leptin receptor gene polymorphism Gln223Arg in our study and control groups. In pediatric AML patients, the expression of the OB-RL was 3.5 fold higher (p>0.05), compare to adult AML patients, and the level OB-RS was found to be nearly the same in both groups. However, both isoforms were expressed in the majority of samples from AML patients. An analysis of frequency distribution of the Gln223Arg polymorphism in the leptin receptor gene in leukemic children showed lack of differences between the AML patients and controls. There was no difference in the genotype frequencies between the AML and healthy groups either. However, more than 50% of the AML patients and the controls were carrying Q223R substitution. The prevalence of R/R homozygotes in pediatric and adult AML patients were approximately 21% and 7.69% respectively. Given that this prevalence in control group was 16.9%, a significant fraction of the population may be at risk with this susceptible genotype. Furthermore, 41.6% of AML patients having Q223R substitution were found to express OB-RL and only 25% were expressing OB-RS. The Q223R substitution in exon 6 is in the extracellular region of the LEPR, within C domain, representing a leptin binding site. The change in charge from

neutral to positive could possibly affect the functionality of the receptor with a significant reduction in cell surface expression of the receptor as well as alterations in the signaling capacity including constitutive activation of STAT1 and STAT3 and highly impaired ligand-induced STAT5B activation, whose deregulation is known to promote cell growth and prevent apoptosis in myeloid leukemias. The biological and pathological roles of leptin in leukemogenesis will be clarified by further investigations.

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DECREASED PHLPP EXPRESSION LEADING TO AKT ACTIVATION IN PEDIATRIC ACUTE MYELOID LEUKEMIA

F. Atalar,[1] A. Tekiner,[2] U. Ozbek[3]

[1]Istanbul University DETAE, ISTANBUL; [2]Istanbul University, Institute of Experimental Medical Research Genetics Dept, ISTANBUL; [3]Istanbul University,Institute of Experimental Medical Research (DETAE, ISTANBUL, Turkey

PI3K/Akt signaling is frequently activated in acute myeloid leukemia (AML) patient blasts and strongly contributes to proliferation, survival and drug resistance of these cells. The termination of Akt signaling is under the control of two key proteins: PTEN, a lipid phosphatase, that prevents activation by removing the second messenger that activates Akt, and PHLPP, a protein phosphatase, that inactivates Akt by direct dephosphorylation of the hydrophobic motif. PTEN has proven to be the archetypal tumor suppressor by its effects on the Akt signaling pathway. Nonetheless, there are abundant examples of Akt phosphorylation being elevated in cancer cell lines having wt PTEN. Thus, it is clear that other mechanisms causing elevation of Akt phosphorylation contribute to tumor progression. Gao *et al.* (2005) showed that PHLPP levels are markedly reduced in a number of colon cancer and glioblastoma cell lines. The role of PHLPP, referred to a novel tumor-suppressor, in Akt signaling emerges and makes PHLPP an attractive target for the development of novel anticancer strategies. We have studied the expression of PI3K/AKT pathway genes together with PHLPP, PTEN and caspase-3 in 35 pediatric AML patients by quantitative real-time PCR (qRT-PCR). Results were compared with peripheral blood samples and $CD33^+$ bone marrow cells from healthy donors by using Ct values. In order to characterize PHLPP gene, we have studied the expression of its four major domains (an amino-terminal PH domain, a leucine-rich repeat region (LRR), a PP2C-like catalytic core, and a PDZ binding motif). Our results suggest that AKT was up-regulated in AML patients that were examined (OR=4.4 95%CI=0.04-2.9, p=0.06). PTEN, PHLPP and caspase-3 were found to be decreased in AML patients compare to $CD33^+$ healthy bone marrow cells (3 times (p>0.05), 10 times (p>0.05) and 3 times (p>0.05) respectively). The expression of the four domains of PHLPP were also examined by qRT-PCR, we have detected expression in PH domain, PP2C-like catalytic core domain and PDZ binding domains in AML patients and both control group. However, we could not detect expression of LRR domain in AML patients and the expression of LRR was significantly lower in controls. Our data suggest that PHLPP may play an important role in the pathophysiology of hematopoietic malignancies and the progression of leukemia. The results presented here form the foundation for exploring the role of PHLPP in hematological malignancies and further studies are needed in order to determine the dysregulation of the PI3 kinase pathway which would improve our understanding and treatment of this disease.

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ACTIVATING MUTATION VAL617PHE OF JANUS KINASE 2 GENE AND CXCR4 EXPRESSION IN PH1-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES

M.R. Villa,[1] S. Improta,[1] M.R. Esposito,[1] A. Lucania,[1] S. Russolillo,[1] C. Consales,[2] C. Tiberio,[2] M.T. Polistina,[2] L. Mastrullo[1]

[1]P.O. San Gennaro U.O.C. Ematologia, NAPOLI; [2]U.O.C. Biochimica e Genomica Molecolare P.S.I. Loreto Crispi, ASL NA,[1] NAPOLI, Italy

Background. Ph1-negative chronic myeloproliferative disorders (CMPD) represent a subcategory of hematological malignancies and are characterized by a stem cell-derived clonal proliferation of myeloid cells including erythrocytes, platelets, and leucocytes. Traditionally, Ph1-negative CMPD include Polycythemia Vera (PV), Essential Thrombocythemia (ET), and Myelofibrosis with Myeloid Metaplasia (MMM). The Val617Phe point mutation of Janus Kinase 2 gene (JAK2V617F) is believed to participate in the pathogenesis of Ph1-negative CMPD and occurs in the majority of patients with PV and approximately half of those with either ET or MMM. Disregulation of CXCR4 and disruption of the CXCR4/SDF-1 axis may play a role in the pathogenesis and disease progression of CMPD. *Aims.* We investigated the expression of the chemokine receptor CXCR4 both on circulating and on bone marrow $CD34^+$ cells of patients with CMPD and its association with JAK2V617F mutational status. *Methods.* In our institution we are following 25 patients with PV (14 M and 11 F, median age: 48 years, r.: 39-76 years), 18 patients with ET (9 M and 9 F, median age: 40 years, r.: 29-84 years) and 15 patients with MMM (10 M and 5 F, median age: 50 years, r.: 43-74 years). The diagnoses of Ph1-negative CMPD were based on the Polycythemia Vera Study Group criteria as well as bone marrow biopsy. We used the allele specific polymerase chain technique for detection of Val617Phe mutation in all 58 patients with chronic myeloproliferative syndrome. Surface CXCR4 expression were measured flow cytometrically. *Results.* We measured Val617Phe frequency as 80% (20/25) in PV, 50% (9/18) in ET, and 40% (6/15) in MMM. We found significantly elevated hemoglobin levels and platelet count together with very low serum level of erythropoietin (measured at the time of diagnosis) in Val617Phe-positive polycythaemia vera and essential thrombocythaemia patient groups compared to Val617Phe-negative patients. However, white blood cell count and the frequencies of splenomegaly and other complications (thrombosis, bleeding, transformation to acute leukemia) were not significantly different between the mutation-positive and negative groups. The expression of CXCR4 on circulating $CD34^+$ cells was significantly reduced in patients with MMM as compared to normal controls and patients with PV and ET. Interestingly, by analysing immunophenotypic pattern of bone marrow $CD34^+$ cells we found in 10 out of 58 CMPD patients (i.e. 3 PV, 2 ET, 5 MMM) an over-expression of CXCR4 (as defined by CXCR4 mean fluorescence intensity ratio thresholds of more than 5). This subset of patients showed significantly higher levels of bone marrow blast cells and serum lactate dehydrogenase (LDH). No statistical association was found between JAK2V617F mutational status and the CXCR4 expression. *Conclusions.* The non-invasive mutation analysis of the Janus Kinase 2 Val617Phe is suitable for routine laboratory application and helps the differential diagnosis of chronic myeloproliferative syndrome. However current informations on disease-specific prognostic relevance of JAK2V617F are inconclusive, while our results warrant further investigation into the role of CXCR4 in CMPD and suggest that CXCR4 should be incorporated into the risk assessment of CMPD patients.

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INCREASE OF LYMPHOPLASMOCYTOID CELLS IN THE BONE MARROW OF PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA UPON IMATINIB MESYLATE TREATMENT: RELATIONSHIP WITH CLINICAL/HEMATOLOGICAL RESPONSE

A. Poggi,[1] I. Pierri,[2] A. Catellani,[2] F. Olcese,[2] R. Grasso,[2] M. Clavio,[2] M. Gobbi,[2] M.R. Zocchi[3]

[1]National Institute for Cancer Research, GENOA; [2]University of Genoa/Department of Clinical Hemathology, GENOA; [3]Scientific Institute San Raffaele, MILAN, Italy

Background. In the last years, tyrosine kinase inhibitors, as imatinib mesylate (Gleevec, Novartis, formerly known as STI571) have become the first line treatment of Chronic Myelogenous Leukemia (CML) and of a rare form of gastroenteric stromal cancer. It has been reported that in the latter case, the response to the drug *in vivo* is mainly due to immunocompetent cells, able to produce cytokines with antineoplastic activity. *Aims.* In this study, 20 CML patients, prior and during treatment with imatinib mesylate, underwent bone marrow (BM) aspirates every 6 months, to analyze: morphologic and phenotypic analysis, cytogenetic and biomolecular evaluation, compared to peripheral blood. *Methods.* Plasma from BM and peripheral blood (PB) was also recovered for evaluation of cytokines able to induce B lymphocytes differentiation, such as interleukin (IL)-4, IL-6 (whose receptor is CD126), IL-3, IL-10 or IL-21 (by ELISPOT and real time polymerase chain reaction): moreover, the expression of MCP-1, SDF-1, IP-10 and IL-8 were also measured. *Results.* We report that in 14 out of 20 CML patients a significant increase in the percentage of BM lymphoplasmocytoid cells was observed upon treatment with imatinib mesylate, with >10% (range 8-12%) of $CD20^+CD126^+$cells. Among this population, two third of cells coexpressed IgM and one third was IgD^+, while a smaller fraction of $IgM^+CD126^+CD20^-$ (3-4%) or $IgD^+CD126^+CD20^-$ (2-3%) cells was also found. In all these patients SDF1 increased in the BM plasma after imatinib (from 10-80 pg/mlL to 150-450 pg/mL) and its receptor CXCR4 was up-regulated on $CD20^+CD126^+$cells. In some cases (n=4) also IP-10 and its receptor CXCR3 were up-.regulated. No significant increase in transcription and secretion of IL-3, IL-4, IL-6, IL-10, IL-21, IL-8 or MCP1

were observed. The lasting 6 patients had<5% of $CD20^+CD126^+$ lymphocytes (range 2-4%), 2/3 coexpressing IgM and 1/3 coexpressing IgD. All patients with increased number of $CD126^+$ B lymphocytes (n=14) underwent hematologic remission, 10 of them with complete molecular and cytogenetic remission. On the other hand, among the patients with low or undetectable $CD20^+CD126^+$cells (n=6), only 4 underwent hemathological remission and none of them displayed stable cytogenetyc and molecular remission. In two patients relapsed after six months of treatment, the fraction of BM $CD20^+CD126^+$ lymphocytes decreased from 11% and 8% to 7 and 5%, respectively, with undetectable IgM+ $CD126^+CD20^-$ or IgD^+ $CD126^+CD20^-$ cells. *Conclusions.* These data suggest that this population of lymphoplasmocytoid B cells depends on or contribute to the pharmacological response. The increased production of SDF-1 that follows imatinib administration might be responsible for the observed increase in BM lymphoplasmocitoid cells, possibly due to the double proliferative/chemotactic effect of the cytokine on B cells, leading to both redistribution and *in situ* differentiation of $CD20^+$ $CD126^+$ lymphocytes. Finally, this phenomenon might help in monitoring the outcome of disease and the response to treatment.

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A DESCRIPTIVE STUDY OF DEEP VEIN THROMBOSIS (DVT) IN A TERTIARY CARE HOSPITAL IN SRI LANKA

A. Premawardana,[1] A.C.R. Navaratne,[2] W.I.K. Boteju,[3] C.K. Somaratne,[3] B.L.P.P. Balasooriya,[3] R.J.K. Senarath,[3] W. Wijebandara,[3] M.B.S.N. Mandawala,[2] T. Ruwanpathirana,[3] A. Kasthurirathne,[3] B.G.N. Rathnasena,[3] P. Fernando,[3] S. Fernando,[3] P. Wijesinghe[3]

[1]University of Ragama, COLOMBO; [2]NCTH Ragamac, COLOMBO; [3]NCTH Ragama, COLOMBO, Sri Lanka

Background. DVT is thought to be rare in Asian countries yet treatment guidelines have been developed using western data. Incidence, risk factors and guidelines for DVT prophylaxis are not well established for the Sri Lankan population. Though believed to be an effective screening tool for DVT the Well's Clinical Score is not widely used in Sri Lankan hospitals *Aims.* 1) To assess the risk factors and the incidence of DVT in a tertiary care hospital in Sri Lanka. 2) To assess the validity of Well's Score as a referral guide for CDU (Colour Duplex Ultrasound). 3)To assess the usage of Well's Score as a referral guide for CDU. *Methods.* Over a period of 8 months all patients admitted to four units (including one general medical, one general surgical, one Gynaecology & Obstetrics, and one Orthopedic ward) of the North Colombo (Teaching) Hospital: a 1550 bed tertiary care hospital, were screened for asymmetrical limb swelling of more than 2 cm. The latter group were subjected to risk assessment for DVT, Well's Scoring and CDU (Colour Duplex Ultrasound) after obtaining informed consent. *Results.* In the study population of 23274, 93 (0.4%) had unilateral limb swelling of which 12 (12.9%) were CDU confirmed DVT giving an incidence of 0.5 per 1000. Data of the two groups (those with DVT and those with leg swelling without DVT) was compared. Limb swelling lasting for more than two weeks was significantly commoner among DVT patients when compared with those without DVT (75% *vs* 25.9% (χ^2=11.52; p=0.001). Other conventional risk factors such as age >40 years (75% *vs* 80.2%) immobilization more than 3 days (25% *vs* 21%), presence of fracture (8.3% *vs* 9.9%), recent major surgery (25% *vs* 16%), smoking (16.7% *vs* 13.6%), use of Oral Contraceptive Pills (8.3% *vs* 0%), presence of diabetes (8.3% *vs* 12.3%) and hypertension (0% *vs* 11.1%) were not significantly different between the two groups. None of the patients had been evaluated with the Well's score as a guide to refer for CDU by the relevant clinical teams. In 55 (59.1%) subjects Well's score was 0 or less (minimum probability of DVT) and there were no subjects with DVT in this group. All 12 patients with DVT had a moderate or high probability Wells score. Sensitivity and the Negative Predictive Value of the Well's score was 100%. *Conclusions.* Overall incidence of DVT in the study population was lower than in other comparable published studies from Asia. Well's score which was underused by the clinicians is a highly sensitive screening tool for DVT. This continuing study highlights the importance of developing guidelines for DVT based on local epidemiological data. We also recommend the use of Wells clinical score prior to referral for CDU as unnecessary referrals for CDU thus could be avoided.

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NATURAL ANTICOAGULANTS AND FIBRINOLYTIC SYSTEM IN PATIENTS WITH ULCERATIVE COLITIS

M.S.S. Ayaz,[1] M.D. [2]Çakal,[1] B.S. Güney[2]

[1]T Yuksek Ihtisas Hospital, ANKARA; [2]Dade-Behring, ISTANBUL, Turkey

Background. Chronic intestinal diseases characterized by remissions and activation periods. Thromboembolism is an extraintestinal complication of IBD that is one of the most important cause of mortality and morbidity. Both arterial and venous thrombosis can be seen in patients with IBD. *Aims.* The aim of this study is to investigate the association between disease activity and level of natural anticoagulan and fibrinolytic system in patients with IBD. *Methods.* 96 patients with IBD consisting of 74 UC and 22 CD, who were followed up at Türkiye Yüksek i htisas Hospital and 20 healty controls enrolled into the study. We studies PC, PS, ATIII, PAI-1, APCR in patients with IBD and control group. PC, PS, ATIII, PAI-1, APCR were measured automate coagulometer system(Dade-Behring, Marburg Germany). *Results.* It was found that level of all natural anticoagulant proteins was significantly lower in patients with IBD than in healty controls. Levels of natural anticoagulan proteins were similar in control group and patients with UC and CD Who were in remission but significantly lower in patients who had active disease than in control group. Ferequency of APCR was similar in patients with IBD and healty control. An inhibitör of fibrinolysis, PAI-1 level was decreased in patients with both UC and CD especially who had active disease compared to healthy control. We thought that increase in fibrinolysis could be responsable for increased tendency of thromboembolism in patients with IBD. *Conclusions.* In conclusion, increased risk of thrombosis in patients with IBD is multifactorial in etiology. Not only decreased level of natural anticoagulan protein levels but also increase in fibrinolysis may increase risk of thrombosis in patients with IBD. We thought that reversal of disturbances of natural anticoagulant and fibrinolytic system by controlling disease activity can be decreased the risk of thrombosis in patients with IBD.

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DRUG HERBS AND ORAL ANTICOAGULANTS, A PROBLEM IN THE DAILY PRACTICE

J. Peris, L. Garcia Sanchez, B. Pascual, N. Muro, T. Vinuesa, G. Surra

Hospital Municipal Badalona, BADALONA, Spain

Background. The vitamin-K antagonists are drugs widely used as thrombosis therapy and its prophylaxis. Their use must be monitored closely, because these products have a narrow therapeutic index. Numerous interactions with herbs are documented, either increasing or decreasing the anticoagulant effect. *Aims.* We describe the problem that supposes the interactions with herbs in the daily surgery of anticoagulant treatment. Our main objective is to identify these interactions and if they have a significant effect. *Methods.* The two oral anticoagulant drugs available in Spain are acenocoumarol and warfarin. The international normalized ratio (INR) is the laboratory test used to mesure therapeutic efficacy and safety of vitamin-K antagonists. A control test is done every four weeks and if necessary it can be done earlier. One year observational study: interviewing patients with their INR alterated about herbs that they were taking at that moment. Literature review to know which products could interact with oral anticoagulants *Results.* In one year of about 1000 patients that we control in the surgery, we could relate variations in the INR with some herb in 13 cases. In 11 cases the INR was increased with level >4 (range 4-16.16): One of these patients was taking dandelion (Taxacum officinale). Four patients were taking chamomille (Matricaria capensis). These herbs have coumarins compounds. Three patients were taking equinacea (Equinacea purpurea, Equinacea angustifolia). One was taking bilberry (Vaccinium myrtillus). Another one grapefruit (Citrus paradisi). All these three products inhibit different isoenzymes of cytochrome P450 (the acenocoumarol is metabolized by these enzymes). The last patient was taking garlic oil (Allium sativum). Garlic increases the anticoagulant effect. In two cases the INR was decreased with level <2 (range 1-1.37), those patients were taking liquorice (Glycyrhiza glabra), which activate the cytochrome P450 and increases acenocumarol metabolism. *Conclusions.* It is commonly believed that herbal products are inoffensive, this is the reason why mainly of the patients do not take medical advise before starting a treatment with them. However interactions can appear with the usual treatment, and consequently there is risk of complications. If we fix on the vitamin-K antagonists the risk resides on the hemorragic or strokes events, that can appear depending on the interaction. In conclusion, we believe that patients should be educated

about the potential risk of using herbal products while being treated with vitamin-K antagonists, and physicians need to ask questions about the use of herbal products as part of the medical record.

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LOW-MOLECULAR-WEIGHT HEPARINS FOR THROMBOPROPHYLAXIS AND TREATMENT OF VENOUS THROMBOEMBOLISM IN PREGNANCY: REVIEW OF SAFETY AND EFFICACY IN 85 PATIENTS OF OUR CENTER

C. Andón Saavedra, M.F. López Fernández, L. Garcia Iglesias, S. Muñiz Lobato, S. Martín Perez, V. Noriega Concepción, J. Batlle Fonrodona

Complejo Hospitalario Universitario Juan Canalejo, A CORUÑA, Spain

Background. Pregnancy is associated with an increased risk of venous thromboembolism (VTE), and this condition remains an important cause of maternal morbidity and mortality. VTE can occur at any stage of pregnancy but the puerperium is the time of highest risk. Approximately a 50% of gestational VTE is associated with thrombophilia. Low-molecular-weight heparins (LMWHs) are now widely used for the prevention and treatment of VTE in pregnancy, however, evidence for the management of VTE during pregnancy is lacking, and generally guideline recommendations are extrapolated from studies in nonpregnant women. *Aims.* To assess the safety and efficacy of LMWHs for the prevention and treatment of VTE in pregnancy. *Methods.* 98 pregnancies in 85 patients from 1999 until now were retrospectively included. The LMWHs most common used were Dalteparin and Tinzaparin. The patients were subdivided in several groups according to the LMWH indication: a) treatment of VTE, b) thromboprophilaxis in patients with risk factors (previous VTE or thrombophilia), prevention of recurrent pregnancy loss (RPL) or other adverse pregnancy outcome (Table 1). *Results.* The average mean age of women was 31.5 years, 18 patients were older than 35 years. The duration and the time of starting LMWHs therapy depended on the indication. We determined the anti-X level activity in all the cases of the therapeutic group, supporting levels among 0.8-1.2 u/mL, and in the prophylactic group between 0.4-0.8 u/mL. Therapeutic group (Table 1). Twenty pregnancies were included in this group, 4 of these were pregnancies with rethrombotic episodes that were on prophylactic treatment. The previous VTE was associated with : 1 / pregnancy, 2/ oral contraceptive use in a woman being a homozygous mutation of the Factor V Leiden (FVL), 3/ transitory factor, 4/ idiopathic origin. All patients had a normal pregnancy presenting no haemorrhagic complications. Prophylactic group (Table 1). 62 out of 78 pregnancies included in this group had data of trombophilia: hereditary in 47 and acquired in 14. The most common thrombophilic risk factors were FVL heterozygous and the lupus anticoagulant, followed by heterozygosity for PT20210A. This group had two premature deliveries and two dead fetuses. Adverse events were registered in 12 pregnancies: 2 late allergic skin reactions, 1 purple skin, 8 haematoma in puncture area and, one of these women also presented haemorrhage postpartum and one patient presented intraabdominal haemorrhage during *in vitro* fertilization. *Conslusions.* The LMWHs are effective in the treatment and prevention of ETV. In our center the most frequent indication is prophylaxis in patients with hypercoagulability. Their use is not related with adverse events in the fetus; in mothers the most frequent complication is haematoma in puncture area. It is necessary to think about the possibility of haemorrhagic complications. The delivery was safety in all the cases. In the group of women with recurrent pregnancy loss (n=9) the thromboprophylaxis was effective in 7 patients.

Table 1. Principal indication for LMWH use.

INDICATION	Nº PREGNANCIES	DIAGNOSTIC INDICATION
TREATMENT IN ACUTE VTE	20	- Without previous VTE: 16 . with thrombophilia: 5 . without thrombophilia:11
1º trimester : 4 2º trimester : 5 3º trimester : 5 Puerperium : 6		- Rethrombosis pregnancy with prophylaxis: 4 . with thrombophilia: 1 . without thrombophilia: 3
THROMBOPROPHYLAXIS	78	- Previous VTE : 15 - Thrombophilia : 36 - Previus VTE and thrombophilia : 17 - Thrombophilia and abortus: 9 - Another causes: 2

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THROMBOSIS IN ABDOMINAL VESSELS AND THROMBOPHILIC RISK FACTORS.: A RETROSPECTIVE STUDY

N. Fernandez Mosteirin, C. Salvador Osuna, M. Torres, N. Padron, A. Godoy, B. Soria, F. Sevil, M. Guillen, J.F. Lucia, M. Giralt

Hospital Universitario Miguel Servet, ZARAGOZA, Spain

Background. thrombosis in the major vessels of abdomen and the resulting portal vein thrombosis (PVT), Budd-Chiari syndrome and mesenteric vein thrombosis (MVT), causes a wide spectrum of clinical pictures ranging from an asymptomatic patient to a patient with acute abdominal pain. This clinical setting has severe clinical consequences and complications. *Aims.* To analyze epidemiology, associated risk factors, clinical presentation, diagnostic mehtods, treatment and evolution of patients anticoagulated due to thrombosis in the major vessels of abdomen in our centre. *Patients and Methods.* We reviewed the medical records and data refered to anticoagulant treatment in patients receiving anticoagulation due to these diagnosis. *Results.* 21 patients were included (11 male/10 female), median age at diagnosis 53 years (range: 24-74), median time of follow up 44 months (7-150). Diagnosis were PVT in 13 patients (62%; 8 male/5 female), Budd-Chiari syndrome in 1 patient (4.8%) diagnosed of Paroxismal Nocturnal Haemoglobinuria (PNH) and MVT in 7 patients (33.3%). Clinical presentation (sings / symptoms): Abdominal pain 17 (80.9%), upper gastrointestinal bleeding 4 (19.05%), vomiting 4 (19.05%), fever 6 (28.5%), leukocytosis 1 (4.8%), no digestive symptoms 1 (4.8%). An abdominal computed tomography (CT) was made in all cases and diagnosis was performed in 20 patients (95.2%). 71.4% (15/21) of cases had recognizable risk factors and these are described in Table 1. No neoplasms were observed (Table 1). Four patients developed intestinal ischemia (19.1%). Surgical treatment was performed in 5 patients (23.8%), four of them the oldest of the serie (>70 years). Anticoagulation was initiated once diagnosis has been stablished in all patients. Repermeabilization of affected abdominal vessels happened in 4 patients (19.1%), three of them the youngest of the serie. Rethrombosis of abdominal vessels was observed in 3 patients (14.3%), 2 of them 3 and 4 weeks after completing anticoagulation treatment, 1 with AFS and 1 with ET. 8 patients (38.1%) developed complications of intraabdominal vessel thrombosis: 5 (23.8%) esophageal and gastric varices; 3 (14.3%) portal cavernomatous transformation. 1 patient (4.7%), the one with PNH developed an ischaemic cerebrovascular event during anticoagulation into therapeutic range. 6 patients (28.5%) developed bleeding complications during oral anticoagulation therapy: 3 upper gastrointestinal bleeding, 1 muscular hematoma, 1 epistaxis and 1 intracranial hemorrhage. None of the patients died due to acute or chronic complications of intraabdominal vessel thrombosis. *Conclusions.* Thrombophilia has a wide range of clinical presentations but abdominal organs probably bear the most important sequelae. Clinical presentation is protean and need a high degree of suspicion in patients who have risk factors, and the diagnosis is made based on imaging studies. In our serie an underlying prothrombotic condition was identified in more than a 70% of the patients, according to data reviewed in literature. The improving in diagnostic tests in thrombophilia research allow us to identify underlying risk factors, and to consider that intraabdominal vessel thrombosis occurs from both a primary thrombophilic condition and a factor that triggers the formation of the patological thrombus.

Table 1.

Risk Factor	n	(%)	Risk Factor	n	(%)
Acquired Thrombophilia	6	28.5	Inherited Thrombophilia	3	14.3
Essential Thrombocytemia (ET)	3	14.3	Protein C deficiency	2	9.5
Chronic Myeloid Leukemia	1	4.7	Heterozygote Prothromobin	1	4.7
Antiphospholipid Syndrome (AFS)	1	4.7	Adominal inflammation	4	19.1
Oral contraceptives	1	4.7	Pancreatitis	1	4.7
Combined Thrombophilia	2	9.5	Splenectomy	1	4.7
AFS+Heterozygote Prothrombin	1	4.7	Inflamatory bowed disease	1	4.7
PNH+Appendectomy	1	4.7	Renal colic	1	4.7

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TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA WITH PETHEMA LPA 99 TRIAL: TUNISIAN SINGLE CENTER EXPERIENCE

R. Jeddi,[1] H. Benneji,[1] K. Kacem,[1] S. Mnif,[2] R. Ben Amor,[1] L. Aissaoui,[1] R. Ben Lakhal,[1] H. Benabid,[1] Z. Belhadjali,[1] B. Meddeb[1]

[1]Aziza Othmana Hospital, TUNIS; [2]Institut Pasteur, TUNIS, Tunisia

The rationale for avoiding AraC in PETHEMA trial LPA 99 was to reduce death in CR without increasing the incidence of relapse. In our Department APL93 trial (with AraC) was used between 1998-2004 for the treatment of 27 patients with confirmed APL.Induction mortality rate was 23% and relapse occurred in 20%, leading to 5-years RFS of 72% and 5-years OS of 69%.Since March 2004 The PETHEMA LPA 99 protocol was adopted for the treatment of newly diagnosed APL with the aim to improve our results. Between March 2004 and February 2008, 30 consecutive patients with morphologic diagnosis of APL were treated with LPA99 protocol which consisted of including ATRA and increased doses of anthracyclin during consolidations and without the use of AraC.The study population was composed of: 12 male and 18 female (sex ratio=0.66).According to GIMEMA-PETHEMA risk group stratification;20 were intermediate risk and 10 high risk (WBC >10×10^9/L). Cytological analysis revealed 25 hypergranular,4 microgrnular and 1 rare basophilic form.Imunophenotyping profile revealed the negativity of CD 34 and HLA DR in 90% (27/30). CD 2 was tested positive in 2 of 4 microgranular form. We noted aberrant expression of B lymphoid markers (CD 19, CD 22) in respectively two patients. Additional cytogenetic abnormalities to t (15, 17) were noted in 30.76%.Of the 29 evaluable patients, 26 achieved hematologic complete remission (89.65%). 1 death due to cerebral hemorrhage occurred before treatment. Induction failure is due to 3 death due to Retinoic Acid Syndrome (RAS).ATRA related complications during induction were:RAS in 6 cases (20%); RAS was *definitely present* in 3 cases and *indeterminate* in 3 cases, other reported ATRA related complications were :scrotal ulcerations (5), perineal ulceration (1), Sweet syndrome (1), and spleen infarct (1). At the end of induction, molecular assays for PML/RARA were carried out for the 26 patients who achieved complete remission. Of these, 30.76%(8/26) tested were positive. Of the 8 patients tested positive for PML/RARA after induction, 7 were among Intermediate risk, and 1 among high risk group. RT-PCR for PML/RARA was tested negative in 96.15%, and in 100% respectively after the second and the third consolidation course. Median follow up is 23 month .23 patients are alive in continuous complete remission, 1 patient aged 13 yr in the intermediate risk group relapsed after 35 month and treated with ATO 0.15 mg/kg and ATRA 25 mg/m^2. 1 patient (40yr) developed secondary CMML with monosomy 7 after 21 month of CR.1 death in CR occurred during the third consolidation course due to septic shock. The 2-years EFS is 92%.The 2-years RFS is 100%,and the probability of remaining alive after 2 years is 96%.Our study show good tolerance and feasibility of the LPA 99 protocol in our department. Recently the joint analysis of results of APL2000/LPA 99 revealed a beneficial role of AraC in high risk group (WBC >10×10^9/L).Remain the problem of predicting the relapses occurring among intermediate and low risk group.

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THE IMPACT OF FLT3 MUTATIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA: TYPE, SIZE AND INTERACTION WITH NPM1 MUTATIONS

N. Tosic,[1] N. Colovic,[2] M. Colovic,[3] B. Zukic,[4] S. Pavlovic[4]

[1]Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia, BELGRADE; [2]Institute of Hematology, Clinical Center of Serbia, BELGRADE; [3]Institute of Hematology, Clinical Center of Serbia, BELGRADE; [4]Institute of Molecular Genetics and Genetic Engineering, BELGRADE, Serbia

Background. Mutations in the fms-like tyrosine kinase 3 (FLT3) gene (internal tandem duplication in the juxtamembrane domain (FLT3-ITD) and point mutation in the tyrosine kinase domain (FLT3-D835)) and the nucleophosmin (NPM1) gene are the most common abnormalities in adult acute myeloid leukemia (AML). FLT3-ITD positive patients have poor prognosis. The presence of NPM1 mutations is associated with favourable outcome and increased event-free and overall survival, but only in cases when they are not in cooperation with FLT3-ITD mutations. *Aims.* We have examined the impact of FLT3 mutations on clinical outcome in 113 adult AML patients. Also, we have investigated the impact of tandem-duplication size and interaction with NPM1 mutations in 12 FLT3-ITD positive patients. *Methods.* For detection of FLT3 mutations (FLT3-ITD and FLT3-D835) we used polymerase chain reaction (PCR) and PCR-RFLP *Methods.* ITD size was determined using sequencing analysis. Amplification of NPM1 exon 12 was carried out using PCR and the products were directly sequenced. *Results.* FLT3 mutations were analyzed in 113 adult AML patients. Twenty patients were found to be FLT3-ITD positive (17.7%). The mutations occurred most frequently in M5 and M0 subtypes of AML. They were mainly associated with normal kariotype. All patients harboring FLT3-ITD had higher number of white blood cells than patients without it (p=0.027). FLT3-ITD mutations were associated with lower complete remission (CR) rate (χ^2=5.706; p=0.017) and shorter overall survival (OS) (Log rank=8.76; p=0.0031). As for disease free survival, the difference between FLT3-ITD positive and FLT3-ITD negative patients was not statistically significant (Log Rank 0.78; p=0.3764). In multivariate analysis, the presence of FLT3-ITD mutations was the most significant prognostic factor for both OS and CR rate (p=0.0287; RR=1.73; 95% CI=1.06-2.82). However, in the group of patients with the intermediate-risk karyotype, the FLT3-ITD was not associated with inferior clinical outcome. FLT3-D835 mutation was found in 4 patients (3.5%). There was no statistically significant difference in OS depending on the presence of FLT3-D835 mutations (Log rank=1.31; p>0.05). Sequence analysis of twelve FLT3-ITD positive patients showed that the size of duplication was between 24-84 bp (median 60 bp). Patients were divided in two groups according to the length of duplication, *short* (<40 bp) or *long* (>40 bp). No significant difference was found among them regarding OS. NPM1 mutations were found in 5/12 (42%) FLT3-ITD positive patients examined. All of them were of type A mutation. According to a multivariate Cox analysis statistically significant lower OS was found only in *short* FLT-ITD positive/NPM1 positive group (p<0.05). *Conclusions.* The presence of FLT3-ITD mutations in our cohort of AML patients is the most significant factor predicting OS. Coincidence of FLT3-ITD and NPM1 mutations is significant (42%). We did not find any statistically significant correlation between size of ITD-duplication and survival. Lower OS was found in patients with association of NPM1 and *short*-ITD mutation (<40 bp). To confirm this trend of statistical significance, a larger number of patients is required.

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CHEMOKINE RECEPTOR EXPRESSION BY CIRCULATING T CELLS DERIVED FROM ACUTE LEUKEMIA PATIENTS WITH SEVERE CHEMOTHERAPY-INDUCED CYTOPENIA EXPRESS A WIDE RANGE OF CHEMOKINE RECEPTORS

A.M. Olsnes, E. Ersvaer, A. Ryningen, O. Bruserud

Haukeland University Hospital and the University of Bergen, BERGEN, Norway

Background. Normal T cells can mediate antileukemic T cell effects after allogeneic stem cell transplantation, and T cell targeting immunotherapy is also considered for patients receiving conventional chemotherapy. Antileukemic T cell reactivity is most effective in patients with a low leukemia cell burden, and this burden is expected to be lowest early after transplantation/chemotherapy when patients are cytopenic. Local T cell recruitment will then be essential for the efficiency of the antileukemic T cell response. *Aims.* The aim of the study was to describe the chemokine receptor profile of circulating T cells for AML patients with severe therapy-induced cytopenia. *Methods.* The expression of CCR1-5, CCR7 and CXCR2-4 on T cells from peripheral blood samples of cytopenic AML patients and of healthy volunteers was analyzed by flow cytometry. We compared the chemokine receptor expression by T cells derived from healthy individuals, from AML patients with therapy-induced cytopenia after conventional chemotherapy and from acute leukemia patients transplanted with stem cell grafts from HLA-matched family donors. *Results.* Chemokine receptor expression was investigated in 20 samples derived from patients receiving conventional intensive chemotherapy for acute leukemia and eight samples derived from allotransplanted patients. The patients were compared with a group of 12 matched healthy controls. The receptor expression profiles for the three groups are very similar. We defined low expression as <15% positive cells, 15-50% as intermediate expression and >50% positive cells as high expression. When comparing the median expression all three groups showed a very similar profile with CCR1low, CCR2low, CCR3low, CCR4intermediate, CCR5intermediate, CCR7 low/intermediate, CXCR2low, CXCR3intermediate, CXCR4high. There was a considerable variation in receptor expression between individual patients and healthy controls. The variation ranges were generally largest for those receptors that showed a relatively high median expression. Furthermore, there were no statistically significant correlations between the expressions of single chemokine receptors. We also investigated

whether there were any correlations between chemokine receptor expression and clinical parameters, i.e. induction *vs* consolidation cycles, duration of cytopenia, and time of sampling, lymphocyte level or acute phase reaction at the time of sampling. However, no statistically significant differences were observed. When investigating a group of unselected AML patients the leukemia cells usually showed constitutive release of chemokine ligands for all these receptors and ligand release could be increased by the PKC agonist PEP005. *Conclusions.* We conclude that the chemokine receptor profiles of circulating CD3-positive T cells from healthy individuals, AML patients with chemotherapy-induced cytopenia and from allografted acute leukemia patients show no qualitative differences and only minor quantitative differences. T cells showed a broad chemokine receptor expression profile and AML cell chemotaxis seems possible since primary AML cells usually release ligands to these receptors.

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COULD NON MOBILIZATION BE AN ADVERSE PROGNOSTIC FACTOR FOR LEUKAEMIA RELAPSE IN ACUTE MYELOID LEUKAEMIA?

A. Nosari, C. Vanelli, M. Montillo, L. Marbello, A. Molteni, V. Mancini, M. Riva, R. Corneo, L. Intropido, L. Pezzetti, M. Nichelatti, R. Cairoli, E. Morra

Niguarda Cà Granda Hospital, MILAN, Italy

Background. Several studies have reported data on factors influencing mobilizaton of PBSC in non-myeloid neoplasms. On the contrary, there are very few studies on the efficiency of PBSC mobilization in patients with acute myeloid leukaemia (AML). Particularly it is unknown if a failure of mobilization may have a prognostic value, and if it may influence the choice of allogeneic bone marrow procedures in intermediate cytogenetic risk patients. *Methods.* We retrospectively analyzed 96 consecutive AML patients, age <60 yrs, in first complete remission after two courses of consolidation therapy with HD-ARAC, scheduled for stem cell harvest. G-CSF (5 µg/kg/day) was started on day +10 from the beginning of chemotherapy to induce mobilization. Of 96 AML patients in first remission 21, submitted to allogeneic bone marrow transplantation, were not considered in the statistical analysis. *Results.* Seventy five patients were evaluated with Kaplan-Mayer test followed by logrank test. Cytogenetic risk and FLT-3 positivity were similarly distributed between patients who mobilized or not. In 26 of these 75 patients harvest was not performed because the number of $CD34^+$ circulating cells remained below 20×10^6/µL. In the 49 patients in whom the harvest was obtained ($CD34^+$ cells $>2\times10^6$/kg body weight), a median of 2 aphereses (range 1-8) were performed, resulting in a median collection of 5.7×10^6 $CD34^+$ cells/kg (range 2-18.3). The median follow-up of our population was 23 months (range 7-121). The 3-years OS was 52%, not statistically different between mobilizer and non mobilizer patients (p=0.72). The majority of relapses were seen in the first year of observation. In the whole series of 75 patients the median 3-years DFS was 58%; in both groups of the mobilizer and non mobilizer patients the median DFS was non significantly different (p=0.22). However, non mobilizers seem to relapse progressively over time, while mobilizers show a plateau of DFS over 50% at a median follow-up of 23 months (Figure 1). Twentynine patients underwent autologous bone marrow transplant with good hematopoietic recovery. *Conclusions.* Effective harvest was feasible for 65.3% of all 75 patients. Non mobilizers didn't show a significantly higher risk of relapse and/or reduced DFS. However, mobilizers seem to reach a plateau of DFS after the first year. A more numerous population needs to verify these results.

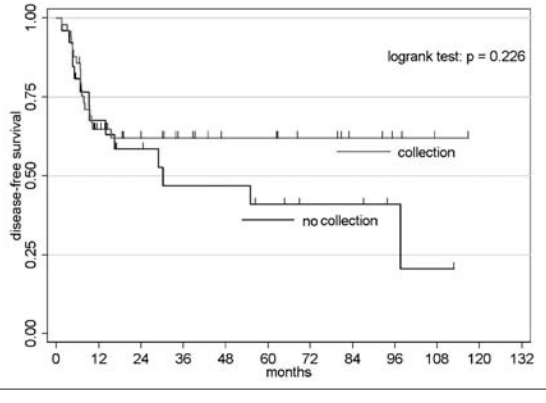

Figure 1.

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TREATMENT FEASIBILITY, CLINICAL OUTCOME AND PROGNOSTIC FACTORS OF ACUTE MYELOID LEUKEMIA IN THE ELDERLY

J. Doyen,[1] C. Bouiller,[1] E. Chamorey,[1] L. Gastaud,[1] S. Raynaud,[2] J. Thariat,[1] A. Thyss,[1] F. Peyrade[1]

[1]*Centre Antoine-Lacassagne, NICE;* [2]*CHU Archet, NICE, France*

Background. Acute myeloïd leukemia mainly occurs in the elderly. Many patients are denied conventional therapy because of its potential toxicity. *Aims.* A retrospective study was conducted to analyse efficacy and toxicity of conventional treatment, clinical outcome and death prognostic factors in the elderly. *Methods.* We retrospectively reviewed the charts of 62 patients in our institution between June 1999 and November 2007. Demographic and treatment data were collected: age (≥75), performans status (PS) (≥2), infection and blood cell count at diagnosis, cytogenetics, bone marrow blast count, prior history of haematological disease and treatment protocol. Univariate (Log-Rank) and multivariate analyses (Cox model) were performed to determine independent death prognostic factors. *Results.* Median age was 69.9 years (range 59-86). Fourty-five percent of patients had a prior history of haematological disease, 39% had unfavourable cytogenetics (according to SWOG/ECOG criteria). Fifty-two percent received one line of conventional anthracyclin-aracytin based regimen. Thirty-five percent received two lines. Response assessment demonstrated 48% major remissions (26 complete remissions (CR), 2 partial remissions (PR) according to NCI criteria) and 52% (n=30) non-responding patients. 6.5% (n=4) of patients had best supportive care only. There were 13.8% (n=8) of treatment-induced death (TID). 82.1% (23/28) of responders received consolidation and of whom 5 were further treated with intensive chemotherapy followed by autologous bone marrow transplant. No TID occurred during consolidation or transplant. Median overall survival was 8.2 months (CI [5.95-15.01]). Median survival by sub-groups was 17 months for responders, 45 months for those who received intensive chemotherapy followed by autologous bone marrow transplant and 1.8 months in the best supportive care arm. Prognostic factors on univariate analysis were: unfavourable cytogenetics (p=0.018), infection (p=0.024), grade 3-4 anemia (p=0.00037), bone marrow blast count superior to 30% (p=0.00666). Unfavourable cytogenetics (p=0,041) and infection (p=0.05) were independent prognostic factors on multivariate analysis. *Conclusions.* Our experience shows that conventional treatment is often feasible and can improve clinical outcome. Infection and unfavourable cytogenetics can identify bad prognosis patients. Prospective studies are required to confirm the reliability of such prognostic factors in other independent subsets of patients.

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MULTIPLE BIOMARKER USE FOR EVALUATION OF TREATMENT-RELATED CARDIOTOXICITY IN ACUTE MYELOID LEUKEMIA. A PILOT STUDY

J.M. Horacek,[1] M. Tichy,[2] L. Jebavy,[1] R. Pudil,[1] M. Ulrychova,[1] P. Zak,[1] J. Maly[1]

[1]*Charles University Hospital, HRADEC KRALOVE;* [2]*Faculty of Military Health Sciences, HRADEC KRALOVE, Czech Republic*

Background. Cardiotoxicity is a potentially serious complication of hematooncology treatment. From cytostatics, anthracyclines (ANT) represent the greatest risk for cardiotoxicity. Various *Methods.*including biochemical markers have been recommended for monitoring of cardiotoxicity. Data on using new cardiac biomarkers in this context are very limited. *Aims.* In our pilot study, we evaluated treatment-related cardiotoxicity in acute myeloid leukemia (AML) with multiple biomarkers of cardiac injury: myoglobin, CK-MB mass, cardiac troponins (cTnT, cTnI), heart-type fatty acid binding protein (H-FABP) and glycogen phosphorylase BB (GPBB). According to the available literature, the last 2 mentioned biomarkers (H-FABP, GPBB) have not been investigated in this context, so far. *Methods.* Twelve AML patients (mean age 51.3±10.7 years, 7 females) treated with 3-6 cycles of ANT-based chemotherapy (CT) were studied. All biomarkers were measured at the baseline (before CT), the day after first CT (cumulative ANT dose 124.7±21.9 mg/m^2), the day after last CT (total cumulative ANT dose 479.8±106.2 mg/m^2) and 6 months after completion of treatment (6 months after CT). Values above reference range recommended by the manufacturers (Roche, Randox) were considered elevated. *Results.* The results and cut-off values for all biomarkers are shown in the Table. GPBB increased above cut-off in 2 patients after first CT, in 3 patients after last CT and remained elevated in 2 patients within 6 months after CT. All patients with cTnI or cTnT positivity had elevated GPBB. Other biomarkers remained within the reference range in all patients. *Conclusions.* Our results suggest that

GPBB could be a new promising marker for detection of ANT-induced cardiotoxicity in AML patients and probably superior to cardiac troponins. The predictive value for development of cardiomyopathy in the future is unclear and will be evaluated during a prospective follow-up. Further studies will be needed to confirm our preliminary results and define the potential role of new biomarkers in the evaluation of treatment-related cardiotoxicity in hematooncology.

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Table 1. Elevated biomarkers of cardiac injury in AML patients treated with anthracyclines (n=2).

cardiac biomarkers	before CT	after first CT	after last CT	6 months after CT
myoglobin above 76.0 µg/L	0	0	0	0
CK-MB mass above 4.80 µg/L	0	0	0	0
cTnT above 0.01 µg/L	0	0	0	1 (8.3 %)
cTnI above 0.40 µg/L	0	1 (8.3 %)	1 (8.3 %)	1 (8.3 %)
H-FABP above 4.50 µg/L	0	0	0	0
GPBB above 7.30 µg/L	0	2 (16.7 %)	3 (25.0 %)	2 (16.7 %)

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HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOGENOUS LEUKAEMIA WITH MULTILINEAGE DYSPLASIA

H.J. Kim, W.S. Min, K.S. Eom, B.S. Cho, Y.J. Kim, S. Lee, C.K. Min, S.G. Cho, J.W. Lee, C.C. Kim

Catholic HSCT Center, St Mary's Hospital, SEOUL, South-Korea

Background. Little is known about the appropriate treatment for acute myelogenous leukemia with multilineage dysplasia (AML MLD). In general, the conventional induction chemotherapy (IC) has not shown a comparable effect as in the other subtypes of AML. The role of hematopoietic stem cell transplantation (HSCT) has been suggested, but with no confirmative results reported so far. *Aims.* To evaluate and stress the efficacy of our intensified induction chemotherapy followed by earlier HSCT in adult patients with AML. *Methods.* We retrospectively reviewed medical records of our centre in the period between July, 2003 and May, 2007. Among 801 newly diagnosed AML patients, 68 (8.4%) were AML MLD. The majority (N=43) was categorized as cytogenetically intermediate-risk group according to the MRC criteria. All patients were treated with the standard protocol of this center, consisting '3x7' idarubicin (IDA) plus N4-behenoyl-1-b-D-arabinofuranosyl cytosine (BH-AC) IC, as previously reported (Park HS *et al.* Semin Hematol 1996;33:24-29). Briefly, based on the results of the bone marrow examination on the D+7 of IC, augmentation treatment was added if a patient demonstrated more than 5% leukaemic blasts. Many of them were treated with sequential maintenance chemotherapies (N=7) or HSCT (N=20) following our standard IC, and analyzed outcome finally. *Results.* Thirty-five patients (74%) were in complete remission (CR) with 1~2 induction course(s). Eight (23%) were relapsed early after IC. Twenty patients in CR received autologous or allogeneic HSCT using this centre's total body irradiation containing preparative regimen followed by standard graft-versus-host reaction prophylaxis. Only 2 patients (10%) were relapsed posttransplant, but 3 out of 7 patients who received only chemotherapy eventually relapsed (43%) with median follow-up of 15 months (range, 6-50) (p=0.008). Interestingly, transplant-related non-relapse mortality was none. *Conclusions.* These results suggest that adult patients with AML MLD resulted in comparable CR after this protocol of IC, even in the poor-risk group. Most of all, HSCT rather than maintenance chemotherapy, specifically early after CR, should be considered in this characteristic disease.

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QUANTITATIVE DETECTION OF MINIMAL RESIDUAL DISEASE IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA USING LNA-MODIFIED TAQ-MAN PROBES AND IGH-RQ PCR

H. Francova,[1] Y. Brychtova,[2] M. Doubek,[2] D. Dvorakova,[1] J. Mayer[2]

[1]University Hospital, BRNO; [2]Hemato-Oncology,University Hospital and Masaryk University, BRNO, Czech Republic

Background. Detection of minimal residual disease (MRD) persistence provides essential information on the treatment response in hematologic malignancies and MRD eradication is the final goal of CLL treatment. Therefore, the development of minimal residual disease detection assays on molecular basis with sufficient specificity and sensitivity is crucial. Until recently, the monitoring of MRD was based on two different consensus JH TaqMan probes. JHQ1/4/5 fits to the JH1, JH4, and JH5 segments, and JHQ6 to the JH6 segments, and RQ-PCR specificity may be markedly limited. These probes fit to just about 90% of all JH rearrangements. Recently, more specific approach can be achieved using LNA-modified (Locked Nucleic Acid) TaqMan probes. However, studies regarding LNA probes for MRD monitoring are rare. *Aims.* The aim of this study was to test the improved IgH-RQ PCR approach using six VH specific LNA modified, fluorescently labelled TaqMan probes, and to introduce highly specific MRD monitoring approach which will be applicable for all IgH gene rearrangements. *Methods.* For each patient, clone specific primers were designed, forward oligonucleotide was designed to match either the FWR2 or CDR2 regions of the particular IgVH gene, reverse oligonucleotide fitting to the CDR3 region, and their quantification standards cloned. The fragment of human albumine gene was used as the external standard. Family specific LNA modified TaqMan probes were designed against all six VH1 - 6 families to the framework region 3 (FR3) which recognize a highly conserved germ-line sequence with the highest degree of homology among different family clones and reduced number of somatic mutations in the majority of patients. *Results.* We developed a DNA based IgH quantitative approach using six VH specific LNA modified, fluorescently labelled TaqMan probes corresponding to the framework region 3 (FR3). This method is feasible particularly for patients with IgVH unmutated genes. In the case of hypermutated genes, the patient specific LNA modified probe has to be designed. To test the sensitivity limit of patient individual qPCR assay, serial dilutions of tested DNA into DNA (mix of 6 DNA samples from healthy individuals) were performed (10^{0}-10^{-8}). The sensitivity achieved was of 10^{-6}, what means one order of magnitude higher sensitivity compared to the JH TaqMan probes system. Moreover, the specificity of LNA-DNA based RQ PCR was solely exclusive for IgH rearrangements in all tested individuals contrary to JH specific system. *Conclusions.* LNA-modified probes and IgH-RQ PCR represent highly specific and sensitive method of MRD assessment in patients with B-CLL. In contrast to JH specific TaqMan probe system, this approach of MRD monitoring is applicable for all known IgVH rearrangements. This method is now routinely used for MRD monitoring in patients after stem-cell transplantation for CLL at our department.

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TELOMERE LENGTH EVALUATION IN PATIENTS WITH UNTREATED B-CHRONIC LYMPHOCYTIC LEUKEMIA - CORRELATION WITH OTHER MOLECULAR, CYTOGENETIC AND IMMUNOPHENOTYPIC FEATURES

J. Brezinova,[1] S. Vcelikova,[1] A. Berkova,[2] Z. Zemanova,[2] J. Tajtlova,[2] L. Grosova,[2] E. Cmunt,[3] J. Karban,[3] J. Schwarz,[1] K. Michalova[1]

[1]Institute of Hematology and Blood Transfusion, PRAGUE; [2]General Faculty Hospital and First Faculty of Medicine, Charles University, PRAGUE; [3]1st Medical Department, General Faculty Hospital and First Faculty of Medicine, PRAGUE, Czech Republic

Background. Clinical course of B-chronic lymphocytic leukemia (B-CLL) varies widely in patients. Staging systems of Binet and Rai are commonly used to predict the course of the disease. However, during the recent years, other indicators related to the genetics and biology of B-CLL such as genomic aberrations, immunoglobulin variable heavy chain (IgVH) mutation status, CD38 and ZAP-70 expression are increasingly utilized. In recent studies telomere restriction fragment length (TRF-L) was found as one of new indicators for prognosis. *Aims.* The aim was the evaluation of telomere restriction fragment length in a cohort of patients with previously untreated B-CLL. Results were correlated with interphase FISH (I-FISH), IgVH mutation status and ZAP-70 and CD38 expression. *Methods.* During the year 2007 we examined 74 peripheral blood samples of patients with untreated B-CLL using I-FISH. In 20 of them (9 male, 11 female, mean age 65.7 years) other biological features were studied including TRF-L, IgVH mutation status, ZAP-70 and CD38 expression. Interphase I-FISH analyses were performed using DNA probes from Abbott-Vysis: 1) CLL Probe panel for regions 17p13.1 (gene p53), 11q22.3 (gene ATM), 13q14.3, 13q34 and 12p11.1-q11; 2) LSI IGH dual color break apart rearrangement probe for detection of 14q32 translocations. Telomere length - TRF-L index in kilobases (kbp) was performed by Terminal Repeat Fragment (TRF) method based on Southern blot hybridization with non-radioactive labeled telomeric probe after digestion of target DNA with Hinf I/RsaI restriction enzymes, followed by chemiluminescent detection. We considered TRF-L shorter than 7.5 kbp (mean TRF-L from 45 age matched healthy individuals, Me=38.5 years) as cases with reduced telomeres. ZAP-70 expression

analysis was done by flow cytometry, PCR *touch down* methodology was used for mutational analyses of IgVH genes. *Results.* Deletion of 13q14 was proved in eight patients, in seven of them as a sole abnormality, in one combined with ATM gene deletion. Partial deletion of IgVH gene was observed in three cases, including one patient with additional chromosome 12 trisomy. In the remaining nine patients results of I-FISH were normal. IgVH mutational analysis was performed in 19 patients - in eight patients mutated, in seven patients unmutated IgVH genes were proved and in four patients the germ-line IgVH genes were not identified. Increased ZAP-70 expression was detected in 11 patients, CD38 was positive in six patients. High heterogeneity in terms of TRF-L was found (median 5.35 kbp, range 3.65-15.5 kbp). Reduced telomeres were confirmed in 15 patients: in seven patients in combination with IgVH unmutated status, in 11 patients with ZAP-70 positivity and in seven patients with CD38 positivity. Normal telomere length was observed in five patients, in all of them combined with favorable prognostic features. Detailed results of TRF-L analyses in comparison with other prognostic markers will be presented. *Conclusions.* Determination of independent indicators of clinical course in early stages is a subject of intensive research and TRF-L technique can contribute together with other prognostic parameters to complete the risk profile of B-CLL patients.

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PLASMA LEVELS OF SOLUBLE ENDOGLIN HAVE PROGNOSTIC SIGNIFICANCE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

L. Smolej, C. Andrys, D. Belada, M. Hrudkova, P. Zak, J. Krejsek, J. Maly

University Hospital and Medical School, HRADEC KRALOVE, Czech Republic

Background. Angiogenesis has been studied in chronic lymphocytic leukemia (CLL) since mid 1990s. Given the extraordinary heterogeneity of clinical course of CLL patients, angiogenesis assessment may potentially improve prognostic stratification. Endoglin (CD105), a member of transforming growth factor beta (TGF-beta) receptor family, modulates cellular responses to TGF-beta and is absolutely essential for normal development of vasculature and angiogenic processes. Elevated circulating levels of soluble endoglin (sCD105) have been reported in patients with various solid tumors and several hematological malignancies. However, publications on sCD105 in chronic lymphocytic leukemia are still missing. *Aims.* to investigate the role of sCD105 in chronic lymphocytic leukemia. *Methods.* We measured peripheral blood plasma concentrations of sCD105 using enzyme-linked immunosorbent assay in 79 patients with untreated chronic lymphocytic leukemia (CLL) and 69 healthy donors.

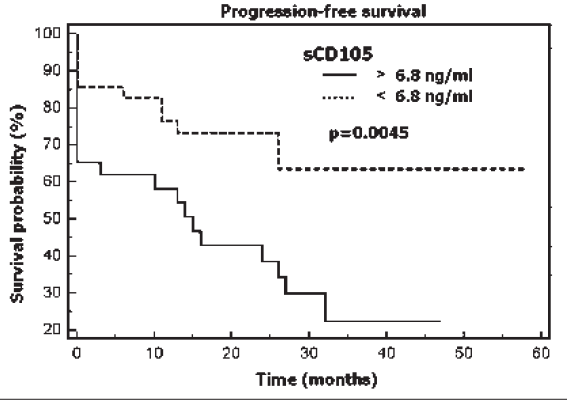

Figure 1. Shorter PFS in patients with high sCD105.

Results. sCD105 was significantly elevated in patients with CLL compared to controls (mean±standard deviation: 6.8±2.1 *vs* 4.6±1.5 ng/mL, 95% confidence interval of mean, 6.4-7.3 *vs* 4.2-4.9 ng/mL, $p<0.0001$). Patients with progressive CLL had higher sCD105 than patients with indolent disease ($p=0.0016$). Soluble endoglin increased significantly with Rai modified stage (Rai low *vs* intermediate *vs* high, $p=0.009$ and $p=0.04$). Progression-free survival was significantly shorter in patients with sCD105 levels above mean (median 15 months *vs* not reached, $p=0.0045$). In a pilot subgroup of 11 patients with serial sCD105 measurements before and after successful fludarabine-based combination treatment regimens, sCD105 levels decreased significantly ($p=0.0098$). There was insufficient number of events to assess the impact of sCD105 on overall survival. Summary: In our study, sCD105 levels were increased in CLL; furthemore, higher sCD105 levels were associated with advanced Rai stages and shorter progression-free survival. Taken together, our data suggest that endoglin may play a significant role in CLL biology and progression; futher investigations in this direction are clearly warranted for better understanding of angiogenic processes and refinement of individual patient´s prognosis in this disease.

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EXPRESSION OF CD72, A PROTEIN INVOLVED IN BCR SIGNALING, DIFFERS BETWEEN ZAP70 POSITIVE AND NEGATIVE CASES OF CHRONIC LYMPHOCYTIC LEUKEMIA

F.P. Careta, R.A. Panepucci, D.M. Matos, W.A. Silva Junior, R. Proto-Siqueira, A.B. Garcia, R.P. Falcao, M.A. Zago

Cepid-Fundherp / FMRP-USP, RIBEIRAO PRETO, Brazil

Background. Absence of mutations in IgVH genes of chronic lymphocytic leukemia (CLL) B-cells defines a patient group with a poorer clinical course. The numbers of CD38 and ZAP70-expressing cells are also used as surrogate markers of mutational status and clinical outcome of CLL patients. All these features relate to a role of BCR signalling in the proliferation and survival of CLL B-cells and establish a link between these markers and the biology of CLL prognostic subgroups. Thus, the identification of additional markers may lead to a better understanding of the molecular basis of this disease and eventually to new therapeutic approaches. *Aims.* Identify differentially expressed genes between unmutated and mutated CLL that may relate to altered signalling of distinct CLL prognostic groups. *Methods.* Six samples from CLL (3 unmutated and 3 mutated) were imunomagnetically separated using anti-CD19 microbeads and a RNA pool of each group was used to generate a transcription profile using serial analysis of gene expression (SAGE). Sampling statistics (Z-test) using the SAGEstat software was carried to select for differentially expressed transcripts ($p<0.01$, fold >2) between mutated and unmutated CLL cases. Flow cytometry was carried on 28 additional CLL samples. The percentage of $ZAP70^+$ cells was calculated on $CD19^+$ lymphocyte gated cells. Percentage of $CD72^+$ cells was determined on lymphocyte-gated cells and compared between $ZAP70^+$ and negative cases (cut-off of 30% ZAP70 positive cells) using a Mann-Whitney test. Three normal blood samples and 2 CLL cases were evaluated for the expression of CD72 on $CD3^+$ T-cells. *Results.* Over 100.000 total tags were sequenced from each CLL SAGE library, roughly corresponding to 25.000 distinct transcripts. A total of 156 transcripts were over-represented on the unmutated CLL SAGE library. A search for genes potentially related to BCR signalling revealed a 4 fold increase of CD72, a specific B lymphocytes glycoprotein involved in proliferation and cellular survival. Using ZAP70 expression as a surrogate marker of unmutated CLL, samples evaluated by flow-cytometry were divided into 12 $ZAP70^+$ samples and 16 ZAP70- samples. The percentage of $CD72^+$ cells on gated lymphocytes significantly differ ($p=0.0044$) between both groups (median 83% and 63%, respectively). Expression of CD72 was restricted to $CD19^+$ cells, since less than 2% of the $CD3^+$ T-cells were $CD72^+$ in the normal and CLL cases evaluated. Since the percentage of $CD19^+$ cells on the $ZAP70^+$ and negative group did not differ ($p=0.1536$, median of 82% and 74%, respectively), differences can be attributed to a higher expression of CD72 on $CD19^+$ cells of $ZAP70^+$ cases. *Summry and Conclusions.* We show that CLL cases of worst prognosis, as defined by ZAP70 expression, display a significantly higher number of CD72 positive cells than the ZAP70 negative cases. Since CD72 is known to transmit survival and proliferation signals to B-cells in a BCR dependent or independent manner, we propose that the CD72 protein may participate in the altered signaling of CLL B-cells, related to bad prognosis. Supported by FAPESP, CNPq and FINEP.

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THE *IN VITRO* EFFICACY OF DEXAMETHASONE MEDIATED CYTOTOXICITY ON CHRONIC LYMPHOCYTIC LEUKAEMIA CELLS IS SIGNIFICANTLY IMPROVED WITH THE ADDITION OF THALIDOMIDE

C. Butt,[1] J. Bailey,[2] G. Eagle,[2] D. Allsup,[2] J. Greenman[2]

[1]University of Hull, HULL; [2]Postgraduate Medical Institute and Hull York Medical School, HULL, UK

Background. Chronic lymphocytic leukaemia (CLL) is an incurable haematological disease that is difficult to treat due to the variability of disease progression as well as differing responses to treatment regimes. Although thalidomide monotherapy has been shown to be ineffective both *in vivo* and *in vitro*, in combination with other treatments, such as fludarabine, it has shown *in vivo* potential in CLL and other haematological malignancies. *Aims.*The aim of this project was to investigate the effects of combining thalidomide with other, already established, CLL

treatment regimes *in vitro*, in an attempt to find a novel therapeutic approach that could be used in patients with features of poor prognosis. *Methods.* Cells from 25 cases of CLL were incubated for 72 hours with dexamethasone at 3 concentrations (10, 5 and 2.5 µg/mL), both with and without thalidomide (100 µg/mL). The annexin V: FITC assay was used to assess cell viability. *Results*. Analysis of cell death showed elevated levels using a combination of dexamethasone and thalidomide, as compared with dexamethasone alone, at concentrations of 10 and 5 µg/ml. Median cell lysis for dexamethasone alone and in combination with thalidomide at 10 µg/mL was 15% (interquartile range (IQR) 0-45%) and 16% (IQR 0-62%), and cell lysis for 5 µg/mL was 15% (IQR 0-38%) and 17% (IQR 0-54%), respectively. Wilcoxon Signed Rank analysis showed that thalidomide significantly increased cell death at 10 and 5 µg/mL, p=0.035 and p=0.034, respectively. *Conclusions.* On analysis of the associated clinical parameters, all 4 patients with p53 dysfunction/17p deletion showed a cell lysis higher than the median, both with and without thalidomide. In summary, the cytotoxic effects of dexamethasone were significantly improved when used in combination with thalidomide, including in patients with poor prognostic features. This could form the basis of a novel therapeutic approach in CLL.

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ASSOCIATION BETWEEN THE PROLIFERATIVE RATE OF NEOPLASTIC B-CELLS, THEIR MATURATION STAGE AND UNDERLYING CYTOGENETIC ABNORMALITIES IN B-CELL CHRONIC LYMPHOPROLIFERATIVE DISORDERS

S. Quijano,[1] S. Quijano,[1] A. López,[1] A. Rasillo,[1] S. Barrena,[1] M.L. Sánchez,[1] J. Flores,[1] C. Fernández,[1] J.M. Sayagués,[1] C. Salvador Osuna,[2] N. Fernández,[2] M. González,[3] P. Giraldo,[2] M. Giralt,[2] M.C. Pérez,[2] J.M. Martin Antoran,[4] L. Perdiguer,[5] J. Díaz Mediavilla,[6] M. González Silva,[7] J. Serena González,[8] C. Cerveró,[9] J.L. Guerra[10], R. Butrón[10], M.C. García[11] J. Almeida,[1] A. Orfao[1]

[1]Centro de Investigación del Cáncer Universidad de Salamanca, SALAMANCA; [2]Servicio de Hematología, Hospital Miguel Servet, ZARAGOZA; [3]Servicio de Hematología, Hospital Universitario de Salamanca, SALAMANCA; [4]Servicio de Hematología, Hospital Rio Hortega, VALLADOLID; [5]Servicio de Hematología, Hospital de Alcañiz, TERUEL; [6]Servicio de Hematología, Hospital Ruber Internacional, MADRID; [7]Servicio de Hematología, Hospital La Línea, CÁDIZ; [8]Servicio de Hematología, Hospital San Jorge, HUESCA; [9]Servicio de Hematología, Hospital Virgen de la Luz, CUENCA; [10]Servicio de Hematología, Hospital Punta de Europa, CÁDIZ; [11]Servicio de Anatomía Patológica Hospital Universitario de Salamanca, SALAMANCA, Spain

Background. Limited knowledge exists about the impact of specific genetic abnormalities on the proliferation of neoplastic B-cells from chronic lymphoproliferative disorders (B-CLPD). *Aims.* To analyze the impact of cytogenetic abnormalities on the proliferation of neoplastic B-cells in 432 B-CLPD patients, grouped according to diagnosis and site of sampling, in comparison to their normal counterparts. *Methods.* A total of 432 untreated patients (260 males and 172 females with a mean age of 66±13 years; range: 19 to 95 years), newly diagnosed with B-CLPD, were included in this study. In all cases, diagnosis was established according to the WHO criteria with the following distribution: B-cell chronic lymphocytic leukaemia (B-CLL), 210 cases; hairy cell leukemia (HCL), 7 patients; mantle-cell lymphoma (MCL), 39; splenic marginal zone B-cell lymphoma (SMZL), 16; MALT lymphoma (MALT-NHL), 20; follicular lymphoma (FL), 71; diffuse large B-cell lymphoma (DLCL), 19; Burkitt lymphoma (BL), 14; and lymphoplasmacytic lymphoma/Waldenström macroglobulinemia (LPL/WM), 36 cases. Immunophenotypic analyses, DNA cell content measurements and interphase fluorescence *in situ* hybridization (iFISH) studies were performed on EDTA-anticoagulated PB (39%), BM (43%) and fine-needle aspirated (FNA) lymph node (LN; 18%) samples. Analysis of the cell cycle distribution of normal B-cells was performed on a total of 20 samples -10 BM, 5 PB and 5 reactive LN- using DRAQ5 (Cytognos, Salamanca, Spain) with the following combination of monoclonal antibodies: CD45-FITC/CD19-PE, CD38-FITC/CD19-PE, CD20-FITC/CD23-PE and CD38-FITC/CD20-PE, respectively. *Results.* Overall, proliferation of neoplastic B-cells highly varied among the different B-CLPD subtypes, the greatest numbers of proliferating cells being identified in DLBCL and BL. Compared to normal B-cells, neoplastic B-CLPD cells showed significantly increased S+G2/M-phase values in MCL, B-CLL, BL and some DLBCL cases. In contrast, decreased proliferation was observed in FL, LPL/WM and some DLBCL patients; HCL, SMZL and MALT-lymphoma patients showed S+G2/M phase values similar to normal mature B-lymphocytes from LN. Interestingly, in B-CLL and MCL significantly higher percentages of S+G2/M cells were detected in BM *vs* PB and in LN *vs* BM and PB samples, respectively. In turn, presence of 14q32.3 gene rearrangements, t(8;14) and DNA aneuploidy, but not other cytogenetic changes, were associated with a higher percentage of S+G2/M-phase cells among LPL/WM, FL and B-CLL cases, respectively. In addition, among B-CLPD patients as a whole, a significant association was observed between a worse performance status (ECOG≥2; (p<0.0001), presence of infiltration of ≥2 extranodal sites (p=0.001), thrombocytopenia (<100×10^9/L; p=0.01), increased LDH serum levels (p<0.001) and a higher tumor cell proliferation. Similarly, a significantly higher proliferation (p=0.03) was observed among stage B and C *vs* stage A B-CLL cases and histological grade III *vs* grade I (p=0.003) and II (p=0.001) FL patients. *Conclusions.* The proliferative rate of neoplastic cells from patients with B-CLPD is highly heterogeneous, such variability reflecting their maturation stage, and to a certain extent also unique specific underlying genetic abnormalities, at the same time it is associated with the clinical and biological behaviour of the disease.

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EPSTEIN-BARR VIRUS (EBV) INFLUENCES AID (ACTIVATION-INDUCED CYTIDIN DEAMINASE) EXPRESSION LEVELS IN BURKITT LYMPHOMA

R. Hassan, F.E. Felisbino, C.G. Stefanoff, M.H.M. Barros, I. Zalcberg Renault

Instituto Nacional de Cancer - INCA, RIO DE JANEIRO, Brazil

Background. Activation-induced cytidin deaminase (AID) is an indispensable enzyme for immunoglobulin somatic hypermutation (SHM) and class switch recombination. AID overexpression may be involved in the pathogenesis of autoimmune diseases and B-cell leukemias/lymphomas. EBV is a gammaherpesvirus which establishes latency in memory B cells, driven by expression of viral genes mimicking important physiological B signals. It was shown that during the establishment of latency, LMP1 signaling maintains AID expression, while EBNA2 downregulates it during EBV-driven B cell growth. *Aims.* To investigate the relationship between AID expression and EBV in Burkitt lymphoma (BL) primary tumors. *Methods.* Sixteen BL patients (0-16 years) were included in this study. Four cases were AIDS-related BL. Eight cases were EBV-associated, as demonstrated by EBER-ISH and PCR. EBV latency was investigated by immunohistochenistry (LMP1, LMP2A, EBNA2 and ZEBRA) and RT-PCR. AID quantitative expression was studied by Taqman® RT-PCR, in an ABI7700 platform (Applied Biosystems). Quantitative estimations were performed twice, using the DDCt method, GAPDH as endogenous gene and 5 PBMC pooled samples from normal donors as calibrator. Immunoglobulin heavy chain (IGH) SHM analysis was performed by sequence analysis of 8-15 cloned rearrangements using MacVector 7.1.1 software. Results: Normal donors showed expression levels between 0 and 2.05. Average cycle threshold (Ct) were 38.86±0.31 for AID and 23.90±0.06 (ΔCt 14.96± 0.32) for GAPDH. 1/16 studied tumours did not show AID expression above physiological levels while the remaining 15 showed variable expression levels, between 77 and 1756.3 times higher than calibrator, distributed around an average of 442.9±486 and a median of 246.9. IGH mutation frequency varied between 0 and 8.4% and 6/11 cases showed evidence of ongoing mutation. AID expression levels were not related to SHM frequency or to the presence and extension of ongoing mutation. Most of the EBV- cases expressed low levels of AID RNAm (median 145.12, range 77-881), while EBV$^+$ cases expressed significantly higher, although variable AID levels (median 299.9, range 120-1187) (Mann Withney Test p= 0.028). The presence of EBV was also significantly associated with AID high expression levels regarding the median value of the entire group (246.9) (Fisher's Exact Test, p=0.03). AID levels of AIDS-BL (all EBV$^+$) (median 255, range 211-1187) were not different from the entire EBV$^+$ group. AID levels were not related to EBV latency of EBV particular gene expression. *Conclusions.* The pattern of AID expression exhibited by BL is characterized by a wide although smooth range, suggesting the concurrence of multiple factors. Our study, with primary EBV$^+$ and EBV- BL tumors arisen in the same geographic environment, shows that EBV can influence AID expression independent on the expression of LMP1 and EBNA2 proteins. Whether AID expression in EBV$^+$ BL is associated to an immune response to the virus or to EBV cell manipulation warrants further experimental studies. Work supported by the Swissbridge Foundation (Switzerland) and FAPERJ and CAPES (Brazil).

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CLINICAL AND HEMATOLOGICAL CORRELATION WITH THE PRESENCE OF JAK2 (V617F) MUTATION IN NEGATIVE BCR/ABL MALIGNANT MYELOPROLIFERATIVE DISORDERS

M. Closca, A. Lupu, V. Teleanu, O. Ciocan, A. Ciobanu, C. Saguna, A. Colita

Coltea Hospital, BUCHAREST, Romania

Background. Ninty percent of patients with polycitemia vera, fifty to sixty percent of patients with essential trombocytemia and fifty percent with agnogenous myeloid metaplasia harbor Jak2 (V617F) mutation. The impact of this mutation on clinical phenotype is still debated. The aim of this study is to identify other specific molecular abnormalities associated with the patogenesis of negative BCR/ABL malignant myeloproliferative disorders, particularly detection of JAK2 V617F mutational status and to evaluate the correlation between the presence of the mutation and both clinical and hematological features design and methods. JAK2 V617F mutation was detected through sequentiation, allele-specific PCR, restriction PCR and quantitative TaqMan PCR on 110 patients diagnosed with chronic myeloproliferatives disorders in our center, according to WHO criteria. *Results.* Older patients presented progressively higher percentages of the V617F allele. Splenomegaly and microvessel related symptoms were significantly more frequent among patients with JAK2(V617F) mutation. Increasing mutant allele load correlated with higher frequency of arterial thrombosis at diagnosis. Statistical analysis also showed that $V617F^+$ patients had higher leucocytes, hemoglobin levels and thrombotic events. *Conclusions.* The JAK2(V617F) mutant allele burden contributes to determinate the clinical phenotype in patients with myeloproliferatives disorders. Therefore, detection of the Jak2 V617F mutation can affect not only the diagnosis, but also the management of myeloproliferative disorders.

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INCIDENCE OF C-KIT AND FLT-3 MUTATIONS IN PATIENTS WITH DIFFERENT SUBTYPES OF ACUTE MYELOID LEUKAEMIA

F. Zaker,[1] M. Mohammadzadeh,[1] M. Mohammadi,[2] K. Alimoghadam[3]

[1]Iran university of medical sciences, TEHRAN; [2]Iran university of medical sciencess, TEHRAN; [3]Shariati Hospital, TEHRAN, Iran

Background. FLT3 and c-Kit are members of the class III receptor tyrosine kinase (RTK) family. These mutations result in autonomously leukemic cell proliferation and unfavorable prognosis. However, the data concerning the incidence and associations with patients characteristics vary in different studies. *Aims.* Our aim from this study was to set up of molecular diagnosis and screening of these mutations in AML patients with differents subtyps. *Methods.* All adult patients diagnosed in main haematology center in Tehran. Peripheral blood or bone marrow of 212 patients were screened. PCR assay was performed to detect FLT3-ITDs. D835 point mutations of the FLT3 gene were detected with PCR followed by digestion with EcoRV of PCR product for wild type. Non fully digested products were considered as mutated. All products runned on 8% PAGE. Exon 8 mutations in c-kit receptors were analysed by PCR. Products were run in%10 CSGE and results were documented. We also studied c-kit exon 17 (D816) point mutations with RFLP technique and AatII enzyme on PCR products of these patients. Products runned on 8% PAGE. In some cases sequencing have been detected. *Results.* The median age of onset was 47±12 (range from 17-75) years. FLT3-ITD were detected in 18% and D835 mutations in 6% of patients. According to FAB classification, rate of FLT3 mutations were found higher in acute promyelocyte leukaemia and characterized by the T-15-17. D835 mutation was found in patients with M3, M2 and M5 type of AML. Evidence to date suggest that PML/RARA is insufficient for leukomogenesis. Potential candidate include mutations of FLT3, which could confer a proliferative advantage thereby complementing the differentiation block induced by PML-RARA. A positive correlation with high presenting WBC > 20000/micl (58%) and high percentage of circulating blast cells was demonstrated in ITD positive patients ($p<0.05$). Exon 8 mutations of c-kit were diagnosed in 1.3% of AML patients (confirmed by sequencing method) and 4.7% of patients showed D816 mutations with different findings in subtypes of AML. C-kit mutations demonstrated especially in M2&M4 cases. *Conclusions.* In this study we demonstrated that the FLT3 mutations is a frequent molecular lesions in AML patients with incidence of 24%. The presence of ITD were associated significantly with M3 morphology. Mutations of c-kit resulted in 5.6% of AML (one patient had both mutation together) which was significantly associated with M2 and M4 subtypes. These data showed that 30% of AML patients had mutations in RTK. Thus, these data provide a rationale for evaluation of inhibitors as a component of induction therapy and it may help to select better treatment.

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UNCOMMON PRESENTATION FEATURES OF THE T(8;14)(Q11.2;Q32) TRANSLOCATION IN ACUTE LYMPHOBLASTIC LEUKAEMIA

J. Kelly,[1] C. Bermingham,[1] P. Carty,[1] A. O'Marcaigh,[2] H. Enright,[3] D.R. Betts[1]

[1]National Centre for Medical Genetics, DUBLIN; [2]Our Lady's Children's Hospital, DUBLIN; [3]The Adelaide and Meath Hospital, DUBLIN, Ireland

Background and Aims. The t(8;14)(q11.2;q32) translocation represents a rare but non-random event in acute lymphoblastic leukaemia (ALL) that is seen in both childhood and adult on-set cases. The translocation results in a rearrangement of IgH on 14q32 and hence constitutes one member of the emerging group of translocations that involve this locus in ALL. Almost 50% of reported cases will arise in either Down syndrome (DS) associated disease or in t(9;22)-positive disease. We present an additional two cases that further strengthen this association but illustrate that the translocation need not present in a simple reciprocal manner. *Methods.* Presentation bone marrow aspirates were cultured according to recognised procedures and analysed by both conventional G-banding and fluorescence *in situ* hybridisation (FISH) using commercially available probes that were hybridised according to the manufacturers instructions. *Results.* From a series of over 400 cases of ALL analysed at presentation two were found to contain a t(8;14)(q11.2;q32) translocation in either a balanced or unbalanced form. Case 1 was a DS 18-year-old female with pre-B ALL who presented with a der(14)t(8;14) as the sole clonal aberration. FISH analysis with an IGH probe confirmed the unbalanced nature of the rearrangement. Case 2 was a 49-year-old female with B-cell ALL, who presented with a t(9;22) as the sole aberration in a minor stemline clone. The major sideline clone contained a t(8;14). Notably the der(8) showed a deletion of the p arm and in the majority of metaphases was present as two copies. Analysis at subsequent time points in this case demonstrated only the presence of the t(9;22)-stemline clone. *Summary and Conclusions.* This report further reinforces the association of the t(8;14)(q11.2;q32) with both the t(9;22) and DS-associated ALL. In both cases a standard der(8) was absent. We would therefore, speculate that further cases of this translocation exist that may have been simply referred to as an add(14q). Case 2 would also raise the question of how important this rearrangement is in the disease process given its subsequent disappearance but the persistence of the leukaemia. However, more cases are still needed so as to further understand its potential relevance to the disease process and prognosis.

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DETECTION AND MONITORING OF MINIMAL RESIDUAL DISEASE FOR RARE MLL REARRANGEMENTS IN INFANTS' ACUTE LEUKEMIA USING QUANTITATIVE REAL-TIME PCR ASSAY

A. Tsaur,[1] A. Ivanova,[2] S. Kovalev,[3] A. Misurin,[4] M. Suchkova,[4] A. Kustanovich,[5] Yu. Yakovleva,[2] A. Popov,[6] E. Shorikov,[2] L. Savelyev,[6] O. Aleinikova,[5] L. Fechina[2]

[1]Research Institute of Cells Technologies, YEKATERINBURG, Russian Federation; [2]Regional Children's Hospital, Research Institute of Cell Technologies, EKATERINBURG, Russian Federation; [3]Ural State University, EKATERINBURG, Russian Federation; [4]National Research Center for Hematology, MOSCOW, Russian Federation; [5]National Center for Pediatric Oncology and Hematology, MINSK, Belarus, Republic of Belarus; [6]Ural State Medical Academy, EKATERINBURG, Russian Federation

Background. MLL-EPS15 and MLL-MLLT10 are detected relatively rare among MLL rearrangements in infants. Moreover, due to high variability of breakpoints in these fusion genes (FGs) precise real-time PCR detection and quantification is extremely important. *Objective.* To develop quantitative real-time PCR (qRT-PCR) assay for detection and minimal residual disease (MRD) monitoring of MLL-EPS15 and MLL-MLLT10 FGs. *Methods.* Bone marrow samples were obtained from 2 infants with ALL (patient 1 and 2) within the Russian-Belarusian multicentral study MLL-BABY and from 1 infant with AML (patient 3). Parents' informed consent was signed in all cases. Treatment and diagnostics procedures have been approved by Federal and Institutional Ethics boards. In patients 1 and 2, who have had BI immunophenotype, t(1;11)(p32;q23) was found by G-banding and confirmed by FISH with LSI MLL dual color probe (Vysis, USA). Cytogenetics did not reveal any chromosomal

translocation in patient 3 with MLL-MLLT10, moreover split-signal FISH for MLL gene was not informative. Total RNA was extracted from the nucleated cells. Quality and integrity of RNA was estimated using 2100 Bioanalyzer (Agilent Technologies,USA). Only RNA with RNA integrity number more than 4.0 was taken into next steps. Detection of both FGs was done by multiplex PCR (N.Palisgaard *et al.*,1998). Afterwards, we used two forward primers and two Taqman probes located on the MLL (exons 7 and 9) (M. Jansen *et al.*, 2005) in combination with reverse primer localized on MLLT10 exon 10 (N.Palisgaard *et al.*,1998). The same MLL primers and probes were used with reverse primer located on EPS15 exon 3. PCR products were sequenced and cloned into pGEM plasmids. Standard curves were constructed using 10-fold dilutions of two different plasmids containing the fragment of MLL-EPS15 and MLL-MLLT10 FGs, respectively. qRT-PCR was performed using principles of Europe against cancer (EAC) protocol (J.Gabert *et al.*, 2003). ABL was used as control gene. Sensitivity was estimated according EAC recommendations. Samples of 30 patients with other MLL rearrangements and germ line MLL were estimated for the excluding false-positivity. *Results.* In patients 1 and 2 specific MLL-EPS15 PCR products were detected using MLL exon 9 primer. In patient 1 sequence analysis showed fusion between MLL exon 9 and EPS15 exon 2, in patient 2 MLL exon 10 fused with EPS15 exon 2. In patient 3 MLL exon 9 connected with MLLT10 exon 10. Sensitivity of qRT-PCR assay for MLL-EPS15 run up to 1E-5, but in case of MLL-MLLT10 only level 1E-4 was achieved. Coefficient of variation is up to 25%. There was not detected any non-specific amplification in MLL-EPS15-negative, MLL-MLLT10-negative samples. *Conclusions.* Our qRT-PCR assay can be used for simple and rapid quantification of MLL-EPS15 and MLL-MLLT10 FGs. It is highly sensitive, specific and precision. We were able to successfully monitor MRD in all available patients' samples. Due to small size of exons 10 and 11 of MLL gene the approach of using primer and Taqman probe localized on exon 9, known to be the most frequent breakpoint region of MLL, can be widespread and can significantly improve diagnostics of rare MLL rearrangements.

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AUTOLOGOUS BONE MARROW PROGENITOR CELL TRANSPLANT IN PARKINSON'S DISEASE. A COOPERATIVE MULTICENTER STUDY

E. Novoa,[1] F. Perez Chavez,[2] R. Morales Aceves,[3] R Rangel Guerra,[2] M. Soto Valdez,[2] M.A. Medina,[4] A. Perez Chavez,[5] R. Cazares,[6] A. Ortega,[2] R. Caride[7]

[1]Ministry of Health, MONTEVIDEO, Uruguay; [2]UANL, Servicios Medicos, MONTERREY, Mexico; [3]Universidad de Guadalajara, PUERTO VALLARTA, Mexico; [4]Cellther Program, MONTEVIDEO, Uruguay; [5]UANL, Anestesiology Dept., MONTERREY, Mexico; [6]UANL, Patologia Clinica, MONTERREY, Mexico; [7]Police Hospital, MONTEVIDEO, Uruguay

*Background.*The most common cause of parkinsonism is idiopathic Parkinson's disease, a neurodegenerative disease, first described by an english physician Dr. James Parkinson in 1817. Current therapy can not avoid progression. *Aims.* 1) Control of clinical symptoms and signs of the disease, and quality of life. 2) evaluate other aditional therapeutic effects, 3) evaluate side effects of the ABMD-PC transplant in this group of patients. *Methods.* from october 2007 to january 2008, 15 patients were evaluable to be included on this protocol with neurological diagnosis of idiopathic parkinson's disease. 10 men and 5 women. Median age was 70 years old (59-87). For the initial evaluation score and follow up the UPDRS (Unified Parkinson Disease Research System) scale was employed. The control group was the same cohort of patients in the six months before ABMD-PC transplant. Local anaesthesia was employed in 7 patientes with xilocaine 2% for harvest and transplantation in the gastrocnemius muscle. General anaesthesia with Propofol was received for the rest of patients (8/15). Cell concentration was obtained by gradient of density. Mobilization with filgrastim was employed, 5 ug/kg/weight daily (two doses) before transplantation (48 hs). Unmanipulated autologous bone marrow derived progenitor cells were injected in one of the lower limbs in 2 mL aliquots. The mean number of transplanted mononuclear cells was $2{,}2\times10^9$/kg body weight.*Results.* the procedure mortality rate was 0%. The only complication of this treatment was local hemathoma in the transplanted leg (3%). 73% of patients showed a positive answer to treatment with desappear of IPD symptoms; in the first transplanted patients, until for 120 days. *Conclusions.* autologous bone marrow derived progenitor cell transplant, by the Conzi-Fortunato effect, can be performed safely and appears to be a benefical complementary therapy for patients with idiopathic Parkinson's disease. www.cellther.org

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IMPORTANCE OF JAK-2 IN ERYTHROPOIETIN-INDUCED PROLIFERATION OF TUMOR CELLS

B. Udupa, M. Bose

Univerisity of Arkansas for Medical Sciences, LITTLE ROCK, USA

Background. Erythropoietin (EPO) activates multiple signaling pathways by interacting with erythropoietin receptor (EPOR). Signaling through EPOR in response to EPO involves the activation and phosphorylation of several proteins like Janus kinase-2 (JAK-2)/Signal Transducer and Activator of Transcription (STAT) and Ras/mitogen activated protein kinase (MAPK). *Aims.* In the present study we examined the functional status of EPOR associated with JAK2 pathway in a rat pancreatic tumor cell line, AR42J cells. *Methods.* Jak-2 and other cell signals were detected quantitatively by Western blotting and cell proliferation was measured by a commercially available WST-8 cell counting kit. *Results.* Our results showed that 5 mU/mL EPO significantly enhanced the proliferation of these tumor cells (0.94±0.01) at 48h, when compared with untreated cells (0.68±0.01). JAK-2 phosphorylation was increased by 30% at 5 min which increased and sustained up to 60% at 4 h. Phosphorylation of extra cellular regulatory kinase 1/2 (ERK1/2, a component of MAPK) was rapid and reached maximum at 5min (3 folds over control) whereas c-Jun NH2 terminal kinase 1/2 (JNK1/2, second component of MAPK) was activated to a maximum extent of 2 folds over control at 30 min. Pretreatment of cells with 2.5 μM of JAK-2 inhibitor significantly suppressed EPO induced proliferation. Further this treatment completely inhibited EPO induced phosphorylation of ERK1/2 and JNK1/2 as well. *Conclusions.* These results provide the evidence for the significant role of JAK-2 and indicate that activation of JAK-2 is required to initiate EPO enhanced proliferation of tumor cells with induction of phosphorylation of ERK1/2 and JNK1/2. EPO is being used routinely for the treatment of anemia associated with the chemotherapy in cancer patients and this may likely increase the chances of tumor growth in this manner. This aspect has to be taken in to account for any treatment involving EPO.

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METHYLATION OF THE VON HIPPEL-LINDAU TUMOR SUPPRESSOR GENE IS A FREQUENT EVENT IN PLASMA CELL NEOPLASIAS

E. Hatzimichael, K. Maki, A. Dasoula, L. Benetatos, E. Lambri, A. Vassou, M. Syrrou, K. Bourantas

University Hospital of Ioannina, IOANNINA, Greece

Background/Aims. The Von Hippel-Lindau (VHL) tumor suppressor protein, through its oxygen-dependent polyubiquitylation of hypoxia-inducible factor (HIF-1a), plays a central role in the mammalian oxygen-sensing pathway. In the absence of VHL, HIF-1a becomes stabilized and is free to induce the expression of its target genes, many of which are important in regulating angiogenesis, cell growth or cell survival. It has been suggested that bone marrow hypoxia is lessened during multiple myeloma (MM) progression and that myeloma-associated angiogenesis is functional. Aberrant promoter methylation causes repression of gene transcription and represents a mechanism for tumor suppression gene inactivation. Aim of this study was to investigate whether the VHL gene is methylated in patients with plasma cell neoplasias and whether there is any correlation with clinical parameters. *Patients and Methods.* Methylation status of the VHL gene promoter was studied prospectively in 29 patients with MM and 3 patients with Waldenström's macroglobulinemia (WM); 16 male, 14 female, median age 66 years. Using the Durie and Salmon staging system patients' disease stages were as follows: smouldering MM 4/29 patients, IIA 6/29 patients, IIIA 5/29 patients and IIIB 7/29 patients. Samples were taken at diagnosis in 26/32 patients, at high plateau phase in 3/32 while in 3/32 patients samples were available both at diagnosis and when disease progression occurred. Genomic DNA was isolated and bisulphate modification was performed using commercially available kits (QIAmp DNA mini kit, Qiagen and EZ DNA methylation kit, Zymo Research respectively). The methylation-specific polymerase chain reaction (MSP) with primers for methylated and unmethylated alleles of the VHL gene promoter was employed to study its methylation status. Human male genomic DNA universally methylated for all genes (Intergen Company, Purchase, NY) was used in all experiments as positive control for methylated alleles. *Results.* Methylation of the VHL promoter was found in 3/3 patients with WM and in 15/29 patients with MM at diagnosis; in one of them methylation was also found during the course of the disease despite the patient having received treatment. Two patients with MM, who were not methylated

for this gene at diagnosis, were found to be methylated when progression, accompanied by extramedullary disease, was noted. No statistically significant correlation was found with disease stage, bone lytic lesions or presence of extramedullary disease. *Conclusions.* Methylation of the VHL promoter seems to be a frequent event in plasma cell neoplasias. Whether it represents an early or late event of the disease merits additional study.

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INVESTIGATION OF THE ANTITHROMBOTIC EFFECT OF AR-H067637, THE ACTIVE METABOLITE OF THE ORAL ANTICOAGULANT AZD0837, USING HUMAN BLOOD IN A FLOW CHAMBER MODEL *IN VITRO*

M. Elg, H. Zachrisson

AstraZeneca, MÖLNDAL, Sweden

Background. AZD0837 is a new oral anticoagulant which is bioconverted to its active form AR-H067637, a reversible and selective direct thrombin inhibitor. During the early stages of new anticoagulant development, bridging antithrombotic results from animal studies to the human situation facilitates decision-making regarding the dose to be investigated in the first human studies. *Aims.* In this study the antithrombotic effect of AR-H067637 was evaluated in a flow chamber model developed by Badimon *et al.*[1] using pig aorta and human whole blood. *Methods.* Blood was drawn from healthy subjects into citrate-containing tubes. AR-H067637 was added at blood concentrations from 0.01 to 10 μmol/L. Denuded pig aorta pieces were used as the thrombogenic surface in the flow chamber. The blood was drawn for 5 minutes through the flow chamber with a shear of 220-s which is comparable with venous flow rate. The thrombus formed inside the chamber was degraded by plasmin, and the degradation product of fibrin, D-Dimer, was measured as an indirect measure of thrombus size. Platelets attached to the thrombus were lysed and P-selectin was used as a measure of the amount of platelets within the thrombus. Activated partial thromboplastin time (APTT) and prothrombin time (PT) were used to measure the anticoagulant effect of AR-H067637. *Results.* When using D-Dimer levels as a measure of thrombus size, the IC50 for the antithrombotic effect of AR-H067637 was 0.48 μmol/L. AR-H067637 appeared to have no effect on P-selectin content in the thrombus. APTT and PT were shown to be concentration-dependently prolonged on treatment with AR-H067637. *Conclusions.* AR-H067637, the active metabolite of AZD0837, concentration-dependently prevented thrombus formation on the denuded pig-aorta.

Reference

1. Badimon L, et al. J Lab Clin Med 1987;110:706-18.

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EFFICACY AND SAFETY WITH DEFERASIROX (EXJADE®), A ONCE-DAILY ORAL CHELATOR IN THALASSEMIC ADULTS

V.E. Vlachaki,[1] S.H. Haralambidou,[1] V.P. Perifanis,[1] G.S. Spanos,[2] I.T. Tsatra[1]

[1]Hippokration General Hospital, THESSALONIKI; [2]Eurodiagnosis, Diagnostic Center, THESSALONIKI, Greece

Deferasirox (Exjade®, DFX) is a once-daily oral, iron chelator approved for the treatment of transfusional iron overload in adult and pediatric patients. It was licensed in Greece in January 2007. The aim of this study was to present the efficacy and safety of DFX in Greek thalassemic adults. *Methods.* DFX has been administered at 20-30 mg/kg/day in 7 patients with β-thalassemia major (age: 23-55y) and in 2 with thalassemia intermedia (age: 25y) for one year. Efficacy was monitored via serum ferritin (SF) every three months. MRI T2* measurements for liver and myocardial iron loading has been assessed pretreatment in all patients and after one year in 4 patients until now. Safety was assessed by the incidence and type of adverse events. *Results.* Baseline. All 7 patients were transfused every two weeks. Serum ferritin was 1735,857±1317,003 (627-4240 ng/mL) while in 4 patients cardiac T2* was 33,75±6,39 (27-41ms and liver T2* was 4,065±4,9 (0,86-11,5ms). The 2 patients with thalassemia intermedia had serum ferritin 889±9,8 (882-896 ng/mL), cardiac T2* 36,5±3,53 (34-39 ms) and liver T2* 3,7±1,83 (2,4-5 ms). After one year. In 7 thalassemia major patients and in 2 with intermedia thalassemia, serum ferritin was 1128,286±913,397 (54-2650 ng/mL) and 638±175,36 (514-762ng/mL) respectively. The mean reduction in ferritin was 34% and 28% respectively. In 4 patients cardiac T2* was 33,875±6,536 (26-39,5 ms) and liver T2* was 6,725±9,544 (1,4-21 ms). We did not find any difference in cardiac T2* while we found no significant statistically increase in liver T2* (p=0,06). Adverse events were minor, mainly nausea or diarrhea. Serum creatinine was increased in one patient. Decrease of DFX dose improved creatinine level. Another patient presented skin rash which disappeared with antihistaminic pill. All patients continue to receive DFX treatment. In conclusion this promising new drug might improve compliance and hence the life expectation in thalassemic patients.

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INFLUENCE OF HELICOBACTER PYLORI INFECTION ON IRON STATUS IN PATIENTS WITH GASTRITIS

Z.W. Grotto, C. Alvarenga, G. Montes, R. Zeitune

State University of Campinas, UNICAMP, CAMPINAS, Brazil

Background. The role of H.pylori (HP) infection as a possible cause of iron deficiency anaemia (IDA) is not completely established in the literature. Some mechanisms are invoked to explain the relationship between HP gastritis and IDA, as occult gastrointestinal bleeding, competition for dietary iron by the bacteria, and an abnormality of the gastric juice composition with consequent impaired iron absorption. The objective of the present study was to evaluate the possible association between HP infection and anaemia in a group of Brazilian adult patients with gastritis. *Patients and Methods.* We have studied 94 patients submitted to endoscopy and gastric biopsy requested for gastritis investigation. Complete blood cell counts including mean reticulocyte volume (MRV) as an early indicator of iron deficiency were measured using an automated blood counter. Serum iron, total iron binding capacity and serum ferritin were performed to detect iron depletion. Gastritis was diagnosed on the basis of the histological findings and HP presence was detected by haematoxylin and eosin staining. C-reactive protein (CRP) was measured as an indicator of inflammatory activity. *Results.* Gastritis was confirmed in 83 of 94 individuals; 71 patients were HP^+ and 12 HP^-. Thirteen patients presented anaemia, 10 were HP^+ and 3 HP^-. Three anaemic patients did not show gastritis at histological exam. According to laboratorial parameters 6 patients were considered to have IDA, 3 patients were classified as heterozygous beta-thalassemia and 3 patients presented other causes of anaemia. Four iron deficient patients were HP^+ and 2 were HP^-. There were no significant differences between groups HP^+ and HP^- concerning haemoglobin levels, mean corpuscular volume, MRV, transferrin saturation and serum ferritin determinations. CRP levels were similar in gastritis and normal groups and among HP^+ and HP^- patients. *Conclusions.* According to our results IDA incidence is not higher in patients with gastritis or HP^+ than in normal population. The association between diminished iron stores and HP positivity has been showed in epidemiological studies conducted over diverse geographic areas, but especially in children and using HP seropositivity as indicator of HP infection. Our data are in agreement with other results who found that the HP infection did not relate to haemoglobin level or iron deficiency.

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IMMUNE GRANULOCYTOPENIA IN SIBS

N.A. Finogenova, E.A. Mamedova, T.V. Polovtseva, M.N. Vasilyeva

Federal Clinical Research Center of Pediatric Hematology, Oncology and Immunolog, MOSCOW GSP-,7 Russian Federation

Background. In the early childhood immune granulocytopenia is one of the most frequently diagnosed forms. Etiopathogenetic mechanisms of this disease are studied insufficiently. *Aims*. Studied features of course of immune granulocytopenia in sibs. *Methods.*There are three families under our supervision where children in one generation had all immune granulocytopenia. In all the cases the clinico-hematological picture in dynamics was followed up, investigated anti-neutrophil antibody (ANAB) in the blood serum in granulocytotocix test. *Results*. In the first family there are 2 girls, hetero-ovular twins. At the debut of the disease the condition is serious, localized purulent infection (LPI), one child has osteomyelitis of the left hip joint, abscesses of soft tissues - minimum level of neutrophils - 20/mm^3, the other has purulent lymphoadenitis, abscesses of soft tissues -minimum level of neutrophils - 200/mm^3. The both girls ANAB are revealed in titers 1:2-1:64. By the present time the duration of disease is 2,5 years, remission is not reached. In two other families granulocytopenia's course proceeded most seriously in the first children, these children had not been examined hematologicaly before admission to clinic, inoculations were made in accordance with age.

Whereas the next children had easier course of disease that was caused by early revealing of granulocytopenia, follow-up by hematologist from the first months of life, and regular medical check-up, with rejection of preventive inoculations. In the second family there are three sibs - two boys and a girl. The senior boy is ill beginning from age of 6 months, in the clinical presentation - LPI mastitis, recurrent otitis, revealed ANAB in titers 1:2-1:4, minimum level of neutrophils 40/mm^3, full clinico-hematological remission is reached in 4,5 years. Two other children had granulocytopenia proceeded easier without LPI, ANAB are not revealed, minimum level of neutrophils 400/mm^3 and 200/mm^3 accordingly, remission is reached in 2 years in one and 1,5 years in other child. In the third family there are 2 sibs. The senior girl is ill beginning from age of 8 months, medium hard clinical picture without LPI. ANAB were revealed in titers 1:4-1:8, minimum level of neutrophils - 50/mm^3 antigranulocyte. Remission is reached in 3 years. In her younger brother ANAB were not revealed, minimum level of neutrophils - 300/mm^3. Remission is reached within a year. *Summary and Conclusions*. Presence of immune granulocytopenia in sibs testifies about the genetically-determined mechanisms of its occurrence, early revealing and regular medical check-up in children with granulocytopenia makes easier the course of the disease and faster beginning of remission.

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THE EFFICACY OF RITUXIMAB IN PATIENTS WITH SPLENECTOMIZED REFRACTORY CHRONIC IDIOPATHIC THROMBOCYTHOPENIC PURPURA

S. Pasa,[1] A. Altintas,[2] T. Cil,[3] R. Danis,[4] O. Ayyildiz[2]

[1]Dicle Universitesi Faculty of Medicine, DIYARBAKIR; [2]Dicle University Faculty of Medicine Department of Hematology, DIYARBAKIR; [3]Dicle University Faculty of Medicine Department of Oncology, DIYARBAKIR; [4]Dicle University Faculty of Medicine Department of Nephrology, DIYARBAKIR, Turkey

Background. and *Aims.* The most difficult problem a physician encounters is the management of patients with idiopathic thrombocytopenic purpura (ITP), who has persistent severe thrombocytopenia after failure of initial treatment with glucocorticoids and splenectomy. Most of the patients refractory to corticosteroids and splenectomy will become refractory to other available agents, such as intravenous immunoglobulin (IVIg), danazol or chemotherapy. *Patients and Methods*. In this study, we investigated the effect of rituximab on 17 splenectomized refractory chronic ITP patients. *Results.* We showed that the anti-CD20 antibody, rituximab, induces a clinically significant response in severe chronic ITP patients, who are unresponsive to other therapeutic options. After sixth months, 10 out of 14 responders were still maintaining their durable and significant platelet responses (platelet counts > 50×10^9/L), without requirement to any other ITP medication. *Conclusions.* We suggest that, rituximab is an effective treatment option in splenectomized refractory or relapsed ITP patients. Rituximab was well tolerated without severe side effects.

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FIVE YEARS EXPERIENCE IN USING PARTICLE GEL IMMUNOASSAY SPECIFIC TO HEPARIN/PF4 FOR THE DIAGNOSIS OF HEPARIN-INDUCED THROMBOCYTOPENIA

S. Tadic,[1] N. Antonijevic[2]

[1]National Blood Transfusion Institute, Belgrade, BELGRADE; [2]Clinic for Cardiovascular Disease, Clinical Center of Serbia, Belgrade, BELGRADE, Serbia

*Background.*The major complication in the therapeutic or prophylactic use of heparin in medical treatment is type II heparin-induced thrombocytopenia (HIT II), a unique form of drug-induced immune-mediated thrombocytopenia. Clinically, HIT II is characterized by thrombocytopenia which paradoxically is associated with thrombosis,(ie., deep vein thrombosis, pulmonary embolism, and venous gangrene), in about 50% of the cases. In the pathogenesis of HIT II, the formation of platelet factor 4 (PF4)/heparin complexes seems to be the major determinant. The binding of PF4 to heparin induces antibody production directed against a neoepitope created by the three-dimensional assembly of PF4 and heparin. Subsequently, PF4/heparin-antibody complexes induce platelet activation, followed by the shedding of platelet microparticles, thrombin generation, and the involvement of endothelial cells. Laboratory testing in the diagnosis of HIT II is based on either the immunological detection of antibodies directed against the PF4/heparin complex or the functional platelet-activating potential of the emerging immuncomplexes. *Aims.* To evaluate the particle gel immunoassay (ID-H/PF4 test) as a technically easy, rapid, and routine immunological assay for HIT. *Methods.* The ID-Heparin/PF4 antibody test (ID-H/PF4), uses the standard methodology and equipment of the ID-Micro Typing System (DiaMed, Cressier sur Morat, Switzerland). The test has adopted the ID microcolumn system used for red cell serology by using special red-dyed, high-density polystyrene particles coated with PF4-heparin complexes that serve as a solid phase. Patient serum and PF4-heparin-coated microbeads are added to the incubation chamber of the microcolumn card, and after a 5-minute incubation, the card is centrifuged. A strong positive result is indicated by the agglutinated microbeads remaining at the top of the column. *Results.* During five years (from 2003 to 2007), 95 serum samples from consecutive patients suspected to have heparin-induced thrombocytopenia, were investigated in the Department of Immunohaematology-National Blod Transfusion Institute in Belgrade. In 32,6% (31/95) of the patients, ID-H/PF4 test showed a positive results. *Conclusions.* The ID-Heparin/PF4 antibody test (ID-H/PF4) appears to be a useful tool for rapid detection of heparin-induced antibodies. Prospective clinical studies with a large number of patients are underway to clarify the diagnostic usefulness of ID-H/PF4.

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EFFECTIVE BUT DELAYED RESPONSE TO RITUXIMAB IN PATIENTS WITH REFRACTORY ITP:SINGLE CENTRE EXPERIENCE

M. Gleeson, K. Kelly, D. Gamaleldin, P. McEvoy, P.T. Murphy

Beaumont Hospital, DUBLIN, Ireland

Background. Immune Thrombocytopenic Purpura (ITP) is a disorder characterised by anti-platelet auotantibody formation and increased platelet destruction. Second line therapies for ITP include Rituximab, a monoclonal antibody targeting the CD20 antigen of B-cells. Previous Studies have suggested that overall response rates to Rituximab are in the range of 52-54%. *Aims.* To assess the response rates of 11 patients with refractory ITP who were treated with Rituximab at our institution from 2004 to 2007. *Methods.* We performed a retrospective analysis to assess response rates of all patients with refractory ITP treated with Rituximab at our centre. All patients had received Rituximab 375 mg/m^2 weekly for 4 weeks.

Table 1.

Response	1 MONTH	2 MONTHS	3 MONTHS	6 MONTHS
Complete Response (CR)	0 (0%)	2 (18%)	2 (18%)	3 (30%)
Partial Response (PR)	3 (27%)	2 (18%)	1 (9%)	3 (30%)
Minor Response (MR)	2 (18%)	1 (9%)	3 (27%)	0 (0%)
No Response (NR)	6 (55%)	6 (55%)	5 (45%)	4 (40%)

Based on platelet counts at 1, 2, 3 and 6 months from the start of Rituximab, we classified patients into response categories at these intervals. Complete response (CR) was defined as a PLT count ≥100×10^9/L. Partial response (PR) was defined as a PLT count between 50 to100×10^9/L. A minor response (MR) was defined as a PLT count <50×10^9/L but increased by 50% from baseline. 3 patients had a baseline PLT count of ≥50×10^9/L but required higher platelet counts for anticoagulation or surgery. Here we defined CR if PLT count increased ≥100×10^9/L and PR if the platelet count increased ≥80×10^9/L. *Results.* 11 patients were assessed, 6 female and 5 male. The mean age was 50. All patients previously received steroids and intravenous immunoglobulin (IVIG). Other previous treatments included Anti-D (n=3), Danazol (n=1) and splenectomy (n=1). During Rituximab treatment 3 patients (27%) required maintenance steroids and 2 (18%) required maintenance IVIG. One patient required maintenance Anti-D three months after commencing Ritux-

imab. Patients were followed for a median of 10 months (range 5-42 months) post Rituximab. Rituximab therapy was well tolerated with no severe adverse events.The response to treatment is summarised in Table 1. No CR occurred until 2 months and 60% had either a CR or PR at 6 months. 5 patients (45%) have a sustained response to date. 2 have a sustained complete response, both are female and aged 26 and 32 years. 4 (36%) required further treatment: splenectomy (n=3), IVIG (n=1), Anti-D (n=1), thrombopoietin receptor agonist (n=1). *Conclusions.* After 6 months the overall response rate (CR and PR) was 60%, which is similar to the overall response rate of 55% observed in a recent meta-analysis. The response to Rituximab was delayed, with no CRs at one month and the best response at 6 months. Although the 2 patients with sustained CRs are young females, larger studies are needed to establish predictors of response to Rituximab. Following Rituximab only 4 patients required further treatment to date. Rituximab may therefore be used to defer splenectomy perhaps indefinitely in some patients and is becoming an established second-line therapy in refractory ITP.

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THE EFFECTS OF LOSARTAN AND VALSARTAN ON PLATELET AGGREGATION AND HEMATOLOGICAL PARAMETERS IN PATIENTS WITH NEWLY DIAGNOSED ESSANTIAL HYPERTENSION

I. Yavasoglu, I. Yavasoglu, M. Unubol, B. Acar, G. Kadikoylu, Z. Bolaman

Adnan Menderes University, Medical School, AYDIN, Turkey

Background. Angiotensin II receptor blockers (ARBs) have antiproliferative, antihypertensive, and preventive effects against atherosclerosis. There are controversial *in vitro* effects of ARBs on platelet aggregation in several experimental studies. *Aims.* To evaluate *in vivo* effects of losartan and valsartan on platelet aggregation with ADP, collagen, epinephrine, ristocetin and other hematological parameters. *Methods.* Fourty-three patients (19 female and 6 male, mean aged 54±8 years for losartan, 11 female and 7 male, mean aged 51±7 years for valsartan) with newly diagnosed essential hypertension were enrolled to this study. The patients with the tendency to hemorrhage and thrombosis, diabetes mellitus, and use of anti-platelet and anti-coagulant drugs were not enrolled to the study. The patients were treated with 100 mg/day losartan and 160/mg valsartan, respectively. Before the treatment and after 2 months, whole blood parameters (Beckman Coulter, USA) and platelet aggregation tests with collagen, epinephrine, ristocetin, and ADP (Chrono-log, 570 blood aggregation systems, 2 West Park Road, Haverton,PA, 19083-4691, USA) were performed. Two-paired t test was used for comparison of pre- and post-treatment values of two groups. Values of $p<0.05$ were accepted as significant. *Results.* At the end of the study, both drugs lowered systolic and diastolic tension ($p<0.001$). Moreover both drugs decreased platelet aggregation with ADP, collagen, epinefrin, but these decrements were not significant ($p>0.05$). Only losartan statistically significicantly decreased platelet aggregation with ristocetin ($p=0.027$). While losartan decreased hemoglobin and hematocrit levels ($p=0.02$), both drugs did not change other hematological parameters ($p>0.05$). *Conclusions.* Losartan decreased platelet aggregation with ristocetin, hemoglobin and hematocrit levels. These hematological parameters of losartan may be useful for atherosclerosis, inhibiting platelet aggregation and decreasing viscosity, in addition to antihypertensive effect. But larger studies are necessary to confirm this drug's preventive effects.

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RISK OF CATARACT, DIABETES AND RENAL FAILURE AMONG PATIENTS WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

I. Bennett,[1] U. Forssen,[1] C. Enger,[2] J. Nelson[1]

[1]GlaxoSmithKline, PHILADELPHIA; [2]I3 Drug Safety, ANN ARBOR, USA

Background. ITP is a disease caused by inadequate platelet production as well as increased platelet destruction. Morbidity in adult patients with ITP has seldom been studied and ITP remains poorly described. *Aims.*To examine the prevalence and incidence of cataract, diabetes and renal failure among patients with chronic ITP compared to a non ITP population. *Methods.* In a retrospective analysis, we explored a large U.S. health insurer database comprising medical claims in a geographically diverse health plan. The plan provides fully insured coverage for physician, hospital and prescription drug services. Gender distribution was similar in the plan but compared to the US population but elderly individuals were under represented. Patients were determined as having chronic ITP if they: a) had at least two physician claims separated by at least six months with ICD-9 CM diagnosis code 287.3x for primary thrombocytopenia, b) had at least 12 months of continuous enrollment prior to the date of the diagnosis code eligibility, and c) were 18 years or older between January 1, 2000 and September 30, 2006 with follow-up continued through December 31, 2006. Cataract and renal failure were defined using ICD-9 CM diagnosis codes while prescription for antidiabetic drugs was used to define diabetes. Prevalence of cataract, diabetes and renal failure was determined in the 12-month period prior to ITP cohort entry. Incidence of co-morbidity during an average 15 months of follow-up post-ITP cohort entry was calculated after excluding individuals with pre-existing disease. A comparison group comprising individuals from the same plan who did not have ITP was selected and an adjusted incidence rate ratio (IRR) and 95% Confidence Interval (CI) of occurrence of cataract, diabetes and renal failure comparing these to the ITP patients was estimated using Poisson regression. *Results*. Among the chronic ITP patients (N=3131), the prevalence of cataracts and diabetes was high (11.1% and 11.6%, respectively) and the prevalence of renal failure was also high (6.4%). During the follow-up period, 240 patients with ITP had a diagnosis of cataract (incidence rate of 559 per 10,000 person years [PY]), 106 had a diagnosis of diabetes (incidence rate of 232/10,000 PY), and 180 had a diagnosis of renal failure (incidence rate of 374/10,000 PY). After adjusting for age, gender, history of hypertension, steroid use and interferon treatment, the adjusted IRR comparing the ITP cohort to the non-ITP cohort for diabetes was 1.61 (95% CI: 1.31-1.96) and for renal failure was 2.14 (95% CI: 1.82-2.51). After adjusting for age, gender, history of hypertension, and other factors, the IRR for cataract was 1.18 (95% CI: 1.03-1.35). *Summary.* ITP patients had a high prevalence of pre-existing medical conditions, namely cataract, diabetes and renal failure. After cohort entry, these patients went on to have a high burden of disease from diabetes and renal failure, especially compared to patients without ITP.

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DASATINIB ALONE CAN ALLOW MRD NEGATIVE COLLECTION IN PATIENTS WITH PH-POSITIVE ALL: A CASE REPORT

M. Cimminiello,[1] M. Pizzuti,[2] D. Vertone,[2] I. Attolico,[2] M. Poggiaspalla,[2] A. Magaldi,[2] T. Grippo,[3] C. Musto,[3] A. Olivieri[2]

[1]Hematology, POTENZA; [2]Ematologia Ospedale San Carlo, POTENZA; [3]U.O. di Medicina Trasfusionale Ospedale San Carlo Potenza, POTENZA, Italy

Patients with Philadelphia chromosome (Ph)-positive acute lymphoblastic leukemia (ALL) generally have a rapid disease course and a poor prognosis. Recent experiences suggest that imatinib is effective in Ph-positive ALL. Dasatinib, a novel tyrosine kinase inhibitor (NTKI), has previously induced responses in patients with imatinib-resistant or -intolerant Ph-positive ALL. Moreover recent experiences suggest that previous treatment with NTKI did not increase transplant-related toxicity. We observed a 52 - year - old male, with splenomegaly, elevated leucocyte count (47.4×10^9/L), anemia and trombocytopenia. Bone marrow aspiration and cytogenetic analysis allowed to diagnose a *de novo* Ph-positive ALL and molecular analysis confirmed the p190 BCR-ABL transcripts. This patient was admitted in a GIMEMA multicenter prospective study aimed to evaluate the efficacy of Dasatinib alone as first line therapy in ph-positive ALL; Dasatinib was administered orally, at 70 mg twice-daily, for three months, with a careful evaluation of haematological and extrahematological toxicity. Peripheral blood-cell mRNA was collected and analyzed for BCR-ABL and for the level of expression by quantitative reverse transcriptase polymerase chain reaction (Q-RT-PCR). Dasatinib induced rapid complete hematologic remission (CHR); we did not observe severe non-hematological. The molecular analysis of Minimal Residual Disease (MRD), performed by Q-RT-PCR in BM samples, showed a rapid, progressive decrease from 0.002% at day +22, to 0.0005% at day +57, up to 0.0001% at day +84. As the protocol design provided that patients obtaining CHR after 3 months of Dasatinib should have be assessed for High-Dose Therapy (if eligible), we activated a MUD research and, as no related donors were available, the patient received a consolidation course with HAM (sequential high-dose Ara C/mitoxantrone chemotherapy) followed by G-CSF. Chemotherapy was well tolerated and the quick haematological recovery was associated by an elevated spike of $CD34^+$ cells, allowing to collect 25×10^6 $CD34^+$ cells/kg in a single leukapheresis, at day +126 (6 weeks after the Dasatinib discontinuation). The MUD research did not allow to find any HLA full compatible donor, so we decided to plan an autologous transplantation for this patient after a second consolidation course with chemotherapy. Allogeneic stem cell transplantation (SCT) remains the only poten-

tially curative option in patients with ph-positive ALL, and long-term survival rates with SCT are markedly increased when these patients are in complete remission. On the contrary no relevant benefit has been reported in these patients with Autologous transplantation, except for those patients receiving MRD negative Autograft. In this first experience Dasatinib showed to be not only able to obtain a CHR, but it also achieved a complete clearance of MRD, evaluated by Q-RT-PCR, and in the stem cell collected. Moreover the great amount of $CD34^+$ cells mobilized suggests that Dasatinib do not impair the mobilization of normal stem cells in these patients. If these preliminary findings will be confirmed, the incorporation of Dasatinib into induction and consolidation regimens prior to SCT should be considered and the option of a less life threatening option such as autologous transplantation, could be also considered especially for those patients lacking an HLA-compatible donor.

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RARE, BUT LIFE-THREATENING METABOLIC COMPLICATION OF ACUTE LYMPHOBLASTIC LEUKEMIA

M. Serban, M. Bataneant, S. Arghirescu, I. Hortensia, E. Boeriu, C. Petrescu, D. Mihailov, J. John, S. Hanini

University of Medicine and Pharmacy Victor Babes,Timisoara, TIMISOARA, Romania

Background. The spectrum of metabolic anomalies that can occur during acute lymphoblastic leukemia (ALL) is dominated by hyperkalemia, hyperuricemia, hyperphosphatemia, aso. Hyperglycemia is accounted only for a minority of cases, but sometimes expressed by life-threatening evolution. *Aims.* Our retrospective study aimed at evaluating the frequency, the clinico-biological expression, the nature and dynamics depending on the primary disease and on the specific therapy of hyperglycemia resulting during ALL. *Methods.* The analysis was performed on 125 patients diagnosed with ALL, treated and followed-up in a period of 10 years in our clinic; besides hemato-immuno-cytogenetic investigation, the patients (0-18 years of age) were controlled for glycemia, and those with hyperglycemia were analysed for HbA1c, fructosamine, insulinemia, amylasemia, uric and lactic acid, and blood gas parameters; HLA-type and viro-serological profile were also checked. *Results.* Hyperglycemia was observed in 16 (12.8%) patients: 3 with impaired glucose tolerance and 13 with a biological profile of diabetes; in 5 of them, hyperglycemia was present at the onset or at the relapse of the disease, without any association with the therapy, and 11 developed hyperglycemia undergoing treatment with corticosteroid±asparaginase. Therapy dependent hyperglycemia was mild or moderate, requiring insulin therapy only in 3 cases; the other 5 patients with therapy independent hyperglycemia presented severe keto-acidosis, one leading to coma; insulin (0.5-1 IU/kg/d) being mandatory for a period of 3-24 days in all cases for the metabolic control. In all cases, insulinemia was within or above normal ranges; high amylasemia and amylasuria was reported only in one case. HLA-type and viro-serological profile for hepatitis, cytomegalovirus and Epstein Barr virus were not different from the controls. *Conclusions.* The intimate nature of hyperglycemia secondary to ALL is still ignored. The insulin resistance seems to be the main mechanism, induced by enzymatic changes of neoplastic cells of ALL.

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FLOW CYTOMETRIC MRD DETECTION IN CHILDREN WITH B-LINEAGE ALL AT THE END OF ALL MB 2002 REMISSION INDUCTION TREATMENT PROTOCOL

A.M. Popov,[1] T. Verzhbitskaya,[2] G. Tsaur,[2] E. Shorikov,[2] L. Saveliev,[1] L. Fechina[2]

[1]Ural State Medical Academy, EKATERINBURG; [2]Regional Children's Hospital, Research Institute of Cell Technologies, EKATERINBURG, Russian Federation

Background. The flow cytometry application for monitoring of minimal residual disease (MRD) in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) bases on leukemia-associated immunophenotype detection. But aberrant antigens' expression, detected at the time of primary diagnostics can be modulated by chemotherapy, especially during remission induction. This instability of immunophenotype is termed *immunological shift*. Its consideration is necessary for successful MRD monitoring. *Aims.* To evaluate the proportion and level of MRD-positivity at the end of ALL MB 2002 protocol remission induction. To investigate immunological shift in children with BCP-ALL occurring during this treatment. *Methods.* Tumor blasts' antigen profile in bone marrow of 36 children with BCP-ALL treated by ALL-MB 2002 was investigated at the time of primary diagnostics. Bone marrow specimens were examined at the end of remission induction on day 36 by 4-6-color flow cytometry. Only 35 patients who have achieved complete remission were included in to analysis. ALL MB 2002 protocol design has been previously described (A. Karachunsky *et al.*, SIOP 2005). *Results.* 13 of 35 patients were MRD-positive at day 36. MRD level ranged from 0,013% to 5,570% (mean 0,93±0,44%). In four cases MRD-positivity was higher than 1%, in four patients intermediate MRD level (0.1-1%) has been detected. and The rest cases were weakly MRD-positive (0,1-0,01%). Postinduction changes in immunophenotype of tumor cells, ranged from 1 to 5 comparing to the initial data were noted in all positive cases. In 6 patients CD19 hyperexpression was noted. CD10 downexpression was detected in 10 children. In one patient CD34 hyperexpression was found. CD34 expression downmodulation was noted in 7 cases. Moreover, residual leukemic cells lacked CD34 completely in four patients. Interestingly, in two cases, primary weakly CD10-positive (23% and 25% positive blasts respectively), tumor cells lost this marker on day 36. MRD in both cases was presented by blasts with pro-B phenotype. In contrast, in similar case CD34 was gone on day 36 and only CD10-positive residual tumor cells were presented. In 4 of 35 primary CD38-positive cases expression of this marker decreased during remission induction, although in two other children CD38 was hyperexpressed during remission induction. In some patients also CD45 and CD20 expression increased comparing to primary diagnosed level. In all cases except three CD58 expression was stable. In patients with primary myeloid antigens' coexpression, CD13, CD33 or CD15 were also high at day 36. *Conclusions.* During ALL-MB 2002 remission induction therapy significant immunological shift in patients with BCP-ALL can occur. CD10 downexpression, CD20 and CD45 hyperexpression, CD58 and myeloid antigens' stable expression were the most frequent findings in our series. Consideration of this immunophenotypic changes is necessary for successful MRD monitoring.

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EXPRESSION OF CD66C AND CD25 IN ACUTE LYMPHOBLASTIC LEUKEMIA AS A PREDICTOR OF PRESENCE OF BCR/ABL REARRANGEMENT

T. Owaidah,[1] F. Alrawas,[2] M. Al khayatt,[2] N. Elkum[2]

[1]King faisal specialist hospital & RC, RIYADH; [2]King faisal specialist hospital &RC, RIYADH, Saudi Arabia

Background. Expression of myeloid or T cell lymphoid on precursor B cell lymphoblastic leukemia which referred to as apparent expression is quite common phenomenon, however, there is ongoing contraversion regarding its clinical significance. CD66c is a myeloid marker is very frequently has aberrant expression in precursor B cell ALL, CD66c expression was found on cases of childhood and adult ALL with strong correlation with nonrandom genetic changes BCR/ABL. The association with positivity for Philadelphia chromosome has been the subject of debate. Anther leukemia associated marker CD 25 (interleukin-2 receper- IL2R- a Chain) which is not present on the surface of resting T or B lymphocytes but is rapidly expresse following activation. The frequency of CD25 expressing lymphoblasts was found to be significantly higher in BCR/ABL-positive than BCR/ABL-negative patients. *Aims.* to study the prediction rate for presence of BCR/ABL rearrangement in leukemias expressind CD66c and CD25. Materials and *Methods.* In a cohort of 103 patients diagnosed with Precursor B cell acute lymphoblastic or biphenotypic leukemia at our institute between Feb 2003 and September 2006 were studied for expression of CD66c and CD25 at presentation and we evaluated frequency of expression of either or both in BCR/ABL positive cases. *Results.* Out of the 103 studied patients there were 38 adult (>14yr) and 65 child with age range (2-63 years) and median age (13.3 yr). The majority of patients 96 (93%) had (Pre B ALL) and 7 patients were diagnosed as acute biphenotypic leukemia based on (EGIL) scoring system. The myeloid antigens CD13 ,CD33 and CD15 were positive in 41(40%) , 37(36%) and 18 (18.5%) respectively. Surface CD66c was expressed on 70 (68%) and CD25 was expressed by 33 (32%) of the cases while both were expressed together on 29 (28%). BCR/ABL was positive in 18/103 patients. All cases with BCR/ABL positive were positive for sCD66c and CD25 with P value of >0.0009. *Conclusions.* The use of CD66c and CD25 leukemia associated antigens can predect the BCR /ABL mutaion in Pre B ALL.

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CD11C (LEU-M5) EXPRESSION IN CLL PATIENTS: RELATIONSHIP WITH CLINICAL AND PATHOLOGICAL FEATURES AND POSSIBLE INFLUENCE ON PROGNOSIS

A.E.K.F. Esther,[1] M. Morado,[2] R. De Paz Arias,[3]
Y. Moatassim de la Torre,[3] M.A. Canales-Albendea,[3]
A. Varela Gonzalez,[3] F. Hernández-Navarro[3]

[1]University Hospital La Paz, Madrid, Spain., MADRID; [2]University Hospital La Paz., MADRID; [3]University Hospital La Paz, MADRID, Spain

Background. The absence of a definitive pattern of surface molecules in CLL contributes to its heterogeneous clinical behaviour. CD11c/CD18 (Leu-Camc, p150,95) is a beta2-integrin that is primarily expressed in hairy cell leukaemia although it can be finding in other lymphoproliferative disorders like CLL. The prognostic implication of CD11c in CLL and its correlation with a higher WBC, advanced Rai stage, younger age or lymphadenopathies is a controversial issue. *Aims.* The biological and clinical heterogeneity of CLL has prompted our objective to detect new immunophenotypic risk factors like CD11c in order to demonstrate that its expression could distinguish a more aggressive form of disease. *Methods.* We have prospectively analyzed 34 cases from January 2007 to January 2008 to investigate the association between highlighting of CD11c positivity in CLL patients and clinical-pathological features. The CD11c expression was correlated with clinical, morphologic and immunologic variables like Rai and Binet stage, doubling time, splenomegaly, laboratory test, cytogenetic alterations, CD38 expression, ZAP-70 and autoimmune haemolytic anaemia (AIHA). Descriptive statistics for all variables and non-parametric test (U de Mann-Whitney, p value <0.05) in study of correlation were employed. *Results.* Thirty-four patients were diagnosed with CLL by peripheral blood or bone marrow (89.3% and 10.7%, respectively) according to immunophenotypic score (3/5 3.6%, 4/5 7.1%, 5/5 89%). The mean age was 66 years (SD 13.8), and the male to female ratio was 26 to 8. All patients were classified according to the Binet (A 78.3%, B 8.7%, C 13%) and Rai classifications (0 26.5%, 1 26.1, 2 26.1%, 3 4.3%, 4 4.3%). The clinical variables included splenomegaly (23 pts: 69.6% neg; 30.4% pos). AIHA (14 pts: 78.6% neg; 7.1% pos), prior treatment (24 pts: 91.7% neg; 8.3% pos) and laboratory tests like LDH (25 pts: 84% normal; 16% high), b2microglobulin (22 pts: 59.1% normal; 40.9% high) and immunoglobulin level (21 pts: 66.7% normal; 33.3% low). The lymphocyte doubling time was known in 25 patients (<12 months 22 pts). The immunophenotypic analysis of CD38 expression (28 pts: 32% pos; 68% neg) and ZAP-70 was positive in 6 out of 10 patients anakyzed. Finally, the cytogenetic alterations were determined by FISH and according to these results 14 pts and 2 pts were scored as good or poor prognosis, respectively. The evidence of association between CD11c expression (mean: 56.6% LB,R 9.6-97% LB; MFI mean: 120, R 11-347) with well-known prognostic parameters was only statistically significant for doubling time variable. *Conclusions.* Our study provides additional evidence of CD11c as a developing marker of lymphocytic proliferation for future immunophenotypic analysis related to prognostic in CLL patients. However, the short-time follow-up and low number of cases are important limitations and positive findings must be interpreted with caution. Longer follow-up with higher number of patients is necessary to confirm these preliminary results.

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THE MINIMAL RESIDUAL DISEASE IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS IN MODERN THERAPY KIROV SCIENTIFIC RESEARCH INSTITUTE OF HEMATOLOGY AND BLOOD TRANSFUSION OF ROSMEDTECHNOLOGIES, KIROV

T.P. Zagoskina, O.V. Malykh, N.V. Isaeva, G.A. Zaitceva, M.N. Horobrykh

Kirov Scientific Research Institute of Hematology and Blood Transfusion, KIROV, Russian Federation

Background. Last years approaches to therapy chronic lymphocytic leukemia (CLL) have changed. The most effective in CLL treatment are fludarabine-containing regimens with inclusion monoclonal antibodies to antigenes CD20 and CD52. Achievement of complete long remissions, including molecular and immunophenotypic became the primary objective of therapy. *Aims.* The purpose of this study was the estimation of the minimal residual disease (MRD) in first line CLL patients after therapy under programs: fludarabine with cyclophosphamide (FC); rituximab, fludarabine, cyclophosphamide (RFC); alemtuzumab monotherapy. *Methods and results*. 159 CLL patients are included in research, among the patients: men 94 (59%), women 65 (41%); in a stage A - 10 (6%), in a stage B - 94 (59%), in a stage C - 55 (35%) patients. The age of patients ranged from 34 to 76 years (a median - 58 years). The patients were divided into 3 groups. The first group included 80 patients who received the program of therapy FC (4-8 courses, a median - 6 courses). The second group included 69 patients who received RFC therapy, not less than 6 courses. The third group included 5 patients who received alemtuzumab monotherapy within 12 weeks. The results of research have shown, that in the first group the overall effect is achieved in 64 (80%) patients. Among them, in 22 (28%) patients complete remission (CR) was developed. Among patients of the second group the overall response was observed in 66 (96%) patients. Among them, in 48 (70%) CR was achieved. Among 5 patients of the third group, in 80% CR was observed. In CLL patients in CR period MRD was estimated after chemotherapy and immuno-chemotherapy with flow cytometry method of lymphocytic elements of peripheral blood and a bone marrow. In 68% patients, received RFC, it was defined $D19^+/D5^+/CD23^+$ cells <0,01% and $CD19^+/CD5^+/CD20^+$ <0,01% from all B-lymphocytes. At the same time only in 14% of the patients who received therapy FC, it is revealed $D19^+/CD5^+/CD23^+$ <0,01% and $D19^+/CD5^+/CD20^+$ <0,01% from number B- lymphocytes. In 80% of first line CLL patients after alemtuzumab treatment $CD19^+/CD5^+$ and $D19^+/CD5^+/CD23^+$ was <0,01% of all B-lymphocytes. *Conclusions.* Thus, in CLL patients treated with modern therapy FC, RFC and alemtuzumab allow to achieve CR with MRD eradication. Immunophenotypic remissions are achieved mainly with the use of purine analogs in combination with monoclonal antibodies.

1201

RITUXIMAB THERAPY FOR CHRONIC LYMPHOCYTIC LEUKEMIA-ASSOCIATED AUTOIMMUNE HEMOLYTIC ANEMIA: AN UPDATE

G. D'Arena,[1] L. Laurenti,[2] M. Tarnani,[2] C. Califano,[3] M. Dell'Olio,[1]
S. Capalbo,[4] M. Annunziata,[5] N. Di Renzo,[6] S. Storti,[7] G. Rossi,[1]
F. Ferrara,[5] N. Cascavilla[1]

[1]Casa Sollievo della Sofferenza Hospital, SAN GIOVANNI ROTONDO; [2]Hematology Department, Catholic University of Sacred Heart, ROME; [3]Hematology Unit, Umberto I Hospital, NOCERA INFERIORE; [4]Hematology Department, University, FOGGIA; [5]Hematology and Stem Cell Transplantation Unit, AORN A. Cardarelli, NAPLES; [6]Hematology Unit, V. Fazzi Hospital, LECCE; [7]Hematology Oncology Unit, Catholic University, CAMPOBASSO, Italy

Background. Autoimmune hemolytic anemia (AIHA) is the best known autoimmune complication of chronic lymphocytic leukemia (CLL), occurring in 10-20% cases. Steroids are the first-line treatment choice, while different immunosuppressive agents are also used for steroid-refractory disease. In recent years the anti-CD20 monoclonal antibody rituximab has been used for the therapy of steroid-refractory AIHA and autoimmune thrombocytopenia, either idiopathic or in association with CLL. *Aims.* In 2006 we reported our experience on 14 patients with CLL-associated AIHA (Amer J Hematol 2006). Hereby we updated those results. Patients and *Methods.* Eighteen patients (9 M; 9 F; meange age 67 years; range 54-87 years) with CLL-associated AIHA were seen at our Institutions and treated with rituximab. Results. They developed a direct antiglobulin test positive AIHA at a mean time of 59 months (range 0-218 months) from the diagnosis of CLL. In 3 cases AIHA was diagnosed at the same time as CLL. Only 1 patient had fludarabine-related AIHA. All patients received steroids as first-line treatment. At a mean time of 51 days (range 1-210 days) from the diagnosis of AIHA all patients received rituximab at a dosage of 375 mg/sqm/weekly for 4 weeks. All patients except 3 (2 died of cardiac failure or sepsis soon after the third cycle and 1 HCV-positive patients experienced a rise in serum amino transferases) completed the scheduled four programmed cycles. First injection side effects of rituximab were minimal. All but 3 patients showed an increase in haemoglobin levels in response to rituximab (mean value 3,5 g/dL; range 0,7-10 g/dL) and a reduction in the absolute lymphocyte count and lymph nodes and spleen volume. Ten patients required packed red cell transfusions before starting rituximab; 6 no longer needed transfusions just after the second cycle and another patient after the fourth cycle. Six patients (33%) were considered to fully respond and 7 (39%) only responded partially. At a mean follow-up of 27 months, 11 patients were still alive, 7 of them transfusion-free. *Conclusions.* Our results prove that rituximab-induced B-cell depletion is an effective and well-tolerated alternative treatment for CLL-associated AIHA.

1202

MUTATIONAL STATE AND CD38 ARE INDEPENDENT PROGNOSTIC FACTORS FOR TIME TO PROGRESSION IN EARLY STAGE CHRONIC LYMPHOCYTIC LEUKAEMIA

F. Rossini,[1] A. Perego,[1] F. Biella,[2] A. Soccodato,[3] F. Sciarini,[2] S. Pezzatti,[3] S. Besana,[2] S. Cammarota,[3] C. Colombo,[2] D. Perego,[2] P. Brambilla,[2] E.M. Pogliani[3]

[1]S. Gerardo Hospital, MONZA; [2]Clinical Pathology Desio Hospital, DESIO; [3]Haematology Unit. S.Gerardo Hospital, MONZA, Italy

Mutational state and CD38 are independent prognostic factors for time to progression in early stage chronic lymphocytic leukaemia. *Background.* both immunoglobulin heavy-chain gene mutational state and CD-38 positivity were recently identified as predictive factor for time to progression in early stage untreated CLL. *Aims.* whis study was aimed to evaluate the prognostic impact of these two different prognostic factors to time to progression (TTP) in early stage CLL patients followed in our Center. *Methods.* we studied 157 patients (91 M; 66 F); median age was 65.4 (range 36-85). They all had a stage A CLL at diagnosis. Median follow-up was 65.8 months (range 7-254 months). The following cut-off values were used: CD38 >20%; VH unmutated: >98% homology with germ-line sequence. Patients were treated when symptomatic or when progressive disease developed. 53 pts were treated at a mean of 27.5 months (range 1-96) from diagnosis. *Results.* 104 pts are still off therapy after a mean follow-up of 61.6 months (range 7-216). Initial hemoglobin level was 15.3 for untreated pts and 13.5 for treated pts; initial absolute lymphocyte count was 12.4 for untreated pts and 29.5 for treated pts. Initial platelet level was 206.2 for untreated pts and 200.8 for treated pts. 42 pts were unmutated (25 treated) (59.5%), 115 were mutated (28 treated) (24.3%). 47 pts were CD38 positive (19 treated) (40.4%), 110 were CD38 negative (34 treated) (30.1%). At univariate analysis, both mutational state and CD-38 are independent prognostic factors for TTP. Unmutated pts had a TTP of 54.2 months *vs* 147.8 months for mutated pts (p<0.001). CD38 positive pts had a TTP of 57.2 months *vs* 134.7 of CD38 negative pts (p=0.007). When the two factors were combined, three groups of pts with statistically significantly different TTP (p<0.0001) were seen (Figure 1): group 0 (mutated and CD38 negative): n= 88; TTP 151.7 months; group 1 (unmutated or CD38 positive): n=49 TTP 73 months; group 2 (unmutated and CD38 positive): n=20 TTP 32.2 months.*Conclusions.* both mutational state and CD38 can identify poor prognosis pts among early stage (Binet A) CLL. When the two factors are combined, a subgroup (12.3% of patients) with particularly short TTP can be identified.

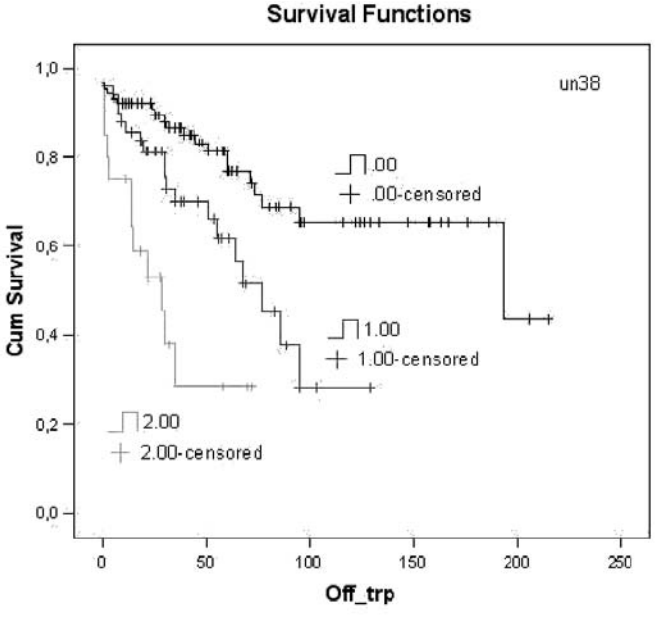

Figure 1.

1203

FLUDARABINE AND CYCLOPHOSPHAMIDE (FC) VERSUS CYCLOPHOSPHAMIDE, VINCRISTINE AND PREDNISONE (CVP) AS FIRST LINE TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL). SINGLE CENTER EXPERIENCE

H. Ionita,[1] L. Chreveresan,[1] R. Mihaescu,[1] I. Ionita,[1] A. Isac,[1] M. Cheveresan,[2] D. Oros,[1] M. Iordache,[1] I. Musta,[1] N. Basa,[1] M. Delamarian[2]

[1]University of Medicine and Pharmacy Victor Babes, TIMISOARA; [2]County Hospital Hematology Departament, TIMISOARA, Romania

Background. The introduction of fludarabine into the treatment of chronic lymphocytic leukemia (CLL) has improved the complete remission rate (CR), overall response rate (OR) and progresion free survival (PFS) compared with alkilator based regimens. Its synergistic action with cyclophosphamide has demonstrated significative advances as front line therapy in untreated CLL patients. *Aims.* To evaluate the response rate, time to disease progression and survival of the patients of FC (arm A)*vs* CVP (arm B) as first line CLL treatment. *Methods.* Starting from 2004, 74 untreated patients with CLL, 49 male and 25 female, were randomised into the two treatment arms, each 37 patients. The diagnosis of CLL was established according to the criteria of International Workshop on CLL (IWCLL)1989. The median age was 68,5 years, range (44-75), ECOG performance status 0-II with high risk category (RAI stage III - IV or RAI stage I - II if they have at least one of the followings: one or more of the disease related symptoms, progressive marrow failure, massive splenomegally or lymphadenopathy or progressive lymphocytosis). Arm A received: Cyclophosphamide 250 mg/m^2 iv D1to D3 and Fludarabine 25mg/m^2 D1 to D3. Arm B received: Cyclophosphamide 400 mg/m^2 iv D1 to D3, Vincristine 1,4 mg/m^2 D1 and Prednisone 100 mg/m^2 D1 to D5. Cycles to be reapeted every 21 days. Hematological toxicity was recorded according to the NCI-WC for diagnosis and treatment and evaluation of response was done according to the NCI-WC response criteria. Were excluded from the study patients with stable or progressive disease after 3rd cycle. While PR and CR cases continued to 6 cycles of the same treatment. To confirm the response to treatment were performed Bone marrow biopsy and immunophenotyping. *Results.*Twenty six patients had stage IV, 28 patients had stage III and 20 had stage II. The median TLC was 93×10^9/L, the median lymphocyte count was 75×10^9/L, the median hemoglobin level was 8,9gr/dl, the median platlets count was 130×10^9/L. Bone marrow biopsy showed diffuse pattern in 82% (61 pts) and the median lymphocyte in the bone marrow was 90,5%. Complet clinical remission was reported in 21/37 patients in Arm A (56,75%) compared to 8/37 patients in Arm B (21,62%) p=0,15. Confirmed CR by bone marrow biopsy was reported in 11 patients in Arm A (29,73%) and only in 12 cases in Arm B (32,43%). Partial response with nodules was reported in 7 patients (18,92%)in Arm A and 4 cases (10,81%) in Arm B. Median time to progression was 27 month in arm A and 8 month in arm B (p=0.03). In therms of haematological toxicity in Arm A 6 patients developed grade IV neutropenia and received G-CSF treatment while 2 patients developed severe anemia (grade III and IV) that reqiured red blood cell transfusion. Two patients developed a transient febrile neutropenia of unknown origin, which require hospitalization. Mild extra-hematological toxicity consisting of nausea and vomiting occurred in 7 patients during the treatment in boath Arm A and B. *Conclusions.* The combination of FC is able to induce higher response rate at the level of bone marrow biopsy. The hematological and extrahematological side effect were mild and manageable. There was a statistically significant difference in time to disease progression in favor of FC regimen .

1204

THE STROMAL CELL DERIVED FACTOR-1 PROMOTE MULTI- LOCALIZATION OF MYELOMA CELL IN BONE MARROW

S. Abroun,[1] F. Yousefpour,[2] M. Nikogoftar[3]

[1]Department of Hematology, School of Medical Sciences, THEHRAN, Iran; [2]Dept. of Oral and Maxillofacial Surgery School of Medicine, Yamaguchi, UBE, Japan; [3]Iran Blood Transfusion Organization, TEHRAN, Iran

Multiple Myeloma (MM) is a progressive disease characterized by the expansion of malignant plasma cells in the bone marrow (BM) with bone lesion as one of myeloma pathogenesis. Myloma cells have multi localization potential in bone marrow (BM) by induces multi bone lesion. In order to clarify the pathogenesis of MM it is an important to understand the molecular events of primary myeloma cells vivo. In BM

microenvironment myeloma cells closely contact to bone marrow stromal cells that are necessary for the survival and proliferation of myeloma cells and it resemble that the presence of stromal cell has been necessary for survival and pathogenesis of myeloma cell. Stromal cells secret so many cytokines. Interleukin-6 (IL-6), a growth factor for myeloma cell *in vitro* and *in vivo*, insulin-like growth factor-I (IGF-I), and stromal cell-derived factor (SDF)-1a are secreted by stromal cells, SDF-1 ubiquitous secreted by stromal cell. The expression of CXCR4, as receptor of SDF-1 is necessary for cells response to SDF-1. In this study we cultured primary myeloma cells and myeloma cell lines which expressed CXCR4 on cell surface as previously described for several days. SDF-1 supports the survival of primary myeloma cells as well as myeloma cell lines, induced the phosphorylation of ERK, AKT and also degradation of IkB follow by activation of NF-KB. We founded that SDF-1 reduced the expression of syndecan-1 (CD138) as good marker of plasma cell distinguished. Syndecan-1 is a adhesion molecules that can bound to collagen and fibronectin. In other hand it we founded that after SDF-1 treatment, increased the matrix metalloproteinase (MMP)-9 in gene and protein levels. It well understood that activation of MMP-9 in cancer cells accelerate cancer cell metastasis. These results suggested that, stromal cells by secretion of SDF-1, first: recruit myloma cells to BM, second: support myeloma cell survival and proliferation, third: reduced myeloma cell adhesion to BM scaffold, forth: by induction of MMP-9 accelerate to myeloma cell metastasis and new localization in the same bone. Still the molecular mechanism of SDF-1 to enhanced the MMP-9 expression in this study is not clear, although it had been reported NF-kB has influence on MMPs expression, however the exact mechanism remain to elucidate.

1205

INVESTIGATION OF PLASMA CELL DYSCRASIAS WITH LOW LEVEL PLASMA CELL ASPIRATE INVOLVEMENT

F. Hodkinson, L. Galligan, S. Drain, M.A. Catherwood, T.C.M. Morris, M.B. Drake, P.J. Kettle, H.D. Alexander

Belfast City Hospital, BELFAST, Northern Ireland

Background. Genetic heterogeneity is a hallmark of plasma cell dyscrasias. Specific acquired cytogenetic aberrations identify subsets of patients with poorer prognosis; however, the patchy distribution of plasma cells (PC) within the marrow and variable quality of bone marrow aspirates compromises the success rate of FISH analysis in MGUS and multiple myeloma. Therefore, cell-targeting *Methods.*are essential for the reliable detection of cytogenetic aberrations in aspirates with low level PC involvement. This study investigated the routine application of interphase FISH in CD138 purified PC in plasma cell dyscrasias. In addition, relationships between cytogenetic aberrations and biochemical, and biological prognostic indicators were explored. *Aims.* To evaluate the success rate of FISH in CD138 purified plasma cells from aspirates with <10% PC involvement; to determine the prevalence of cytogenetic aberrations in MGUS and myeloma patients and identify patient subsets with poorer prognosis. *Methods.* Bone marrow aspirates, from 175 individuals identified as having a clonal plasma cell population by morphology/flow cytometry, were purified using CD138 magnetic microbead autoMACS system (Miltenyi Biotec). Cytospins were prepared from the CD138-positive fraction and analysed by interphase FISH. *Results.* Eighty-five (49%) of aspirates had <10% PC involvement (as determined by morphological assessment of aspirate smears), of which 41 were subsequently diagnosed with myeloma, 37 with MGUS, and 7 with relapsed myeloma. Of 175 aspirates, 108 had a sufficient number of PC to proceed with FISH (71% of cases with <10% PC involvement and 84% of cases with >10% PC involvement). Currently, 70/108 cases have been analysed by interphase FISH, with an 81% success rate. For the 57 cases with available FISH data, 6 were MGUS (2F/4M) and 51 were myelomas (19F/32M). Beta-2-macroglobulin (β2M), free light chain (FLC) ratio and paraprotein (g/l) were significantly higher in the myeloma patients compared to MGUS (p<0.05). There was no significant difference in the prevalence of cytogenetic aberrations between MGUS and myeloma patients; however increased prevalence of monosomy 13q in myeloma patients approached significance (p=0.052). β2M was significantly lower in cases with amplification of chromosome 11 (p=0.020) and t(11;14) (p=0.017). While not reaching statistical significance, logistic regression analysis indicated a higher prevalence of males, amplification of chromosome 11, monosomy 13q and del(p53) in CD56-negative cases compared to CD56-positive cases. *Conclusions.* Few centres routinely perform conventional and/or interphase FISH on aspirates with <10% PC involvement; which accounts for 25% of new myeloma cases at this centre. However, this study has demonstrated a high success rate for obtaining potentially clinically relevant information from aspirates with <10% PC involvement by utilising CD138 purification prior to interphase FISH analysis. Additionally, this has enabled further dissection of MGUS and myeloma patients with regards to cytogenetics. As expected MGUS and myeloma patients could be distinguished by biochemical indicators of disease activity. Although the prevalence of cytogenetic aberrations in MGUS and myeloma patients, and associations between biological/biochemical and cytogenetic prognostic indicators with CD56 expression did not reach statistical significance, a larger cohort would provide clarification.

1206

CD200 EXPRESSION ON MYELOMA CELLS AND ANTITUMOR IMMUNITY

C. Conticello,[1] R. Giuffrida,[1] L. Adamo,[2] S. Ragusa,[2] L. Memeo,[1] A. Chiarenza,[3] A. Romano,[3] R. De Maria,[1] R. Giustolisi,[1] M. Gulisano,[2] F. Di Raimondo[3]

[1]Istituto Oncologico del Mediterraneo, CATANIA; [2]IOM Ricerca, CATANIA; [3]Department of Biomedical Sciences, Hematology Section, University of Catania, CATANIA, Italy

Immune evasion in cancer plays a pivotal role in the failure of natural host antitumor immune response. CD200, initially described as the Ox-2 tumor antigen, is a cell surface ligand involved in regulating immune system. It is expressed on a variety of cell types, including myeloid cells, endothelium, ovarian cells, placental trophoblasts, and neurons where its interaction with CD200 receptors (CD200Rs) negatively regulates immune and inflammatory responses. Recent studies have identified CD200 as a downstream target of RAS/RAF/MEK/ERK activation in melanoma. In addition, CD200 expression was previously described on B cell lineage malignancies, and acute myeloid leukaemia. Moreaux *et al.* showed the correlation between CD200 mRNA expression and reduced event-free survival (14 months *vs* 24 months) in patients with MM, independently from known adverse prognostic factors. Here we show that CD200 protein is over-expressed in 30 human myeloma samples. These cells expressed ERK but a low percentage (30%) expressed the phosphorilated form(p-ERK). In addition, we observed that CD200 MM cells have a reduced immunogenicity in Mixed Lymphocyte Reaction cultures in comparison with normal lymphocytes (CD25 expression < 50%). We hypothesize that MM CD200 expression may suppress antitumor response and that anti-CD200 treatment might be therapeutically beneficial for treating CD200-expressing tumors.

1207

HIGH-DOSE MELPHALAN AND AUTOLOGOUS STEM CELL TRANSPLANTATION FOR AL AMYLOIDOSIS PATIENTS

M. Michael,[1] E. Kastritis,[2] S. Delimbassi,[1] M.C. Kyrtsonis,[3] C. Papadimetriou,[2] I. Apostolides,[1] P. Panayiotidis,[3] N. Harhalakis,[1] G. Pangalis,[3] M.A. Dimopoulos[2]

[1]Evangelismos Hospital, ATHENS; [2]Alexandras Hospital, ATHENS; [3]Laiko Hospital, ATHENS, Greece

Introduction. Several reports suggest that light chain (AL) amyloidosis patients treated with high-dose melphalan and autologous stem-cell transplantation (HDM/ASCT) may achieve a long term survival, with 10-year survival rates reaching up to 25%. Patients who are eligible for HDM are usually younger and have less organs involved by amyloid however treatment related mortality rates may reach up to 25-30% in some series. The aim of this retrospective analysis was to clarify the clinical characteristics, toxicity and long-term survival of AL patients treated with HDM/ASCT in three Greek centers. *Patients and Methods.* Diagnosis of primary AL amyloidosis was based on positive Congo red staining, immunohistochemistry and the presence of typical clinical and laboratory features. Definition of organ involvement and treatment response was based on established criteria published by Gertz *et al. Results.* between 1999 and 2006 12 patients underwent HDM/SCT for AL amyloidosis. Six patients received HDM as first-line treatment and another six at relapse. Median age was 51 years; seven were males and lambda-light chain was involved in 11 (91%) patients. The median bone marrow infiltration before HDM/SCT was 11% (range, 1-50%) and four patients had been previously diagnosed with multiple myeloma. The median time from diagnosis to ASCT was 7 months (range, 1-59 months) and the median number of chemotherapies received before transplant was one (range, 0-2). One organ was involved in 6, two in 4 and three or more in 2 patients respectively. Heart was involved in three (25%) cases, kidneys in 11 and liver in one. Four patients had symptoms of periph-

eral neuropathy and another 4 patients presented with soft tissue involvement. All patients received peripheral stem cell graft and were mobilized with G-CSF alone while conditioning regimen delivered was melphalan 140 mg/m^2 All patients engrafted (median time, 12 days) and toxicity was mainly hematologic (grade IV) and mucositis (grade II), both manageable, while transplant related mortality (TRM) reached 25% (3/12). Among nine patients who survived longer than day 100, 4 achieved complete hematologic response, 4 partial responses and one did not respond. Organ response was achieved in 7 patients-including two patients with heart involvement. Two patients who achieved an organ response relapsed after 4 and 10 months respectively. With a median follow-up of 29 months overall survival has not been reached while the estimated 5-year survival rate is 75%. *Conclusions.* Despite the small patient sample HDM/SCT proves an effective treatment option for AL patients with almost 2/3 showing durable hematologic and organ responses which is translated to prolonged survival. Strict selection criteria for patients suitable for transplantation can significantly decrease TRM to less than 25%.

1208

MODIFIED TOTAL THERAPY 3 FOR MULTIPLE MYELOMA OUTSIDE ARKANSAS - A SINGLE CENTER EXPERIENCE

H. Nativ-Magen, R. Ram, A. Gafter-Gvili, O. Shpilberg

Rabin Medical Center, PETAH-TIQVA, Israel

Background. Incorporating novel agents with an anti-myeloma activity, such as thalidomide and bortezomib to chemotherapy and autologous stem cell transplantation (ASCT), has become the corner stone for the treatment of multiple myeloma. This comprehensive approach has demonstrated high rate of complete remission, shown to be the key for maintaining prolong survival and event free survival. However, opinions regarding the inclusion of novel agents in first line chemotherapy are divided and have been issue for debate. For the last 3 years we adopted a modified approach of an intensified regimen, previously published as Total Therapy 3 (TT 3) by the Arkansas' group. Our modification included: A. Administration of a single novel agent during induction due to limited availability. B. A single ASCT in cases of CR or VGPR after the first transplant C. Patients did not receive consolidation chemotherapy. *Aims.* This study aims to evaluate the efficacy and safety of modified TT3 outside clinical trial in a multiple myeloma clinic of a tertian medical center. *Methods.* Files of all patients treated according to a modified Arkansas' protocol were retrospectively analyzed. We collected patients' and disease's characteristics and adverse events during treatment milestones. Patients were graded for response to treatment and overall survival was evaluated.

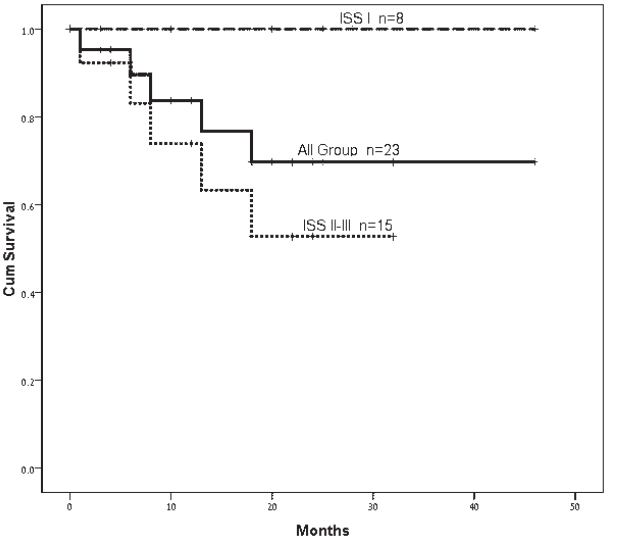

Figure 1. Kaplan-Meier plot of OS according ti ISS.

Results. Twenty three patients were analyzed. Seventy three percent of the patients achieved very good partial remission (VGPR) after receiving combination of chemotherapy and novel antimeyloma agent. Eighty six percent of the patients achieved at least VGPR after tandem ASCT. Two years overall survival was estimated to be 70%. Patients with stage I according to the International staging system (ISS) had 2 years overall survival of 100%, while those who were stage II-III had 2 years overall survival of 53% (Figure 1). Among baseline characteristics of patients, only the deletion of chromosome 13q was associated with a reduced response to chemotherapy, which disappeared after tandem ASCT. Three patients died during treatment: one patient with secondary amyloidosis had fatal arrhythmia and two patients due to non-neutropenic septic shock. All three patients had at least stage 3 renal disease. Febrile neutropenia was documented in 27%, 0%, 81% and 71% during first and second course of DTPACE and first and second course of ASCT, respectively. VTE occurred in 9% of the patients during the course of DTPACE. *Conclusions.* Combination therapy with antimyeloma novel drugs, chemotherapy and ASCT for newly diagnosed myeloma is feasible, effective and safe. It seems that this approach is appropriate for high risk myeloma patients (ISS II-III), while low risk patients (ISS I) may benefit from less aggressive first-line regimen. Further randomized controlled trials should assess the optimal combination treatment.

1209

ADVANCED HODGKIN LYMPHOMA IN TUNISIA: SINGLE INSTITUTION TREATMENT RESULTS OF 126 PATIENTS

R. Ben Lakhal, H. Ben Neji, R. Mansouri, L. Aissaoui, R. Jeddi, R. Ben Amor, K. Kacem, N.H. Kraiem, H. Ben Abid, Z. Belhadjali, B. Meddeb

Aziza Othmana Hospital, TUNIS, Tunisia

Introduction. Until 2001 all advanced Hodgkin lymphoma (HL) in Tunisia had been treated with 8 ABVD equivalent regimens (MDH99). Since Marsh 2002 and faced with the unsatisfactory results of advanced HL (40% of progression and relapses) ,the Tunisian HL group introduced an intensified chemotherapy (4 BEACOPP escalated + 4 baseline BEACOPP) in the treatment of High risk advanced HL (international prognostic score IPS ≥3) (MDH 2002). *Methods and Materials.* Between 1993 and 2001, 71 patients (G1) were treated with 8 ABVD equivalent regimen (MDH 99). Between 2002 and 2006, 55 patients (G2) were enrolled in the prospective non randomized study (MDH 2002) in a single institution (Aziza Othmana Hospital Tunis). According to MDH2002: low risk patients (15 patients) received 8 ABVD and high risk patients (40 patients) were treated with intensified chemotherapy. In each group (G1 and G2) the following prognostic factors were analyzed with regard to their impact on overall survival (OS) , relapse free survival (RFS) and event free survival (EFS) at 5 years: Age , sex , B symptoms , histology, mediastinal bulky disease, peripheral bulky mass, number of involved lymph node regions, IPS and remission status after four chemotherapy cycles (induction therapy). *Results.* At 5 years and for G1: OS, EFS and RFS were respectively 69%, 60% and 78% . These rates were respectively at 80%, 66% and 84% and toxic deaths was at 9% for G2. For All patients, induction unsatisfactory response was the most adverse prognostic factor for OS (G1=p=0.001,G2 =p=0.0005) and for EFS (p=0.001) IPS > or = 3 influenced adversely the OS only for the G1 treated with 8 ABVD (p=0,02). In G2 patients treated with intensified chemotherapy (IPS ≥3) had a better RFS (94%) than patients treated with ABVD (73%), p = 0.02. *Conclusions.* In our country , intensified chemotherapy improve the OS and the RFS but the EFS still lower than in developed countries with a high rate of toxic death (9%) and of primary treatment failure (22%) These unsatisfactory results must be improved by a best management of acute toxicity and intensified induction therapy for all advanced stages. The question to be answered: how we must treat BEACOPP refractory HL?

1210

C-REACTIVE PROTEIN (CRP) SERUM LEVELS AT THE TIME OF DIAGNOSIS IN PATIENTS WITH HODGKIN'S LYMPHOMA (HL): CLINICAL AND LABORATORY CORRELATIONS AND PROGNOSTIC SIGNIFICANCE

X. Yiakoumis, G.P Pangalis, M. Angelopoulou, V. Pappis, M.C. Kyrtsonis, S. Sahanas, M. Siakantaris, E. Dimitriadou, S. Kokoris, C. Kalpadakis, E. Plata, S. Masouridis, P. Tsirkinidis, M. Moschoyiannis, E. Chatzileonida, E. Variamis, P. Panayiotidis, G. Vaiopoulos, T. Vassilakopoulos

University of Athens, Laikon General Hospital, ATHENS, Greece

Background. CRP is an acute phase reactant, mainly produced from hepatic cells during inflammation. It is considered an important indicator of systemic inflammatory response and reflects the production of proinflammatory cytokines. Besides its role as a marker of acute inflammation and infection, CRP has been recently used as a prognostic factor for ischemic heart disease and many solid cancers. Its clinical signif-

icance in HL has not been extensively studied. *Aims.* To investigate the association of CRP levels with baseline clinical and laboratory findings in patients with HL at the time of diagnosis and to evaluate the possible prognostic significance of CRP in previously untreated patients with HL. *Patients and Methods.* We determined serum CRP levels in 172 patients with HL prior to any treatment. Patients had no signs or laboratory evidence of infection at the time of CRP measurement. The demographic, clinical and laboratory features of the patients were representative of a series of unselected adult patients with HL. Briefly, the median age of the patients was 30 years (15-79), 60% were males, 61% had early stage disease (Ann Arbor Stage IA/IIA), 66% had nodular sclerosing disease, 19% mixed cellularity, 28% had B-symptoms, 16% 5 or more involved sites, 41% anemia, 45% white blood cell count ≥10×10^9/L, 12% severe lymphocytopenia, 45% erythrocyte sedimentation rate (ESR) ≥50, and 33% serum albumin <4 g/dL. *Results.* Median serum CRP level was 17.225 mg/L (range: undetectable to 290.0 mg/L); 123/172 (72%) patients had CRP levels higher than the upper limit of 5 mg/L. Patients with CRP levels >17.225 mg/L presented more often with advanced stage (Iə, IIə,III, IV, p<0.001), ≥5 involved sites (p<0.001), anemia (p<0.001), white blood cell count ≥10×10^9/L (p<0.001), lymphocytopenia (p=0.009), ESR ≥50 mm (p<0.001), albumin <4 mg/dL (p<0.001), elevated LDH (p=0.02) and b2-microglobulin >2.4 mg/L (p=0.04). No correlation was observed between CRP and age or gender. With 34 events related to disease progression recorded so far, the 4-year progression free survival was 79% *vs* 67% for patients with CR p<17.225 mg/L and >17.225 mg/L, respectively (p=0.03) while the 4-year overall survival was 94% *vs* 84% (p=0.04) [14 deaths recorded]. In multivariate analysis, the inclusion of other variables such as B-symptoms obviated the prognostic impact of CRP. *Conclusions.* An elevated CRP is not always indicative of infection in patients with lymphadenopathy and/or fever. Serum CRP levels are elevated in 3/4 of patients with HL in the absence of infection and are strongly correlated with factors reflecting tumor burden as well as cytokine production and activity (B-symptoms, anemia, ESR, albumin etc). Moreover, increased (above the observed median value) CRP levels at the time of diagnosis of HL were associated with worse outcome, although a potential independent prognostic impact of serum CRP levels needs to be evaluated with the accumulation of more patients and treatment failure events.

1211

EFFICACY AND SAFETY OF INTRATHECAL LIPOSOMAL CYTARABINE INJECTION GIVEN PROPHYLACTICALLY IN PATIENTS WITH HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA: A REPORT OF 24 PATIENTS IN SPAIN

M. Canales,[1] M.J. Peñarrubia,[2] A. Salar,[3] J.A. Diaz,[4] J.J. Ferreiro,[5] S. Ferrer,[6] A. Llorente,[7] D. Caballero,[8] J.A. Garcia-Marco,[9] L. Palomera,[10] J.J. Sanchez-[11] E. Gonzalez-Barca[12]

[1]Hospital Universitario La Paz, MADRID; [2]H. Rio Hortega, VALLADOLID; [3]H. del Mar, BARCELONA; [4]HCU Santiago, SANTIAGO DE COMPOSTELA; [5]H. Donostia, SAN SEBASTIAN; [6]H. Dr. Peset, VALENCIA; [7]H. Joan XXIII, TARRAGONA; [8]HCU Salamanca, SALAMANCA; [9]H. Puerta de Hierro, MADRID; [10]H. Lozano Blesa, ZARAGOZA; [11]H. Morales Messeguer, MURCIA; [12]ICO, BARCELONA, Spain

Background. Patients with diffuse large B-cell lymphoma (DLBCL) who develop lymphomatous meningitis (LM) during or after first-line treatment have a poor prognosis, with CNS relapse occurring within 1 year in approximately 80%. Risk factors have been defined and prophylaxis is recommended in patients with high-risk DLBCL. Liposomal cytarabine (DepoCyte®), a sustained-release preparation of cytarabine for intrathecal (IT) injection, has been shown to be well tolerated and effective in the treatment of LM. Its long CSF half-life allows liposomal cytarabine to be given less frequently than conventional therapy, reducing discomfort for patients and the risks associated with repeated lumbar punctures. *Aims.* The potential of liposomal cytarabine to improve the outcome of prophylaxis against LM is being investigated into a multicenter and open trial in patients with DLBCL no localized older 65 years and younger 65 years with IPI 0-2 in Spain. *Methods.* Preliminary efficacy and safety results are reported in 22 patients (median age 67 years; range 18-78; 14 male) with newly diagnosed DLBCL at high risk of developing LM (defined as the presence of at least one of the following criteria: retroperitoneal mass >10 cm, Waldeyers ring or paranasal involvement, involvement of >30% bone marrow, testicular involvement) who received prophylactic IT liposomal cytarabine during treatment with R-CHOP14 regimen between June 2006 and February 2008 at 11 centers in Spain. Liposomal cytarabine 50 mg was administered during the first day of treatment at first, second and sixth cycles of R-CHOP14 scheme (study days 1, 15 and 71). *Results.* The median number of doses administered was 2,4 (range 1-3). Fifteen patients received corticosteroid as prophylaxis for chemical arachnoiditis: dexamethasone (4 mg IT [n=12] or 8 mg/b.i.d PO [n=2]); 1 patient received IT hydrocortisone (20 mg). The remaining patients did not receive specific corticosteroid prophylaxis for chemical arachnoiditis, only prednisone of R-CHOP therapy, [n=9]. With a median follow-up time of 11,8 months [range (2-20)] no CNS involvement has been observed up to date. No signs of neurological progression or relapsed were observed. Overall, liposomal cytarabine was well tolerated. Five patients experienced minor side effects including headache (Grade 1/2, n=3), nausea/vomiting (Grade 3, n=1) and headache/vomiting (n =1). Nineteen patients hadn´t side effects (n=19). *Conclusions.* These preliminary observations indicate that IT injection of liposomal cytarabine (DepoCyte®) is well tolerated and can be administered effectively and safely in combination with dose-dense regimens. Longer-term follow-up will be needed to confirm these encouraging observations.

1212

CORRELATIONS OF SOME IMMUNOHISTOCHEMICAL PROGNOSTIC MARKERS WITH CLASSICAL PROGNOSTIC FACTORS AND OUTCOME IN DIFFUSE LARGE B-CELL NON-HODGKIN`S LYMPHOMAS - SINGLE INSTITUTION EXPERIENCE

S. Demian,[1] G. Oltean,[2] E. Horvath,[3] Z. Pavai,[3] I. Macarie[2]

[1]University of Medicine and Pharmacy Tg Mures, Ist Medical Clinic, TG MURES; [2]University of Medicine and Pharmacy Tg Mures, Ist Medical Clinic-Hematology, TG MURES; [3]University of Medicine and Pharmacy Tg Mures, Pathology Laboratory, TG MURES, Romania

Background. Diffuse large B-cell lymphomas (DLBCL) are biologically and clinically heterogenous. Immunohistochemistry is now used not only for positive diagnosis but also for prognostic purposes. *Aims.*To study corellations of some immunohistochemical markers positivity with classical prognostic factors and outcome in homogenous group of DLBCL. *Methods.* 28 consecutive newly diagnosed, primary nodal, $CD20^+$ *de novo* DLBCL (centroblastic or unspecified morphology), treated and followed in our department between 2002-2004 were retrospectively selected. There were excluded cases with HIV positivity, Ann Arbor stage I, uncertain (nodal or extranodal) primary site of the disease. Uniformely first-line therapy was the CHOP (cyclophosphamide-epirubicin-vincristine-prednisone) regimen - the standard in our department in mentioned period - and DHAP (dexamethasone-cytarabine-cisplatin) regimen was used as salvage therapy. Tissue microarrays with diagnostic tissue samples were constructed and immunohistochemically analysed, panel: CD79a, CD10, bcl2, bcl6, CD5, CD138, Ki67, p53, CD44s and isoforms v3, v4, v5 and v6. Were considered *positive* cases with immunohistochemical marker expression in >20% of tumoral cells for CD44s and variants and in >10% of tumoral cells for the other studied markers. Results were correlated with *classical* prognostic factors, assessed at diagnosis (International Prognostic Index-IPI, age, gender, Ann Arbor stage, ECOG performance index, B symptoms, presence and pattern of marrow tumoral invasion, bulky disease, serum albumin level, serum lactat dehydrogenase-LDH, eritrocyte sedimentation rate, hemoglobin level), complete remission-CR rate and outcome (overall survival-OS and failure-free survival-FFS, observation period ending january 2008, maxim observation time 71 months, univariate Kaplan-Meier analysis, with log-rank comparison). CR rate after first-line therapy was 55,56%, median FFS 24 months. *Results.* Overall immunohistochemical marker positivity was: CD10-25,93% of cases, bcl2-25,93%, bcl6-33,33%, CD138-7,41%, CD79a-85,18%, p53-37.04%, Ki67-55,56%, CD5-0,0%, CD44s-85,18%, CD44v3-22,22%, CD44v4-29,63%, CD44v5-85,18%, CD44v6-66,67%. CD44v6 was coexpressed with bcl2 predominantly on bcl6 negative cases and correlated with advanced stage, H/I or H-IPI, ECOG≥2. CD44v3 positivity correlates with diffuse pattern of marrow tumoral invasion. Cases with expression of CD10, bcl6 or germinal center phenotype ($bcl6^+/CD10^+/bcl2^-/CD138^-$) displayed better OS and FFS but log-rank test p>0,05. Stage III or IV, IPI H/I or H, ECOG≥2, high LDH, expression of bcl2, p53, Ki67 positivity >50% and activate B-cell-ABC ($bcl6^-/CD10^-/bcl2^+/CD138\pm$) phenotype were associated with significant worse OS and FFS (logrank p<0,05). Applying Fischer`s exact test, complete remission rate correlate positively with CD10, bcl6, CD44v6 positivity, bcl2 negativity and non-ABC immunohistochemically assessed phenotype. *Conclusions.* Immunohistochemistry revealed additional important prognostic informations in DLBCL cases studied. Tissue-microarray technique is not only useful but labour costs profitable to, even for smaller retrospective series studies.

1213

SECOND PRIMARY MALIGNANCIES (SPM).NON HODGKIN LYMPHOMAS (NHL)AND OTHER ONCOHEMATOLOGIC PATHOLOGIES. 91 CASES FROM 2 ONCOLOGIC CENTRES: ONCOLOGY HOSPITAL MARIA CURIE AND HENRY MOORE INSTITUTE, BUENOS AIRES, ARGENTINA

M. Dragosky, S. Alcaraz, I. Annetta, L. Devotto, P. Luchetta, M. Marquez

Oncology Hospital Marie Curie, BUENOS AIRES, Argentina

Are defined as second primary malignancy (SN) those that differ in their histological and molecular characters of the first. A significant increase is reported, constituting at present the 6 th most common malignancies. Starting in the pediatric population, its theme was extended to adults. The number of cancer survivors is growing at a rate of 2% each year. In the etiology are mentioned: longer survival, common etiological factors, environmental exposure, characteristics of the host (genetic predisposition), effects of chemotherapy and radiotherapy, gene mutations, enzyme polymorphism. *Materials and Methods.* Out of a total of 2375 patients with oncohematologic pathology. 91 cases with a second malignancy were reported. Ages: 27 to 87 years, average 66.05, 57 male, 34 female. Location of the first cancer was : breast: 22, colon 9, skin 6(5 squamous, 1 melanoma),prostate, bladder and endometrium: 3, cervix, larynx, tonsils, ovary, thyroid, kidney, testis, vulva, 1. In 39 cases the first malignancy was hematologic: NHL 18 (13 low grade,5 high grade), LLC 10 ,Hodgkin 5, Myeloma 2 ,Myeloproliferative 4 (2 thrombocythaemia, polycythemia 1, 1 myelofibrosis). The survival of the first tumor was 1 to 29 years: <1 year 4, 1 to 5: 25, 6 to 10: 31, 10 to 15: 14, 15 to 20: 11 Over 20: 6. Treatment of the first tumor was:surgery (S)in 19, S + radiotherapy (RT)in 13, S+ chemotherapy (CT): 6, S+RT+CT: 4. CT: 23, CT + RT: 9, RT: 3, without treatment: 3. Location of the second tumor: colon, breast 6, prostate, skin squamous cell, myeloma, 4: lung, kidney, melanoma: 2, rectum uterus, bladder, mesothelioma: 1. In 53 cases the second tumor was hematologic: NHL low-grade 12,high grade 5, LLC: 9, Hodgkin 2, HCL 1, MDS 3, Myeloproliferative (ET:4,PV 2,MF:1). Time elapsed between the 2 tumors: range 6 months to 27 years. Simultaneous (<3 months): 11, <1 year: 5, 1-3: 19, 4-5: 9, 6-10: 15, 11-15: 8, 16-20: 7,> 21:4, no data: 13 cases. 8 patients had a third neoplasia: 2 skin basal cell, 2 breast, 1 NHL T, kidney, lung, neurinoma. *Conclusions.* We emphasize the high incidence of oncohematologic pathology after solid tumors. The average age of 66.05 years in the population is a determining factor in the expectation of a second neoplasm . Monitoring and periodic checks undergo by cancer patients are other factor that allows early detection of occult neoplasia. This population of patients who have successfully overcome a neoplasm should be carefully monitored and a strategy for long-term control should be developed.

1214

DOES PDG-PET ADD ADDITIONAL PROGNOSTIC INFORMATION TO CT STAGING IN LYMPHOMA PATIENTS?

B. Sanchez-Gonzalez, C. Trampal, A. Solano, C. Pedro, J.M. Maiques, A. Alvarez, I. Vollmer, E. Gimeno, E. Abella, F. Garcia-Pallarols, C. Besses, A. Salar

Hospital del Mar, BARCELONA, Spain

Background. 18-Fluoro-deoxyglucose positron emission tomography (FDG-PET) is a noninvasive imaging tool for initial staging and evaluation of treatment response in Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). PET imaging can provide additional information to conventional imaging as computer tomography (CT). *Aims.* The aim of this study was to evaluate the role of PET in improving the staging and if this fact could have an impact in prognosis, in compared to CT. *Methods.* From February 2005 to January 2008, one hundred ten consecutive patients with NHL (49 aggressive, 32 indolent and 3 unclassifficated) and HL (26 patients) were staged according to the Ann Arbor classification with CT and bone marrow biopsy (BMb). As well, all patients underwent to FDG-PET body before treatment. International Prognostic Index (IPI) was calculated following standard staging (IPI-st) and with standard staging plus FDG-PET (IPI-PET) in NHL. *Results.* CT and FDG-PET were concordant in 84 patients (66%). In 37 patients (34%) discordance was observed: in 7 patients FDG-PET was negative, in 11 patients FDG-PET detected more bone lesions, in 11 patients FDG-PET uptake in other lymph nodes, including spleen infiltration, and in 8 patients FDG-PET detected less lymph nodes or organ involvement. However, additional information of FDG-PET changed in staging in 21 out of 37 patients with discordant findings but the IPI risk changed in only 5 patients. Discrepancies CT and FDG-PET were seen in similar proportion in all lymphoma subtypes. Overall survival curves at two years showed no differences between IPI-st and IPI-PET (IPI-st 0-1: 97%, 2-3: 89%, 4-5: 48%; IPI-PET 0-1: 96%, 2-3: 89%, 4-5: 48%). *Conclusions.* FDG-PET can provide additional information to CT in both aggressive or indolent non-Hodgkin lymphoma and Hodgkin lymphoma. CT and FDG-PET were discordant in 30% of the patients but only half of them showed change of stage. IPI risk was modified in few patients with NHL. Inclusion of patients is ongoing.

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THE WANDERING SPLEEN: CASE REPORT AND REVIEW OF THE LITERATURE

G. Fabio, M. Carrabba, E. Volpato, C. Hu, M.D. Cappellini

Università degli Studi di Milano & Fondazione IRCCS Ospedale Maggiore Policlinic, MILAN, Italy

Background. The wandering spleen is a very rare entity. The spleen migrates from its normal location because of congenital or acquired long pedicle, which predisposes the spleen to torsion. Acute splenic torsion is a potentially fatal surgical emergency, and its correct identification represents an imaging challenge. We describe the case of a 34-year-old woman, carrier of G6PDH deficiency and of ectopic spleen. On March 2007 she complained abdominal pain and pyrosis. Hemolytic crisis was suspected out of mild anemia with bilirubin increase. Abdominal ultrasound demonstrated thrombosis of the spleen vein. LMWH was started. Pancytopenia worsened. Abdominal CT-scan showed an enlarged spleen in pelvis with many parenchymal ischaemic areas, a thrombosis of the splenic vein and cavernoma of splenic hilum. A muddle of enlarged and curlying collateral venous vessels occupied the left abdominal side. The patient underwent splenectomy by laparotomy and prolonged anticoagulation prophylaxis. Three weeks post-splenectomy she developed acute abdominal pain and fever. Laboratory tests showed transaminases and LDH rise. We suspected thrombosis of the splenic stump vessels or portal vein and prolonged anticoagulant therapy for three months after surgery. A CT-scan after three months of follow-up showed the complete reabsorbtion of the vessels bandle and no thrombosis. *Aims.* We performed a systematic review to investigate diagnosis, management of acute complications and thrombosis in wandering spleen, before and post-splenectomy.

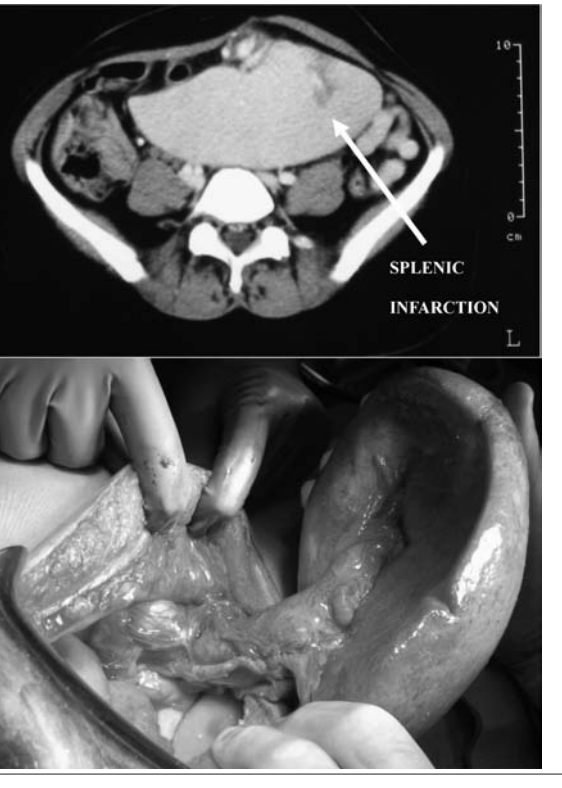

Figure 1. Wandering Spleen: CT-scan and surgical image.

Methods. Computerized literature searches were performed using MEDLINE (PubMed). Headings *wandering spleen* was used for the former search strategy, *splenectomy* combined with *thrombosis* for the latter. We

applied restrinction on the last 10 years of publication and on humans subject. *Results.* International registries or specific guidelines for this rare clinical condition are not available in medical literature. The search *wandering spleen* identified 136 papers , all except 10 were single case reports, half about adults. Presentation symptoms, CT-findings and complication management were detailed. The search *splenectomy and thrombosis* identified 300 papers, mainly cohort of patients with a big heterogeneity of predisposing diseases. We reviewed all cases to identify the correct management. *Conclusions.* Many pitfalls occur on ultrasound and CT: hypertrophy and lateral extension of the left lobe of the liver can be confused with a normally situated spleen. The radiologist may erroneously assume that the absence of the spleen reflects previous splenectomy. The twisted splenic vessels and surrounding fat can mimic a cavernoma. The treatment of choice is splenopexy. In the presence of splenic infarction, splenectomy is required. Because the occurrence of thrombosis is an underappreciated complication after splenectomy, routine postoperative color-doppler could be performed in all post-splenectomy patients for early detection of a portal vein thrombosis. Factors predictive of postoperative thrombosis are spleen weight, operation time, surgical technique itself, and elevated preoperative platelet count. Differences in the shape of the stump may affect the hemodynamic and coagulation state, with an increased clotting risk. A number of investigators recommend the use of postoperative prophylaxis such as heparin, for high-risk patients.

1216

A SHORT ACTIVATED PARTIAL THROMBOPLASTIN TIME HAS NO CORRELATION WITH THE VENOUS DRAW LINE THROMBOSIS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES UNDERGOING PERIPHERAL BLOOD STEM CELL HARVEST

M. Kumar, P. Boraks, D. Camilleri, J. Craig

Addenbrookes Hospital, Cambridge University Hospitals NHS foundation trust, CAMBRIDGESHIRE, UK

Background. Peripheral blood stem cell (PBSC) harvesting after mobilising with chemotherapy is a standard part of treatment of haematological malignancies. Clotting in the venous draw line is one of the occasional complications which necessitate the administration of additional anticoagulation. The level of blood factor VIII can significantly lower the aPTT (activated partial thromboplastin time) which can be a significant risk factor for thrombosis. *Aims.* In order to determine whether a short aPTT increases the incidence of access line thrombotic risk during harvesting and hence the need for additional anticoagulation, we looked into the incidence of access line thrombosis and shortened aPTT pre harvest. *Patients and Methods.*We studied 102 PBSCH in patients with myeloma (n=52 harvests), NHL (n=35), Hodgkin's lymphoma ,(n=3) and others (n=12). Mobilisation was achieved using Cyclophosphamide and G-CSF5mcg/kg. PBSC harvesting was performed using COBE spectra, version 6.1 with an average of 3.2 total blood volumes. The aPTT was measured 1 hour before the procedures. *Results*. A shortened aPTT (normal range 22.5-34.5 seconds) pre harvest was observed in 30/102 (29%) of patients. Thrombosis in the venous access draw line was observed in 9/102 harvests. Out of patients who had access line thrombosis (9), only 2 patients had a shortened aPTT (7%). In the patients who did not have thrombosis (93), 28 had a shortened aPTT (30%). *Conclusions.* A shortened aPTT does not predict the incidence of venous draw line thrombosis.

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PREOPERATIVE VALUE OF PFA-100 IN BARIATIC SURGERY

S. Soler, J.A. Ruano, F. Ramirez, M. Tapia, V.L. Peri, J.M. Bosch, F. Fernandez, J.M. Diaz

Hospital Insular de Gran Canaria, LAS PALMAS DE GRAN CANARIA, Spain

Background and aims. Obesity is considered one of the risk factors for thrombosis, mediated, among other causes, by platelet and vascular endothelial activation . Bariatic surgery is recommended as a radical treatment for obesity when other medical therapies have failed. Despite to this prothrombotic state, from our personal experience and in recent publications, unexpected severe hemorrhages and deaths have been reported with this type of surgery. Leptin is a hormone related to the control of ingested food, and obesity could be connected to a resistance to its action. This hormone is also implicated in many physiological processes, among others, the aggregation of platelet through specific receptors. However, it has been described that there is a population that does not respond to these receptors. This combination of special resistance that occurs in the morbidly obese, coupled with the possibility that there also be associated with platelet resistance, could help to explain the appearance of these cases of unexpected hemorrhage in this type of patients, related to a defect in platelet aggregation. This possible defect could be evaluated by a preoperative study of the platelet function. The PFA-100, a repeatable, available test in this environment, seems to be appropriate. *Methods.* 108 consecutive obese patients about to undergo bariatic surgery were studied. A hemogram, platelet count, quick and PTT were done as well as a platelet PFA-100 study using ADP Collagen and Epinephrine-Collagen cartridges. These patients were compared with 72 controls who were non-obese blood donors. Patients on receiving antiagregant or anticoagulant medication were excluded. A statistical analysis was done applying the student t by mean a SPSS software. *Results.* Concerning demographic data, no differences were found in respect to age (37.22 *vs* 36.51), but in respect to sex, 75% of the patients were women and in donors only 34.23% (p<0,001). There were also differences in taking the medication (30% *vs* 6.45%), which was much more frequent in the obese population, and in the existence of previous hemorrhagic episodes, which surprisingly was more frequent in the controls (1.8% *vs* 8.33%). Concerning analytical data, there were no differences in platelet count, nor in coagulation tests. The average PFA-100 col/adp was 93.13 seconds for the obese group, and 90.46 seconds for the controls. For the PFA-100 col-epi, the averages were 136.14 and 140.007 seconds, both data within normal range. Concerning patients with elongated PFA, 8 patients (7.2%) had PFA col/adp higher than high normal, and 12.7% PFA col/epi. Similar data was obtained with the controls (4.5%) and 12.5%). No significance was found in this test. *Conclusions.* Obese patients undergoing bariatic surgery can have unexpected hemorrhages, whose mechanism could be related to platelet aggregation. PFA-100 pre-surgical study, however, does not confirm platelet dysfunction in these patients, either because there are no anomalies or because it is not an adequate test to evaluate a supposed hemorrhagic tendency in some of these patients.

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MULTIPLE MYELOMA PRESENTED WITH ACQUIRED FACTOR VIII INHIBITOR

I. Sari,[1] M.A. Erkurt,[2] A. Ifran,[2] K. Kaptan,[2] C. Beyan[2]

[1]Pamukkale University Faculty of Medicine, DENIZLI; [2]Gulhane Military Medical Academy, ANKARA, Turkey

Presence of acquired factor VIII (FVIII) inhibitor in patients without hemophilia A is a rare disorder, occurring at a rate of 0.2-1.0 case per million each year. The disease may occur due to several underlying medical conditions such as pregnancy and postpartum status, autoimmune disorders, drug interactions or malignancies. Initial presentation of hematological malignancies associated with acquired FVIII inhibitor is very rare and there is only one documented case for multiple myeloma. Here, we report a second case of multiple myeloma presented with acquired high-titer FVIII inhibitor. A forty-three year old woman was referred to our hematology unit for anemia, and elevated erythrocyte sedimentation rate. Two months prior to presentation, she had had an emergent operation because of ovarian cyst rupture. Also, she had received 14 unit erythrocyte suspensions because of excessive bleeding in perioperative period. At this time, coagulation tests had revealed an activated partial thromboplastin time of 84.4 seconds (normal, 24.0-40.0 s) which could not been corrected by mixing with normal plasma and a factor VIII level of 6% (normal, 70-130%), while previous coagulation tests had been found within normal ranges in 2003. Additionally, other coagulation studies included fibrinogen, fibrin degradation products, von Willebrand factor and factor assays (II, V, VII, IX, X, XI, XIII) had been determined within normal range. These results had been found to be consistent with the presence of acquired FVIII inhibitor. The titer of FVIII inhibitor level had been determined as 10 Bethesda unit/ml. The patient was admitted to our hospital for further evaluation. No positive signs were found in the physical examination. In complete blood count, hemoglobin was 10.6 g/dL, WBC 6.6 $\times 10^9$/L with 74% neutrophils and platelet count 234 $\times 10^9$/L. The erythrocyte sedimentation rate was 110 mm in 1 hr, serum albumin level 2.5 g/dL, globulin level 5.6 g/dL, and C-reactive protein 47.8 mg/L (0-6). Serum protein electrophoresis showed monoclonal band on the gamma region. Serum IgG was 46.60 g/L (7.0-16.0), IgA 0.27 g/L (0.7-4.0), IgM 0.56 g/L (0.4-2.3). The analysis of urine showed kappa light chain paraproteinemia, 338.0 mg/L (<7.1). Also, an IgG-kappa paraprotein was found by immunofixation of urine and serum. The liver and the kidney functions, serum calcium level, and lactic dehydrogenase levels were within normal ranges. Serum ?2 microglobulin level was 3.78

mg/L (0.7-1.8). X-ray films of the bones revealed lytic areas in the skull, pelvis and lumbar vertebras. Bone marrow aspiration showed normal cellularity with 40% plasma cell infiltration. A diagnosis of Ig G-kappa type of multiple myeloma associated with acquired FVIII inhibitor was made and treatment with VAD chemotherapy regimen was started. In conclusion, in patients presenting with severe bleeding, the autoantibodies against FVIII should be considered and coagulation tests should be determined for differential diagnosis of bleeding. Clinicians should be alert for underlying rare neoplastic diseases such as multiple myeloma in the patients with acquired FVIII inhibitor.

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THE GARDNER-DIAMOND SYNDROME IN AN ADOLESCENT GIRL ASSOCIATED WITH ABNORMAL PLATELET AGGREGATION FOLLOWING EXPOSURE TO EPINEPHRIN

Z. Karakas, B. Avci, G. Ozturk, O. Devecioglu

Istanbul University, ISTANBUL, Turkey

Background. The Gardner-Diamond syndrome is a rare disorder characterized by recurrent spontaneous painful bruising in patients with underlying psychosis and neurosis. Despite the presence of other symptoms suggestive of an underlying disorder of primary hemostasis in a large percentage of reported patients, only few patients with Gardner-Diamond syndrome who have had platelet aggregation studies reported. *Aims.* The authors describe a case of Gardner-Diamond syndrome in an adolescent girl who had abnormal platelet aggregation test following exposure to epinephrin. *Case.* This 13-year old anemic girl presented with a 6-month history of episodic painful bruising on limbs and dorsum of hands and feet, epistaxis, metrorrhagia, and psycho-somatic complaints like depression, anxiety, headache, and myalgia. There was no history of trauma, and episodes seemed to be precipitated by stress. Her mother has menometrorehagia. Physical examination at the time of bruising revealed periorbital ecchymosis like Racon Eyes. The lesions were tender to touch. Examinations between exacerbations revealed no unusual bruising on her skin. She did not have laxity of the skin or hyperextensibility of her joints to suggest a connective tissue disorder. Detailed hematologic evaluation showed no coagulation factor deficiencies or vonWillebrand disease. Platelet aggregation following exposure to epinephrine was impaired, while the responses to ADP, collagen, and ristocetin were normal. In psychiatry consultation, it was revealed that she had important family distress, depression attacks and phobias. It was decided to give her a serotonin re uptake inhibitor, fluoxetin (Prozac). Antidepressant and psychotherapy induced improvement in dermatological manifestations clearly. The episodes of painful bruising could be provoked following intradermal injection of washed red blood cells within 96 h. To confirm the diagnosis an intradermal test for autoerythrocyte sensitization was done with concentrations at 10%, 50%, 80%, 100% washed RBCs of the patient on the forearm region with a saline control on the opposite side. Within two hours the patient developed a painful ecchymotic reaction, at the site of the injection, similar to the lesions on her extremities. There was no reaction at the control site. *Conclusions.* The pathogenesis of this rare entity is more likely due to a psychiatric alteration rather than to the immunological mechanisms. Platelet aggregation abnormality occurs during the emotional disorders quickly and causes to local hemorrhage. One of the stress hormones, adrenalin, might be a role for pathogenesis. Gardner- Diamond-syndrome should be included in the differential diagnosis of ecchymotic bleeding.

1220

THE MANAGEMENT OF UNUSUAL ACQUIRED BLEEDING DISORDER SECONDARY TO INHIBITION OF PLATELET FUNCTION- A CLINICAL CHALLENGE

W. Chia, A. Roy, J. Needham, S. Simpson, A. Milne

Basingstoke and North Hampshire Hospital, BASINGSTOKE, UK

Presentation. A 33 years old lady presented with 4 months history of progressive superficial bruising associated with polyarthalgia. The bruises were extensive (up to 15cm), predominantly around the loins and lower limbs. The bleeding was extensive such that she dropped her haemoglobin by >2g/L. *Background.* She was known to have seronegative polyarthritis for 2 years. Previous treatments included hydroxychloroquine, sulphasalazine, citalopram and i.m.depo-medrone. She was intolerant of NSAIDs due to dyspepsia. She had 2 caesarean sections (2000 and 2003), laparoscopic cholecystectomy (2001) and dental fillings without extensive bleeding. There was no family history of bleeding disorder. *Drug History.* At presentation, she was on sulphasalazine which was stopped immediately. Hydroxychloroquine and citalopram were discontinued for 6 weeks prior to admission. She was not on aspirin, NSAIDs or contraceptive pills. *Investigation*. On admission Hb: 82g/L, WCC: 6.5, Platelet: 278. B12, folate, coomb's test, and blood film morphology were normal. Biochemical profile, autoimmune screens, complements, immunoglobulin, CRP, ESR were normal. Clotting screens (including haemophilia and Von Willibrand's disease) anticardiolipid, lupus anticoagulant were normal. Viral, septic screens and whole body CT scans were normal. PFA100 showed 222s (87-167s) collagen/epinephrine, and 123s (59-111s) collagen/ADP. Platelet aggregation studies were abnormal. Platelet nucleotides, platelet factor 3 activity and thromboelastograph were all normal. *Treatment.* Initial working diagnosis was drug induced platelet dysfunction and hence she was treated with twice daily platelet transfusion for 5 days and recombinant factor VIIa (rVIIa) but with no improvement. At that stage, the lab incubated her platelet poor plasma with healthy donor platelet and the PFA100 was found to be abnormal. In view of this result, she was given i.v. immunoglobulin 2 g/kg. This resulted in normal PFA100 and significant clinical improvement. Unfortunately the response was short lived and patient relapsed after 2 weeks with more dramatic bruising. A trial of DDAVP yielded no response and she was commenced on immunosuppression, consisting of prednisolone 40 mg and azathioprine 150 mg daily. The control of bruising was suboptimal and complicated by side effects of steroids. Further treatment with pulse of methylprednisolone yielded no significant improvement. Intravenous immunoglobulin provided further short term improvement. By then, she had 3 months of azathioprine without much response. The azathioprine was stopped and she was given rituximab 375mg/m^2 weekly for 4 weeks. Four weeks later, the bleeding still persisted, requiring admission for blood transfusion. *Discussion*. The clinical course is very aggressive. It is unlikely that patient had any inherited platelet disorder based on her previous surgical history, absence of response to platelet transfusion and rVIIa. Interestingly, when her platelet poor plasma was incubated with healthy donor platelet, the PFA100 remained grossly abnormal. Together with the fact that she responded to i.v.immunoglobulin, it raises the possibility of an inhibitor. This might be related to the underlying seronegative arthritis. Her treatment was predominantly aimed at B-lymphocytes suppression. As there has been limited response to treatment, it has been proposed that the inhibition might be T lymphocytes driven, and hence alemtuzumab could be a treatment option. Further investigations and opinions are being sought.

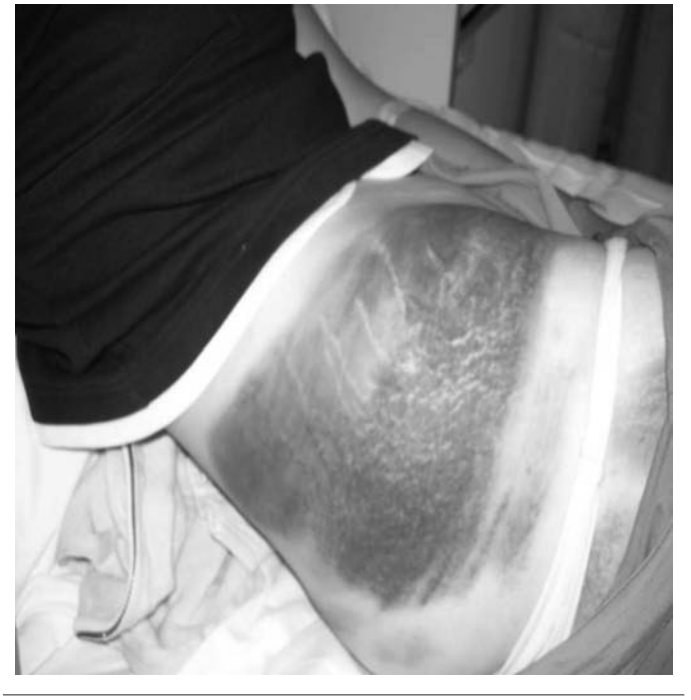

Figure 1. Loin.

1221

EVALUATION OF THE RPR AND TPHA TESTS USED FOR DETECTION OF TREPONEMA PALLIDUM

S. Salvador,[1] A. Lapa,[1] B. Santos,[1] A. Freitas,[1] R. Cortes[2]

[1]Escola Superior de Saúde de Faro, TUNES; [2]Centro Hospitalar do Barlavento Algarvio, PORTIMÃO, Portugal

Background. The Treponema pallidum subsp pallidum is the etiologic agent of syphilis, which is transmitted primarily by sexual contact. The transmission of syphilis can also occur by blood transfusion, for this reason the World Health Organization and the Food and Drug Administration recommended the screening of the disease in all donations of blood. The serological diagnosis of syphilis combines non treponemics tests, like VDRL (Veneral Disease Research Laboratory Test) or RPR (rapid plasma reagin), with treponemics tests, like TPHA (Treponema pallidum hemagglutination assay) or FTA-Abs (fluorescent treponemal antibody absorption). Imumoenzimatics tests (EIA) are a suitable alternative to the combination of screening tests VDRL / RPR and TPHA, presenting similar results. In our Blood-Bank the clinical practice guideline in use for syphilis diagnosis recommend the treponemic test Syphilis EIA II (TA), as a screening test. Positive results are than confirmed by two additional tests: TPHA and RPR. *Aims.* Since EIA Syphilis II (TA) is a reliable screening test, the objective of the present study was to evaluate if the two additional confirmatory tests, for each unit of blood presenting a positive result in a Syphilis EIA II (TA) test, is a real need. *Methods.* Data from 87 samples that reacted positively to the Syphilis EIA II (TA) test and were later confirmed by TPHA and RPR tests were collected from the Blood-Bank database and analysed. The accuracy indices of RPR and TPHA analysis were evaluated, been Syphilis EIA II (TA) used as a reference test for confirmation of RPR and TPHA results. True positives and false negatives results for both tests were determined and sensitivity was also calculated. Since it was not possible to confirm the negative results of Syphilis EIA II (TA) test, the values considered for specificity were obtained from previous studies and by the manufacture's information. *Results.* Of the 87 samples valid for this study, 28 (32.2%) were positive for the RPR test (true positives), while 59 (67.8%) were negative (false negatives). For the TPHA test, 67 (77%) samples were positive (true positive) and 20 (23%) were negative (false negatives). Therefore, the sensitivity of RPR and TPHA tests using Syphilis EIA II as reference test was 32.2% and 77%, respectively. *Summary and Conclusions.* TPHA presented higher sensitivity than RPR and, take into account the specificity values indicated by literature for RPR and TPHA, which ranging between 88.8-98.8%, and 99.6-100%, respectively, it can be concluded that TPHA is the most effective confirmatory test. A new T. pallidum clinical practice guideline should be implemented in our Blood-Bank, using the Syphilis EIA II (TA) test as a screening test and the TPHA test as a confirmatory one. The reduction to only one confirmatory test will have evident economic benefits for the Blood-Bank and will be time saving.

1222

POSITIVE DIRECT ANTIGLOBULIN TEST (DAT) IN NEWBORNS: 3 YEARS EXPERIENCE

S. Kouvardas, H. Kafkoula, K. Mitrousoudis, S. Chaniotaki, M. Xymitiri, A. Xanthaki

Thriasio Hospital, MAGOULA, ELEFSINA, Greece

Background. Haemolytic disease of the newborn is the result of IgG antibodies from the maternal circulation across the placenta into the circulation of the fetus where they react with fetal red cells and lead to their destruction by the fetal reticuloendothelial system. *Aims.* To identify the present antibodies in newborns, after a positive antiglobulin test (DAT). *Materials and Methods.* During a 3 years period, from 01/01/2005 till 31/12/2007, we studied retrospectively 2320 newborns and their mothers. Each newborn was examined for ABO group, Rhesus with phenotype, Kell and DAT. Each mother was also tested for ABO group, Rhesus with phenotype, Kell and indirect antiglobulin test (IAT). After each positive (DAT), we performed the eluate test in order to identify the present underlying antibody. For the laboratorial analysis of newborns the sample used was cord blood and in some cases venous blood. *Results.* At the particular time interval the (DAT) was positive in 87 (3.75%) of newborns and the antibodies found were IgG type immunoglobulin. Anti-A antibody was detected in 58 (66.6%) (newborns of group A with mothers of group O), anti-B antibody in 18 (20.6%) (newborns of group B with mothers of group O), anti-D antibody in one newborn (1.1%) (newborns D+ from D- mothers), anti-C in anotherone (1.1%). The eluates were found negative in 6 (6.8%) newborns and in the remaining 3 (3.4%) no special antibody could be identified. *Conclusions.* We observe that the majority of antibodies in newborns with a positive DAT belong in ABO blood group system and are due to ABO incompatibility (the mother belongs to group O and the newborn to group A or B).

1223

PLATELET GEL FOR HEALING SINUS PILONIDALIS IN PATIENT WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN CHEMOTHERAPY

A. Sau, G. La Barba, S. Pulini, A. Patriarca, A. Recchia, L. D'Arcangelo, A. Spadano, G Fioritoni

Spirito Santo Hospital, PESCARA, Italy

Background. Topical use of platelet gel (PG) is a relatively new technology which may stimulate and accelerate soft-tissue and bone healing; it offers opportunities for treatment of wounds, ulcers, soft-tissues injuries and various other applications in regenerative medicine. The rational to employ this technique is to mimic physiological wound healing and reparative processes. Tissue repair begins with clot formation and platelet degranulation, which release the growth factors (GFs) necessary for wound repair (PDGF, TGF-beta, IGF-I, IGF-II, EGF, FGF-beta). Platelet-derived GFs are biologically active substances that enhance tissue repair mechanisms such as chemotaxis, cell proliferation, angiogenesis extracellular matrix deposition and remodeling. *Aims.* Effectiveness of local homologous PG for healing sinus pilonidalis as a non-invasive method alternative to surgery. *Methods.* A nine year old young female undergoing chemotherapy for acute lymphoblastic leukemia relapsed after hemopoietic stem cell transplantation developed two septic episodes due to Gram-negative bacteria (multiple antibiotic-resistant Escherichia Coli) during the neutropenic period likely from sinus pilonidalis. The sinus pilonidalis was firstly sterilized with topical disinfection and systemic antibiotics. The therapeutic protocol consisted of the once-weekly local application of homologous PG for 4 weeks as follows: 1st day local PG application; 4th day removal a PG and thereafter daily medication with idrogel. *Results.* The pain reduced after the first PG application and sinus pilonidalis completely resolved (Figure 1) after 4 weeks. The patient continued chemotherapy without local and/or systemic bacterial infections. *Summary and Conclusions.* The local application of PG may represent a valuable alternative to surgery. Moreover, the rapid healing achieved with local application of PG allows a reduction in the risk of secondary infection, pain, and need for hospitalization, as well as in the improvement of patient quality of life.

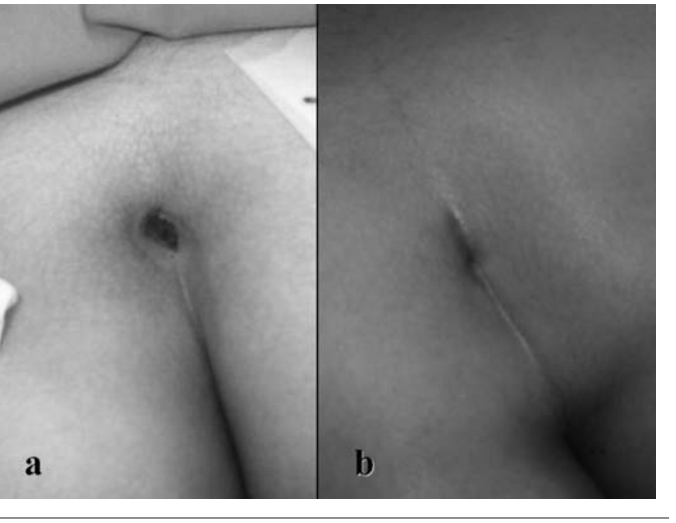

Figure 1. a) at beginning b) after 4 weeks.

1224

SUPPORTIVE CARE OF OUTPATIENTS USING RED BLOOD CELL AND PLATELETS TRANSFUSIONS: SAFETY OF LONG TIME TRANSFUSIONS IN TIMES OF LEUKOCYTE DEPLETION

B. Höchsmann, S. Runzheimer, N. Beckmann, S. Platow, M. Wiesneth, H. Schrezenmeier

Institute for Clinical Transfusion Medicine and Immunogenetics Ulm, ULM, Germany

Background. Long term substitution of blood products becomes more important over the last years. This may be caused by demographic development, increased therapy options in malignant disease or a new restraint in the use of hematopoietic growth factors because of the recently reported side effects. In this context the option of outpatient transfusions is gaining influence to enable an increase in quality of life and cost efficiency. Because of operational availability of blood products and necessary laboratory work, transfusion medicine is predestined for time efficient outpatient transfusions. *Aims.* This study was undertaken to reassess safety and efficiency of leukocyte depleted blood products. *Methods.* Analysis of outpatient contacts, patient characteristics, number and kind of transfused units of red blood cell concentrates (RBC) and platelete concentrates (PC). Ferritin-level, antibodies and CMV-/HBV-/HCV-/HIV -status were checked at least every 6 months during the transfusion therapy. In all cases transfusion efficiency was proofed by blood count and vital parameters as well as by questions for symptoms of anemia and bleeding signs addressed to the patients. *Results.* The criterion for inclusion in follow up analysis - availability of at least two assessments- was met by 199 patients with following patient characteristics: 114 male, 85 female; median age 67 years (minimum: 17 years, maximum: 96 years). Underlying diseases were: hematopoietic disease (n= 124), solid tumours (n= 50), iron deficiency anemia (n=13), renal anemia (n= 2) and other (n=10). 36 patients had stem cell transplantation (SCT), 17 of them had autologous SCT, 16 allogenic SCT and 3 both. 102 patients were CMV-IgG-negative, 179 patients were HBV-negative, 194 patients were HCV-negative and 199 patients HIV-negative at time of first outpatient transfusion. In three patients CMV-seroconversion was observed after a median of 22 RBC and 48 PC. A minimum duration of 12 months transfusion therapy was existent at 55 patients for RBC (median duration: 18 months, maximum duration: 113 months) and at 23 patients for PC (median duration: 19 months, maximum duration 66 months). These patients got a median of 24 RBC (minimum: 4, maximum: 210) and respectively a median of 52 pool-PC (minimum: 2, maximum: 328) and a median of 8 apharesis PC (minimum: 1, maximum: 175). Transfusion reactions were observed two times after RBCs and 7 times after PC. With few exceptions all transfusion reactions could be managed without hospitalisation. During the observation period in five patients allo-antibodies and in three patients HLA-AK were newly diagnosed. 7 patients showed signs of haemosiderosis after long time RBC transfusion therapy. *Summary and Conclusions.* Our data show that outpatient transfusion therapy is safe and efficient even in long term transfusion. Further analysis should elucidate if the observed antibody development is more likely caused by transfusion therapy or the underlying disease. On account to escalation of ambulant supply with blood products increase of quality of life and cost efficiency could be achieved for the individual patient, especially in palliative care situations.

1225

ERYTHROCYTE SEDIMENTATION RATE MEASUREMENT BY TEST-1 REFLECTS INFLAMMATION BETTER THAN THOSE BY WESTERGREN METHOD IN PATIENTS WITH MALIGNANCY, AUTOIMMUNE DISEASE AND INFECTION

C.H. Cha,[1] C.J. Park,[2] Y.J. Cha,[3] H.K. Kim,[4] D.H. Kim,[2] H. Hong,[2] J.H. Bae,[3] J.S. Jung,[4] H.S. Chi,[2] D.S. Lee,[4] H.I. Cho[4]

[1]Asan Medical Center, SEOUL; [2]University of Ulsan College of Medicine and Asan Medical Center, SEOUL; [3]College of Medicine, Chung-Ang University, SEOUL; [4]Seoul National University College of Medicine, SEOUL, South-Korea

Background. Erythrocyte sedimentation rate (ESR) is widely used as screening or monitoring test for acute or chronic inflammatory diseases. Westergren method has been proposed by International Council for Standardization in Haematology (ICSH) as the reference method for ESR measurement. This method requires 60 minutes for ESR measurement and has been performed without quality control. Recently, an automated ESR measurement instrument, TEST-1 has been marketed. This system performs ESR measurement using small amount of blood sample (150 uL) by a micro-agglutination method measuring aggregation capacity of red blood cells, interacting with inflammatory plasma proteins, reducing markedly the analytical time to 20 seconds. More than 5% of TEST-1 ESR values performed at Asan Medical Center showed beyond the limits indicated by ICSH. *Aims.* The aim of the present study is the evaluation of TEST-1 by determining correlations between plasma proteins increasing in inflammatory conditions and TEST-1 ESR values. *Methods.* Subjects were selected from the hospitalized and ambulatory patients of Asan Medical Center. From October 2007 to November 2007 among patients with malignancy, autoimmune diseases or infection, 154 EDTA blood samples (malignancy 69, autoimmune disease 44 and infection 41) that had TEST-1 ESR values ≥20 mm/hr and hematocrit values between 33% and 35% were selected and measured by Westergren method. In each plasma specimen, total protein, albumin and CRP was further measured at Seoul National University Hospital. The fractions of albumin, alpha1-, alpha2-, beta1-, beta2- and gamma-globulin were measured by capillary electrophoresis of plasma protein at Seoul National University Hospital. We determined whether TEST-1 ESR values are within ICSH limits and compared the correlations of Westergren and TEST-1 ESR values with inflammatory plasma proteins each. *Results.* Seventy percent (107/154) of TEST-1 ESR values was lower than Westergren ESR values and 54% (80/149) of TEST-1 ESR values fell within the ICSH limits. For five samples, Westergren ESR values were more than 105 mm, thus determination was not possible whether the TEST-1 ESR values is within the limits. For total samples (n=154) and those of TEST-1 ESR values within the ICSH limits (n=80), linear regression analysis showed a significant correlation between CRP, alpha1-, alpha2- and beta2-globulin and Westergren method, respectively and so did TEST-1 method. TEST-1 method showed higher square of the correlation coefficient (r2) than Westergren method, respectively. For samples of TEST-1 ESR values beyond the ICSH limits (n=69), there was a significant correlation between alpha1-, alpha2- and beta2-globulin and Westergren method and between CRP, alpha1-, alpha2-, beta2- and gamma-globulin and TEST-1 method, respectively. The square of the correlation coefficient (r2) in TEST-1 method was stronger than that in Westergren method. Analyzing according to the diagnosis of patients, TEST-1 method showed higher square of the correlation coefficient (r2) than Westergren method. Particularly, only TEST-1 method was significantly correlated with alpha2- and beta1-globulin in samples of patients with autoimmune diseases and alpha1-globulin in those with infection. *Conclusions.* The correlation of TEST-1 ESR values with plasma proteins increasing in inflammatory conditions was better than that of Westergren ESR values. It is concluded that ESR measurement by TEST-1 reflects inflammation better than that by Westergren method in patients with malignancy, autoimmune disease and infection.

1226

HEAMATOLOGICAL EVALUATION OF VISCERAL LEISHMANIASIS IN YEMENI CHILDREN

G. Al-Alawi, A. Gobah

University of Aden, ADEN, Yemen

Background. In south-east of Yemen visceral leishmaniasis (VL) is endemic in Lahj and Abyan and also in some Northern Areas ; the areas which lack adequate diagnostic facilities. The aim of this study describes the clinical and hematological features in 64 cases of childhood VL. *Methods.* All the children below 12 years of age who were managed as indoor cases from 1st Jan to 31st Dec 2005 were included in this study. The diagnosis of VL was established by demonstration of leishmania parasites in bone marrow aspiration. The demographic information, physical signs at presentations and results of complete blood count were recorded. *Results.* Mean age of the patients was 30 months. 33 were female and 31 were male. Fever was seen in 100% of children with duration before diagnosis was 56 days. Splenomegaly was present in all cases and hepatomegaly in 84.4% with mean enlargement of spleen and liver 9.3 and 3.5 cm respectively. Mean haemoglobin level. WBC and platelet counts were 6.6 g/dL, 358×10^9/l and 71.7×10^9/L respectively. Absolute neutrophil count was $<0.78\times10^9$/L. Mean reticulocyte count was 1.7%. Hypercellular bone marrow in 68.8%. There is positive relationship between parasite load and splenomegaly.and there are reverse relation between parasitemia with hemophagocytosis and frank dyserythropoiesis in bone marrow . *Conclusions.* Hypersplenism , hemophagocytosis, and other changes seen in bone marrow considered the causation of hematological changes in visceral leishmaniasis in children.

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RISK FACTORS FOR FEVER AND INFECTIONS IN NEUTROPENIC PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES

D. Sotiropoulos, A. Syrigou, N. Neokleous, A. Karpouza, A. Bitzioni, E. Tsorlini, A. Anagnostopoulos

G Papanikolaou Hospital, THESSALONIKI, Greece

The infections constitute the most serious problem of patients with haematological malignancies been in aplasia after chemotherapy or preparative conditioning for stem cell of transplantation. The aim of this study is the relation between the sex, the age, the type of the disease or the duration of the myeloid aplasia with the infections that are detected in neutropenic patients after chemotherapy that received for haematological diseases. 147 patients (65 women and 82 men) were studied in 250 cases of myeloid aplasia. In 89 patients the diagnosis was acute lymphoblastic or myeloblastic leukaemia, in 51 non Hodgkin or Hodgkin lymphomas and in 7 multiple myeloma. The treatment they received was accordingly with the protocols of our centre depending on the phase of the disease. The majority of the patients received antifungal prophylaxis and antibiotic therapy for fever or positive culture before the neutropenia period. We performed the following workup of fever: at least three different cultures of blood samples (aerobic, anaerobic, and fungal), cultures of urine and sputum samples per regular intervals and on clinical symptoms special examinations as culture of bronchio-alveolar lavage samples as well as imaging examinations. The t-test was used for the statistical analysis. There was no significant difference between sex and age groups concerning fever and infections. The patients with leukaemia had a higher percentage of fever episodes compared with those who had lymphoma (81% *vs* 60%, $p<0,05$). Gram (+) bacteria were isolated in 45% and 11% of the febrile patients with leukaemia and lymphoma, respectively ($p<0,05$). The longer the duration of the myeloid aplasia, the higher was the incidence of fever, without any significant difference in positive cultures. Remarkable is the fact that from the 72 cases of afebrile patients, 20 (27,7%) had positive cultures for microbes and fungi. The probability of fever in patients that had positive culture is the same compared with the patients with negative cultures. All the positive cultures became negative by administering combined antibiotic therapy based on the antibiogram. 20 patients died from refractory disease (16) or sepsis (4). In conclusion, the sex and the age were not risk factors for the appearance of fever. A risk factor for fever and positive cultures for gram (+) bacteria was the type of the disease. The duration of neutropenia correlated with the positive cultures. Moreover the episodes of fever were not correlated with the fever and the presence of positive cultures in the neutropenic patients. The conclusion, from such type study, could help in the more effective management of infections.

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SICKLE CELL ANEMIA PREVENTION IN NORTHERN ISRAEL

A. Koren,[1] L. Zalman,[1] H. Palmor,[1] R. Bril Zamir,[1] Y. Schneour,[1] C. Levin,[1] D. Filon,[2] A. Openheim,[2] S. Shalev[1]

[1]Ha'Emek Medical Center, AFULA; [2]Hematology Dpt - Hadassah Madical Center, JERUSALEM, Israel

Sickle Cell Anemia (SCA) is a hemolytic anemia caused by a single mutation in position 6 of the β globin molecule. The clinical picture of SCA included vaso-occlusive crises, acute chest syndrome, CNS infarcts, hemolytic and aplastic crises, avascular necrosis of hip, splenic sequestration and others. SCA is autosomal recessive transmitted and beside the homozygous SS form, the combination with other abnormal hemoglobin's like β^+ or β^0 thalassemia, Hgb C or D can cause similar clinical presentation. In Northern Israel about 80 patients with SCA are treated in the hematology units, half of them having Sickle Cell Anemia and the others Sickle Cell β^+ or β^0 thalassemia. Since 1987, a pregnant women screening for β thalassemia is carried on in northern Israel, areas were malaria was present in the beginning of the 20th century. The SCA gene is found mostly among tribes of Bedouin origin. Originally the program design was based in screening red blood call indexes and Hgb electrophoresis performed only in those samples suspected to be β thalassemia carriers. Since 1999 with the introduction of the Variant Hgb Testing HPLC analysis (Biorad, USA), the program design was changed. All the samples were now routinely tested for the detection of abnormal hemoglobin including Hgb S, Hgb C, Hgb D, Hgb O Arab and others. The total number of pregnant women screened in the twenty years since 1987 is 69340. In the period from 1987 till 1998, 51 couples who carried Hgb S were detected (mean 4.25 couples/yr), from 1999, concomitant to the screening program change, till 2006 a total of 63 couples were detected (mean 7.8 couples/yr). A total of 492 prenatal diagnoses for hemoglobinopathies were performed, including 187 in couples at risk for having a offspring with Hgb S. The mean gestational age at the first genetic consultation was 13±4 wks. Only thirteen of those prenatal diagnoses were performed in six women that were referred because a previous affected child. Fifty four of those diagnoses revealed affected fetuses and in four the couple decided not to perform therapeutic abortion and affected babies subsequently born. The economic burden to the health services for giving hospital treatment to SCA patients is high, about £ 5000 per year, and the institution of prevention programs is proven to be cost effective in populations with a high frequency of carriers. Since our program is directed to detect also β thalassemia, a disease that is more frequent in the area covered by the program (>2.5%), the added cost for the prevention of SCA is less significant in spite a low incidence of the S gene in our population which is less than 1%, an incidence that is not considered cost effective for institution of a prevention program.

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COMPARISONS OF REGIMEN COSTS OF BORTEZOMIB AND LENALIDOMIDE FOR TREATMENT OF ADVANCED, RELAPSED MULTIPLE MYELOMA

J. Hornberger,[1] J. Rickert,[1] R. Dhawan,[2] D. Baldesano[2]

[1]Cedar Associates LLC, MENLO PARK; [2]JJPS, RARITAN, USA

Background. Lenalidomide and bortezomib are approved in Europe for treatment of patients with advanced, relapsed multiple myeloma who have received at least one prior therapy. *Aims.* We examined the comparative costs, from a European payor perspective, for a course of therapy in patients with multiple myeloma who have received at least one prior therapy. *Methods.* Data on clinical regimens - including number of administrations, cumulative drug dosages, and incidence of serious adverse events - were obtained from published reports of pivotal clinical trials and detailed summaries on regulatory web resources). Lenalidomide is administered 25 mg orally on d 1-21 of 28 d cycles, in combination with high-dose dexamethasone (HDD), for an average of 44 weeks per course (www.emea.europa.eu/humandocs/PDFs/EPAR/ revlimid/H-717-PI-en.pdf). Bortezomib is administered as 1.3 mg/m^2 on d 1, 4, 8, and 11 of 21 d cycles, for an average of 6.25 cycles per course. Unit costs were obtained from the UK's NHS Trust Reference Cost Schedules (www.dh.gov.uk) and were converted to Euros using prevailing currency exchange rates. *Summary and Conclusions.* Based on published manufacturers' submissions to regulatory agencies on findings of carefully controlled clinical trials, the cost of bortezomib is lower than the cost of lenalidomide. Because of the longer duration of exposure associated with lenalidomide the cost per regimen for bortezomib is approximately one-third the cost of lenalidomide. In those regions where affordability of health care is a highly relevant consideration upon which to recommend uptake of new interventions, such cost analyses provide useful information. Additional information also is needed, however, on the comparative effectiveness of these regimens.

Table 1. Cumulative regimen costs (2007 Euros).

	Lenalidomide + HDD	Bortezomib
Drug	€ 70,364	€ 19,583
Administration	€ 0	€ 4,191
Adverse events	€ 2,038	€ 1,727
Total	€ 72,402	€ 25,501

HDD - high-dose dexamethasone.

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IMMUNE FACTORS ROLE IN HEMOPOIETIC MALIGNANCIES ORIGIN IN PERSONS EXPOSED TO RADIATION AFTER THE CHERNOBYL NPP ACCIDENT

S. Dyagil, Zh.N. Minchenko, V.G. Bebeshko, D.A. Afanasiev

Research Center foe Radiation Medicine, KIEV, Ukraine

Background. Risks of radiation-induced hemopoietic malignancies in remote terms after the Chernobyl NPP accident among the exposed subjects can persist under the undefined yet regularities and dependencies. We suggest here possible role of immune issues in radiation-induced hemopoietic malignancies origin as prognostic marker of genetic predisposition, where HLA-A10; HLA-A28; HLA-B16; HLA-B38; HLA-B35; HLA-DR4 are attributed. Subjects and *Methods.* Immune markers of

radiosensitivity and radioresistance both with ones associated with hemopoietic malignancies were evaluated within acute radiation sickness (ARS) model in human. Twenty-seven ARS grade I-III convalescents exposed to radiation doses of 1-3 Gy were involved along with 30 persons with radiation doses under 1 Gy. *Results*. Immunogenetic survey of patients exposed to less than 1 Gy radiation doses and having hemopoietic disorders revealed in 45% of cases the isolated antigens, their combinations, and separate alleles associated with hematological malignancies risk (HLA-A02; HLA-'35; HLA-'5; HLA-A1; HLA-11) and immune deficiency (HLA-A24; HLA-A9; HLA-'7). Therefore similar associated regularities are surveyed in relationship between immunogenetic component and immuno- and hemopoietic disorders in exposed persons regardless of radiation dose. The mentioned above indicates to a definite risk of hemopoietic malignancies in future being basic for attributing these persons to risk group of hemopoietic malignancies. Presence of HLA-A24; HLA-A02; HLA-A11; HLA-B18; HLA- B35; HLA- B51; HLA-Cw3; DRB111 in HLA-henotype and of DQÄ10101, DQB10501 haplotype are found being the genetic predisposition factors for acute leukemia. At that some alleles, specifically Cw3; HLA- B35, are the biologic markers of increased radiosnsitivity and immunogenetic risk factor of changes in some hemopietic components and of bone marrow syndrome under hemopoietic malignancies due to radiation exposure. Myelodysplastic syndrome has developed in 3 of 27 ARS convalescents and AML - in 1 of them 7-14 years after the exposure. Table 1 shows combinations of radiosensitivity antigens and antigens linked to hemopoietic malignancies origin.

Table 1. HLA-genotype characterization of ARS-convalescents

#	Disease	Antigen A		Antigen ə		Antigen C	
1.	MDS	3*	10**	13	15	4**	-
2.	AML	3*	11*	17	38**	3	-
3.	MDS	10**	11*	8	17	4**	-
4.	MDS	3*	10**	16**	21	3	-

* Antigen responsible for hemopoietic malignancies origin; **Radiosensitivity antigen.

Conclusions. In general the data received suggest that HLA-genetic predisposition is one of hemopoietic malignancy pathways origin being causally linked to radiation exposure. It is actual both at hemopoietic abnormalities and entire pathology levels where carrying the phenotype/genotype radiosensitivity markers increase pathology onset risk. Analysis of isolated antigen and haplotype combinations of immunogenetic HLA-factors carrying in ARS convalescents having hematological malignancies diagnosed 7-9-12 years after the exposure suggest that in all cases the increases radiosensitivity markers are identified in patients phenotypes along with disease-associated antigens.

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EXPRESSION OF STEMNESS MARKERS IN MESENCHYMAL STROMAL CELLS FROM BONE MARROW OF CHILDREN AND ADULT DONORS

M. Choumerianou, H. Dimitriou, M. Martimianaki, E. Stiakaki, M. Kalmanti

University of Crete, HERAKLION, Greece

Background. Bone Marrow mesenchymal stromal cells (MSC) have the ability to differentiate towards multiple lineages of mesenchymal origin. The cells have already been therapeutically applied in various clinical conditions because of their expansion and differentiation capabilities. It has been reported that progression of passages (P) and/or increase of donor age have negative impact on MSC proliferative and differentiation capacities. Pluripotency has been associated with the expression of transcription factors such as Oct-4, expressed in totipotent embryonic stem cell and germ cells. Additionally, Nanog is not only essential for pluripotency and self-renewal of embryonic stem cells, but is also expressed in somatic stem cells that have increased expansion and differentiation potential. *Aims.* To study the expression of Oct-4 and Nanog in early and advanced passages in MSC from children and adults, and assess any correlation with the proliferative properties of the cells. *Methods.* MSC were isolated from bone marrow of children with idiopathic thrombocytopenic purpura (n=7) and autoimmune neutropenia (n=6) and adult donors (n=3). Cells were expanded *in vitro* upto P6. RNA was isolated from each sample and 500ng were used for cDNA synthesis. The expression of Oct-4 transcript was measured with Real Time RT-PCR and Nanog mRNA levels with semi quantitative RT-PCR using GAPDH as normaliser. The doubling time (DT) was calculated and the small-, medium- and large-sized CFU-F colonies were evaluated after Giemsa staining. Statistical analysis was performed with the use of SPSS software. *Results.* The expression of Oct-4 in P2-MSC from children was 0.95 fold higher compared to the respective adult. A minimal difference of 0.06 fold increase was observed in Oct-4 expression when P2-MSC were compared to P6-MSC. The expression of Nanog was not statistically different either between children and adult MSC or between P2-MSC compared to P6-MSC. Neither Oct-4 nor Nanog expression was associated with donor age. Although none of the two transcription factors were associated with the doubling time or the CFU-F capacity of the cells, the doubling time values were lower in children compared to adult MSCs (3.47±1.29 *vs* 4.99±1.10, p=0.057) and respectively higher medium (16.58±4.33 *vs* 4.50±1.64, p-0.024), large (4.25±1.12 *vs* 0.17±0.16, p=0.013) and total (26.75±5.11 *vs* 10.67±7.09, p=0.04) CFU-F count was observed in children P2-MSC. *Summary and Conclusions.* MSC from bone marrow of children and adults, expanded *in vitro*, expressed Oct-4 and Nanog at levels not significantly different. The expression of the two factors was not altered with the progression to P6. The differentiation capacity of adult MSC expressing Nanog should be studied along with genes that may be regulated by its expression. Oct-4 is not correlated to proliferative capacity in our samples and is expressed in adult MSC, therefore its presence solely may not render a cell pluripotent. Its localisation within the cell may play a critical role for its function and it is necessary to define the difference between the two splice variants (OCT4A and OCT4B) and their role.

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THE EFFECT OF CD4$^+$CD25$^+$ REGULATORY T CELLS ON DENDRITIC CELLS

L.S. Zhang, L.J. Li

The Second Hospital of Lanzhou University, LANZHOU, China

Background. CD4$^+$CD25$^+$regulatory T cells(Treg) has been identified recently as a new type of immuno-suppression cells. CD4$^+$CD25$^+$regulatory T cells, are characterized immuno-anergy and immuno-suppression. Their ability is to actively inhibit CD4$^+$CD25$^-$T cells, CD8$^+$ T cells, dendritic cells (DCs), natural killer (NK) cells, natural killer T (NKT) cells, in a cell-to-cell contact manner, and prevent activated CD8$^+$ T and NK from proliferating, and down-regulate costimulators expressed on DC. In short, CD4$^+$CD25$^+$ regulatory T cell play an important function in maintaining immune homeostasis, suppressing all kind of nature and adoptive immune response. The prevalence of Treg in the peripheral blood of many cancer patients is increased when compared with normal individuals. In humans, initial studies have targeted the entire CD4$^+$CD25$^+$ circulating T cell population representing, as in mice, 5-10% of total CD4$^+$ T cells. Dendritic cells (DCs) are the most potent professional antigen presenting cells (APCs) *in vivo*. The mechanism of DC anti-tumor is DC can present tumor antigen peptides to T cells through the high expression MHC-I,II molecules, which can active T cells when associated with the high costimulation molecule expressed on DC surface such as B7-1(CD80),B7-2(CD86) and CD40. *In vitro*, Treg can suppress the function of DC, by the costimulation signals CTLA-4-B7. *Aims*. Does Treg increase in the cancer patients and suppress DC through CTLA-4 way? In order to demonstrate the deduction drawn above, this experiment observed what would happen when co-cultured the Treg and DCs derived from CML patients or normal donors. *Methods.*CD4$^+$CD25$^+$regulatory T cell were separated from peripheral blood of CML patients and normal donors by magnetic cell sorting(MACS) system. Bone marrow mononuclear cells (BMMNCs) derived from CML patients and healthy person were separately induced to DC. Two test groups (CML-Treg /CML-DCs, CML-Treg /normal-DCs) and two control groups(normal-Treg /normal-DCs, normal-Treg /CML-DCs as control)ware designed. The cell phenotypes of Treg and DC were analyzed by flow cytometry, supernate of IL-10 and TGF-β were analyzed by ELISA. *Results*. The purity of sorted CD4$^+$CD25$^+$regulatory T cell was 85~94% with the survival rate of 92~95%. CTLA-4 expression rate on Treg from CML patient and normal donor peripheral blood were 52.4% and 33.2% respectively. The costimulator phenotypes of DC had significant difference between before and after co-culture with Treg(p<0.05). The supernate of IL-10 and TGF-β shown the high level on CML-Treg / CML-DCs group than other three groups (p<0.05). *Conclusions.* We could successfully obtain Treg from peripher-

al blood and DC from bone marrow mononuclear cells. After co-cultured , the costimulators CD80 and CD86 expressed on DC were down-regulated, the cytokines Treg secreted were up-regulated. We concluded that Treg can suppress the function of DC derived from CML,the exact mechanism needs further study.

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DENDRITIC CELLS DERIVED FROM CHRONIC MYELOID LEUKEMIA INDUCED BY LOW DOSE CYTOSINE ARABINOSIDE COMBINED WITH INTERFERON-?

L.S. Zhang, F.X. Song

The Second Hospital of Lanzhou University, LANZHOU, China

Background. Interferon-alpha(IFN-α),an important immunoregulatory cytokine, has been widely used for immunoregulator in chronic myeloid leukemia (CML) since 1980s. Moreover, the patients that achieve above partial cytogenetic response after IFN-α treatment, present obviously more survival superiority than the patients whose IFN-α treatment is ineffective. *Clinical data discovered.* Low dose cytosine arabinoside LD-Ara-C?combined with IFN-α is superior to IFN-α alone, which presents striking cytogenetic remission. One of the mechanisms of IFN-α therapy for CML is possibly related to dendritic cells. DCs, specialized antigen-presenting cells(APCs),play a pivotal role in activating initial T cells,and maintaining cell immune responses. However, LD-Ara-C plays a role in the differentiation of acute myeloid leukemia(AML). Does the superiority of LD-Ara-C combined with IFN-α to CML related to induction of more mature DCs, and then further strengthen the function of DCs? Up to now, no research is available. *Aims.* This study aimed to observe whether the DCs derived from CML were more functional which induced by LD-Ara-C combined with IFN-α *in vitro*, and provide theoretical foundation for clinical therapy. *Methods.*Bone marrow mononuclear cells(BMMNCs) were obtained from bone marrow of CML patients by Ficoll-Paque density gradient centrifugation, and induced with granulocyte-macrophage colony-stimulating factor (GM-CSF),interleukin-4(IL-4),and plus Ara-C and/or IFN-α . Test group was divided into four groups according to the doses of Ara-C(5 ng/mL 10 ng/mL 25 ng/mL 50 ng/mL),Control group just give GM-CSF,IL-4 and IFN-α. The phenotypes (CD1a,CD54,CD83,CD86, HLA-DR) were assayed by flow cytometry (FCM). The mixed lymphocyte reaction(MLR) was evaluated by MTT assay. *Results*. After inducements, BMMNCs showed typical dendritic projections, some of them displayed eccentric and bilobed nuclei with Wright-Giemsa(R-G)staining. The DCs cultured in 5ng/mL group and 10 ng/mL group showed significantly higher levers of CD1a, CD54, CD83, CD86, HLA-DR expression than the other three groups, which had significant difference (p<0.05). 10ng/ml group displayed the highest phenotypes ,which had significant difference than 5ng/ml group (p<0.05). Large part of cells in the 25ng/ml group and all cells in the 50 ng/mlL roup died. MLR was coincide with phenotypes expression of DCs, that is 10 ng/mL group > 5 ng/mL group > IFN-α group > 25 ng/mL group > 50 ng/mL group, which had significant difference (p<0.05). *Conclusions.* The BMMNCs of CML cultured in the presence of LD-Ara-C and IFN-α can be induced into DCs which is superior to IFN-α alone, with morphologic and immunophenotypic characteristics, overexpressed major histocompatibility complex (MHC) molecules, co-stimulatory molecules, and adhesion molecules, and have enhancing MLR. The possible mechanism of LD-Ara-C combined with IFN-α therapy for CML may be related to DCs.

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THE IMMUNOLOGICAL EFFECT OF PD-L1 BLOCKADE ON DENDRITIC CELLS DERIVED FROM CHRONIC MYELOID LEUKEMIA

L.S. Zhang, L.J. Li

The Second Hospital of Lanzhou University, LANZHOU, China

Background Dendritic cells (DCs) are the most powerful professional antigen-presenting cells (APCs) *in vivo* and play a crucial central role in the outcome of several immune responses. DCs have been successfully used in clinical trails to induce tumor-specific immunity. Although the DCs derived from leukemia patients have the similar morphology and immunophenotype with DCs from normal individuals, the immunological function are weaker. Programmed death-1 ligand-1(PD-L1) is a recently identified member of the B7 family molecules and is shown to mediate the inhibition of immune responses. *Aims.* In this study, we have show that the PD-L1-blockade DCs originated from chronic myelocytic leukemia (CML) patients have a more potent ability. *Methods.*Bone marrow mononuclear cells (BMMNCs) were isolated from CML patients by density gradient centrifugation and were cultured in RPMI-1640 culture medium supplemented with recombinant human (rh) GM-CSF and rhIL-4. Then the immature DCs were cultured with or without TNF-α and PD-L1 monoclonal antibodies (mAb). The cells were harvested after 24h,then the morphologic features were observed and the phenotypes were analyzed by flow cytometry, IL-12 concentration were detected by ELISA kits, Mixed lymphocyte reaction were analyzed by MTT assay. *Results*. It was demonstrated that DCs derived from CML-BMMNCs showed the typical morphology. The immunophenotype expressed on DCs, such as CD80, CD86, CD83, HLA-DR, CD1a, were obviously higher after matured and do not be influenced by the PD-L1 mAb. The enhanced T cells proliferation and increased IL-12 production were observed in PD-L1-blockade of mature and immature DCs by PD-L1 mAb(p<0.05). *Conclusions.* This study indicates that the expression of PD-L1 on DCs derived from CML could be upregulated in the presence of GM-CSF, IL-4 and TNF-α. The immunophenotype expressed on DCs and the maturation could not be influenced by the PD-L1 mAb. Not only mature DCs but also immature DCs derived from CML have a more potent ability after using mAb blockade. This maybe provide a new protocol to achieve anti-tumor immunity by DC-based vaccination.

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HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS ASSOCIATED WITH HEMATOLOGIC MALIGNANCIES IN PEDIATRIC AGE GROUP IS AN INDICATIVE OF POOR PROGNOSIS

S. Unal, M. Cetin, F. Gumruk, A. Gurgey

Hacettepe University, Division of Pediatric Hematology, ANKARA, Turkey

*Background.*The literature data on the association of hemophagoytic lymphohistiocytosis (HLH) with malignancies has increased gradually in recent years. The malignancy-associated HLH is usually associated with T-cell lymphoma, natural killer cell leukemia/lymphoma, Hodgkin disease and gastric carcinoma. *Aims.* The primary and secondary HLH cases are quite common in Turkey. The aim of the study is evaluate the clinical characteristics and outcome of patients who had HLH concomitant to a hematologic malignancy or who developed HLH subsequent to the malignancy following initiation of treatment. *Methods.*Between January 1999 and December 2007, 305 patients with hematologic malignancy were diagnosed in Hacettepe University, Division of Pediatric Hematology. Of these children, seven (2.3%) were diagnosed to have associated HLH (acute lymphoblastic leukemia in three, juvenile myelomonocytic leukemia (JMML) in two, T-cell lymphoblastic lymphoma followed by AML-M4 in one, AML-M6 in one). *Results*. One of the patients with JMML had underlying neurofibromatosis-I. The mean age of diagnosis was 7.1 years (3-13) and four were male. Four of the patients had hemophagocytosis concomitant to diagnosis of malignancy. In the rest of the patients, HLH was diagnosed after initiation of treatment, during the course of the disease (two after remission induction treatment, one after BMT). In two of the patients, the site of hemophagocytosis was cerebrospinal fluid and in the rest of the patients the hemophagocytosis was detected in bone marrow. Two patients had associated pulmonary or cerebral fungal infection. Mean serum triglyceride was 197 mg/dl (78-379), mean fibrinogen was 208 mg/dL (100-378) and mean serum ferritin was 1468 ng/mL (269-4005). Cytogenetic analyses of the bone marrow was available in three of the patients and revealed trisomy 8; 47,XXX and t(8;13). Additionally, PTPN11 mutation was positive in another patient. Five of the seven patients (71.4%) deceased during the follow-up. Two patients received specifically HLH-2004 protocol after development of HLH, wheras four received treatment for the primary malignancy. One patient deceased very early without the initiation of any treatment. *Summary and Conclusions.* In conclusion, malignancies are among the underlying etiologies of HLH. Hemophagocytosis may develop not only at the diagnosis of the malignancy, but also during the course of treatment. The higher mortality in patients with concomitant malignancy and HLH makes the determination of HLH in malignancy patients crucial. The association of HLH to malignancy in some of the patients may be related to the heterozygous HLH mutations and this needs to be investigated in further studies.

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DASATINIB IN REFRACTORY KIT-POSITIVE ACUTE MYELOID LEUKEMIA

M. Cioch, A. Dmoszynska, M. Wach

Medical University, LUBLIN, Poland

Introduction. The proto-oncogene c-KIT encodes the receptor tyrosine kinase (RTK) KIT, a member of the type III RTK subfamily. KIT is expressed by blasts in approximately 60% to 80% of acute myeloid leukemia (AML). There are some premises that dasatinib, a TK inhibitor inducing hematologic and genetic remissions in BCR/ABL-positive chronic myeloid leukemia and acute lymphoblastic leukemia may be also effective in KIT-positive AML. The aim of this study was to investigate the capability of dasatinib to induce anti-leukemic action in patients (pts) with refractory AML. *Material and Methods.* Four pts (2 females and 2 males; ranged from 23 to 66 years - mean 47.7) with refractory AML after several course of chemotherapy (from 2 to 4 courses - mean 3) were included in the study. There were following types acc. FAB classification: M0-1, M1-1, M2-1, M5-1. All pts belong to intermediate risk group (normal karyotype). KIT expression was assessed by flow cytometry. Treatment consisted of dasatinib at the dose from 100 do 140 mg/day. *Results.* All pts showed a hematologic improvement, with WBC reduction from mean level 64,7 G/L (from 9.0 to150.0) to mean level 4.5 G/L (from 1.5 to 11.0). Three of these pts were not achieved complete remission (CR). They died after 2 to 5.5 month (mean 3.5 months) beacuse of severe infections. In fourth patient, 23 year old woman, after 4 ineffective courses of intensive chemotherapy the reduction of bone marrow blasts from 40% to 12% was achieved. The joining CLAG-Mit (cladribine, cytarabine, mitoxantrone and filgrastim) regimen resulted in CR. After 5 months of CR duration this pt is qualified to MUD alloBMT. Dasatinib was overall well tolerated. The main complication of treatment was thrombocytopenia needed platelet concentrate transfusions. *Conclusions.* Dasatinib has antileukemic activity resulted in significant peripheral blood blasts reduction in refractory KIT-positive AML. It seems that dasatinib in combination with chemotherapy may be more effective. Further clinical trials are warranted to define the role of dasatinib in therapy of AML.

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BONE MARROW TRANSPLANTATION OR INTENSIVE CHEMOTHERAPY AS CONSOLIDATION FOR INTERMEDIATE RISK GROUP PATIENTS WITH AML? TUNISIAN EXPERIENCE

R. Jeddi, H. Benneji, R. Ben Amor, L. Aissaoui, K. Kacem, R. Ben Lakhal, H. Benabid, Z. Belhadjali, B. Meddeb

Aziza Othmana Hospital, TUNIS, Tunisia

The prognosis of AML patients of intermediate risk group remains problematic since a proportion of this risk group can be cured without stem cell transplantation and related mortality. Our protocol for AML (APL excluded) include one induction course of AraC 200 mg/m^2 d1-d7 + IDA 12 mg/m^2 d1-d3.Patients who achieved complete remission (after 1or 2 cycles) are stratified to 3 risk groups according to cytogenetic stratification of MRC AML 10. Low risk group received consolidation with chemotherapy according to MRC AML 10 (ADE, MACE, and MidAC).While intermediate and high risk group aged less than 40 years and with sibling donor underwent allogeneic stem cell transplantation.Autologous PSC transplantation is only performed for intermediate risk group (< or > 40 yr) after successful harvesting PSC following 2nd and or 3rd consolidation course. 83 adults (36 F, 47 M) with AML were treated in our department between Jan 2003 and Jan 2007.Median age was 41 yr (18-55 yr).Gene expression profile was not performed. Karyotype was informative in 74 cases divided to: Low risk 14.4% (11/74), Intermediate risk 75.7%(56/74), and High risk 9.5% (7/74).Complete remission (CR1) after one course was 69%(58/83). Induction mortality rate was 16.9%(14/83) mainly due to infection (42.8%).9 of the 11 patients who did not reach CR after one course received a second course leading to CR2 of 55.5% (5/9). Among 50 patients treated with chemotherapy; 6 received autologous PSC transplantation.13 patients (8 were of intermediate risk) received allogeneic bone marrow transplantation. For the whole group, 4-years relapse free survival (RFS) was 35%.Median overall survival (OS) is 42 months, and 4-years OS was 48.6%. 4-years OS was 72% (no donor) *vs* 28.7% (donor) (p=0.0383). Post induction mortality rate was (69.2%) in Donor *vs* (24.4%) in No Donor group. In our study Treatment Related Mortality (TRM) was very high with allogeneic bone marrow transplantation, and patients without donor (with majority of intermediate risk group) fare better. Recently, expression gene profile analysis (FLT3) was introduced to improve making the decision of allografting patients of intermediate risk group in Tunisia.

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MONITORING OF MRD IN PERIPHERAL BLOOD OF AML PATIENTS BY QUANTITATIVE ESTIMATION OF WT-1 LEVEL

C. Haškovec, J. Polak, J. Markova, J. Maaloufova, J. Schwarz

Inst Hematol Blood Transf, PRAGUE 2 Czech Republic

Background. About one half of acute myeloid leukemia (AML) patients do not have a specific molecular marker for monitoring minimal residual disease (MRD). 2. *Aims.* Therefore new unspecific molecular markers are searched. One of the very promising candidates is a level of Wt-1 gene expression. The Wt-1 gene has been suggested as a possible marker in bone marrow of AML patients. 3. *Methods.* In our study expression of Wt-1 gene was tested in peripheral blood (PB) of AML patients by quantitative RT-PCR. 4. *Results.* The results obtained in 106 patients monitored for median11 months, showed a good correlation of the Wt-1 level with expression of leukemia-specific genes (PML/RARA, AML1/ETO, CBFB/MYH11) and a clinical course of the patients. Increase of the Wt-1 level in PB proceeded the hematological relapse for several weeks in advance. Moreover, the Wt-1 level after consolidation seems to have a prognostic importance for the patients. 5. *Conclusions.* Therefore, quantitative estimation of Wt-1 level in peripheral blood of AML patients is a suitable molecular marker for monitoring minimal residual disease and a possible prognostic factor in AML patients.

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COMBINATION OF NON-PEGYLATED LIPOSOMAL DOXORUBICIN (MYOCET®) WITH FLAG REGIMEN IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

L. Melillo, G. Rossi, M. Dell'Olio, A. Falcone, M. Nobile, G. Sanpaolo, P. Scalzulli, N. Cascavilla

Casa Sollievo della Sofferenza IRCCS, SAN GIOVANNI ROTONDO, Italy

Background. Combination therapy with anthracyclines in patients with acute myeloid leukemia (AML) is attractive, but it may have significant toxicity. Non-pegylated liposomal doxorubicin (Myocet®) has a longer half-life than standard doxorubicin, lower cardiac toxicity, and comparable efficacy. *Aims.* To assess the efficacy and safety of the combination of Myocet® with FLAG in poor prognosis AML. *Methods.* Between September 2006 and January 2008, 14 AML patients were included (mean age 65±14 years; 9 Males/5 Females). Seven patients were older than 65 years at diagnosis, 3 patients had secondary AML (1 post Idiopathic Myelofibrosis, 2 post Myelodysplasia), 4 patients were in first relapse (2 after transplantation). Mean baseline white blood cells and platelets were 17×10^9/L and 51×10^9/L, respectively. Six patients had comorbidity (5 cardiovascular). Planned therapy was one cycle of i.v. FLAG regimen [fludarabine 30 mg/m^2/day for 5 days, cytosine arabinoside 2 g/m^2/day for 5 days, G-CSF 5 µg/kg/day until neutrophils recovery] to which we added i.v. Myocet® (30 mg/m^2 once on day 1). A consolidation cycle with FLAG+Myocet® was performed in patients with complete response (CR) or a significant partial response (PR). *Results.* After the first course CR was obtained in 8/14 patients (57%), 1 patient achieved PR (7%), for an overall response rate (ORR) of 64%. Median survival time was 10 months, 1-year disease free survival (DFS) (with a follow-up range from 35 to 524 days) was 71% and 1-year overall survival (OS) 38%. No treatment-related toxic death (cardiac, infective, hemorrhagic) was observed. Moreover, neutrophils recovery was seen at median of 20 days and platelet recovery at 23 days. Systemic >grade III toxicity occurred in 3 patients (hypokalemia which resulted in cardiac arrest, hemorrhagic colitis, anasarcatic state), 2 of which with previous cardiovascular comorbidity. No case of cardiac failure was observed. There were 4 episodes of sepsis and 5 of fever of unknown origin. Antibiotics were administered for a mean of 18 days. So far, 6 of them underwent the second cycle without showing any additional toxic effect. *Conclusions.* In this poor prognosis AML patient population, the safety profile of the combination of Myocet® with FLAG was acceptable with no toxic death observed. This new combination shows promising efficacy, but longer follow-up in a wider patient population is warranted.

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PROGNOSTIC SIGNIFICANCE OF THE MULTIDRUG RESISTANCE PROTEINS CO-EXPRESSION IN ACUTE MYELOID LEUKEMIA

O.D. Zakharov,[1] E.Yu Rybalkina,[1] A.A. Stavrovskaya,[1] M.A. Volkova,[1] V.A. Lunin,[2] N.V. Zhukov[1]

[1]N.N. Blokhin's Russian Cancer Research Center, MOSCOW; [2]N.P.Botkin's hospital, MOSCOW, Russian Federation

Background. Conventional induction chemotherapy induces complete remission (CR) only in 65-75% of adults with *de novo* acute myeloid leukemia (AML). *Aims.* We investigated the prognostic significance of multidrug resistance proteins (P-glycoprotein (Pgp), BCRP, MRP1 and LRP) expression on AML blast cells with respect to CR achievement and relapse-free survival. *Methods.* We included in the analysis 42 patients (pts) with *de novo* AML. Expression of multidrug resistance proteins (MDR) on bone marrow blast cells was evaluated by indirect immunofluorescence and flow cytometry before chemotherapy. Expression of MDR proteins was considered as positive if at least 25% of the blast cells were stained by anti-MDR protein antibody. All pts received standard induction therapy (cytarabine, etoposide and idarubicin or daunorubicine, "3+7+7" regimen). *Results.* Blast cells was defined as Pgp-positive in 68.3% of cases, BCRP+ in 48.5%, MRP1+ in 56.4%, and LRP+ in 62.9% of cases. After induction therapy 27 (64.3%) pts achieved CR and 15 pts (35.7%) were resistant to treatment. MDR proteins expression was observed more frequently in resistant group than in a sensitive one (71.4% *vs* 66.6% for Pgp, 62.2% *vs* 46.6% for MRP1, 71.4% *vs* 57.14% for LRP, 54.54% *vs* 45.45% for BCRP, respectively), but the difference was not statistically significant. Expression of all 4 MDR proteins was evaluated in 29 pts (10 - resistant, 19 - sensitive). Blast cells co-expressed 2-4 MDR proteins in 80% of resistant pts (all studied proteins - 30%, 3 of them - 30% and 2 proteins - 20%). In pts who achieved CR co-expression of MDR proteins was observed less frequently - 21%: 3 proteins were expressed in 3 cases (15.7%); all 4 proteins, only in 1 pt (5.3%) with very short CR duration (3 months). Other pts from this group (79%) expressed only one of the proteins studied. The one-year relapse-free survival (RFS) was 33,3%. In the univariate analysis only Pgp expression was negative prognostic indicator for RFS (25% *vs* 53% for Pgp^+ and Pgp^- AML, respectively, $p<0.01$). The co-expression of Pgp/MRP1 or Pgp/BCRP1 also correlate with the worst outcome (RFS for positive and negative cases were 33,3% *vs* 54% and 18% *vs* 44%, respectively, $p<0,01$). Among the pts with the known status of all 4 studied proteins 18,2% were related to the favorable cytogenetic group; 50%, to the intermediate; 31.8%, to the unfavorable group. Blast cells of all pts in unfavorable cytogenetic group expressed more than 1 protein (3 or 4 MDR proteins were expressed in 71,4% of cases). In favorable and intermediate cytogenetic groups blast cells co-expressed MDR proteins in 25% of cases only ($p=0,025$). We didn't reveal any correlation between the expression of any particular MDR protein and the relation to the cytogenetic group. *Conclusions.* Only co-expression of MDR proteins has prognostic value in AML pts receiving standard antracycline-Ara-C regimens. The detection of any single protein with the exception of Pgp didn't influence RFS. Co-expression of 2 and more proteins predict unfavorable treatment outcome in terms of CR and RFS. We observed a correlation between the cytogenetic status and the MDR expression.

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SERUM FERRITIN AS PROGNOSTIC MARKER OF RESPONSE IN ADULT ACUTE MYELOID LEUKEMIA

F. Albano, M. Delia, D. Pastore, M. Giannoccaro, P. Manduzio, V. Fesce, N. Sgherza, V. Liso, G. Specchia

Hematology-University of Bari, BARI, Italy

Background. Recent studies have suggested a link between iron overload and posttransplantation liver toxicity, infectious susceptibility, and even survival in patients undergoing hematopoietic stem cell transplantation for hematological malignancies. *Aims.* To date, there are no data to show that the availability of iron can and does play a critical role in adult acute myeloid leukemia (AML). We report here a study to determine the role as prognostic factor of pre-treatment serum ferritin in adult AML. *Methods.* We studied 30 consecutive adult *de novo* AML patients. For each case included in this study, serum ferritin level was determined at the onset of the disease. The median age of patients (15 males and 15 females) was 57 years (ranged from 16 y.rs to 75 y.rs); according to the FAB criteria the subtypes were: 1 M0, 3 M1, 14 M2, 3 M3, 5 M4, 3 M5b, 1 M6. All cases were treated with standard induction therapy. Student's t-test or the Mann-Whitney test was performed for comparisons of means. A two-tailed Fisher's exact test was used to compare categories. Overall survival (OS) was measured from the time of diagnosis to death or last follow-up visit and it was calculated using the Kaplan-Meier method; the log-rank test was used to compare survival curves. Only p values <0.05 were considered to be statistically significant. The AML patients were subdivided in two groups according to ferritin serum value (<800 *vs* > 800 ng/mL). Results. Fourteen (47%) patients showed a ferritin serum value >800 ng/mL. Compared with <800 ng/mL group, patients with serum ferritin > 800 ng/mL were more frequently non responders to chemotherapy treatment (57% *vs* 25%, $p=0.009$) and they had shorter OS (42 days *vs* the median survival not reached for the < 800 group, $p=0.001$). However, after excluding the M3 patients from the analysis, the differences between the two groups remained (Figure 1). Moreover, patients with serum ferritin > 800 ng/mL showed a trend for a higher frequency of documented infections during induction treatment (36% *vs* 6%, $p=0.07$). It is noterworthy that the patients in complete remission presented at the onset of AML a median ferritin value lower to that associated to the non responders patients (483 ng/ml *vs* 1233 ng/mL, $p=0.03$). *Conclusions.* It is well-known the role of iron metabolism in sepsis and carcinogenesis. The results of our study demonstrated a link between ferritin serum and AML prognosis. Further studies will be required in a large series of AML patients to confirm the useful of serum ferritin as prognostic marker.

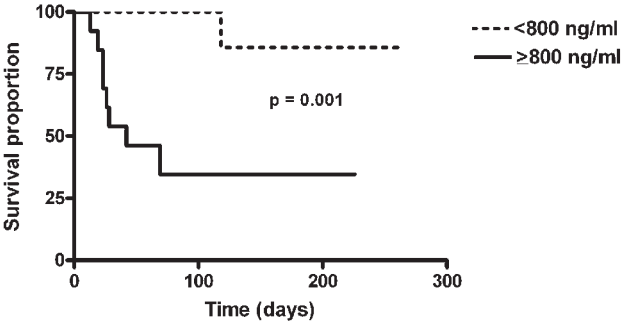

Figure 1.

1242

ANALYSIS OF TREATMENT OUTCOME OF ALL PATIENTS WITH NEWLY DIAGNOSED ACUTE PROMYELOCYTIC LEUKEMIA (APL): NO DIFFERENCE IN SURVIVAL BEFORE AND AFTER ATRA ERA

Z.K. Koristek,[1] J. Schwarz,[2] J. Stary,[3] P. Zak,[4] T. Kozak,[5] J. Mayer,[1] P. Cetkovsky,[2] J. Markova[5]

[1]University Hospital Brno, BRNO; [2]The Institute of Hematology and Blood Transfusion Prague, PRAGUE; [3]Department of Paediatric Haematology and Oncology, Motol University Hospital, PRAGUE; 42nd Department of Internal Medicine - Clinical Hematology, Faculty Hospital, HRADEC KRALOVE; [5]Department of Clinical Hematology, Faculty Hospital Kralovske Vinohrady, PRAGUE, Czech Republic

Background. Acute promyelocytic leukemia (APL) is curable in high numbers of patients. Before introduction of all-trans retinoic acid (ATRA), complete remission (CR) rate using chemotherapy was around 70% but only less than 45% of patients were cured. Induction therapy with ATRA and optimized ATRA-based regimens raised the CR rate in clinical studies up to 90% and 5-year disease free survival to 74%. However, it would be useful and interesting to analyze all the patients regardless of entering any studies. *Aims.* Analysis of treatment outcome of all patients with APL who were diagnosed in 5 cooperating centers in Czech Republic between 1989 and 2006. Patients and *Methods.* APL was diagnosed and confirmed by PCR in 144 patients (64 males, 80 females) with median age of 45.7 years (4.8-79.2). 31 patients were treated before ATRA and the others during 1998-2006. The high risk group ($WBC>10\times10^9/L$) consisted of 31% of patients, 39% of patients had $WBC>510\times10^9/L$ when diagnosed. AML M3 according FAB classification was presented in 80% and M3v in 20% of patients, respectively. All but one patient were positive for PML/RARα, one patient was PLZF/RARα, and one had a combination of PML/RARα and NuMA/RARα. *Results.* Complete remission (CR) was achieved in 96 (69%) of patients. The CR rate in high risk patients was 45% compared with 83% in other patients. Surprisingly, only 50% of patients with age between 30 and 45 years reached CR compared to 71% of patients with age of 45-60 years and 63% of patients older than 60 years. Concentrations of LDH and fibrinogen did not significantly influence the probability of CR in contrast to

performance status (PS). Patients with PS=0-1 (WHO) achieved CR in 84%, patients with PS=2 in 60%, and those with PS=3-4 in only 44%. The overall survival (OS) in 6 years was 55% (43% for high-risk patients). Only 56% of the low-risk patients (according PETHEMA criteria) survived 6 years compared to 70% of intermediate-risk patients. FLT3/ITD mutation did not significantly influence CR rate or OS, however, it is significantly associated with a bcr3 PML/RARα isoform (p=0.002). Surprisingly, we did not found significant difference in 3-years OS of patients treated before (1989-1997) and after ATRA (1998-2006). Overall, 17 (17.7%) of 96 patients relapsed and 7 of these patients are alive and well in CR2. *Conclusions.* The results obtained in treatment of APL using modern ATRA-based protocols are amazing, obviously when reached in consecutive and unselected patients. The aim of our work was to analyze all patients with APL entered not a study but our centers, including patients who died in the first hours and days. We suppose that our treatment results are in general similar to others and presented numbers reflect the fact, that we include into analysis also patients in very bad condition. However, the similar results of APL treatment with and without ATRA and OS of the low-risk patient worse than the intermediate-risk patients should be further analyzed and discussed.

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CLINICAL ACTIVITY OF LUMILIXIMAB AND FCR DOES NOT CORRELATE WITH BASELINE LEVELS OF SERUM CD23, β-2 MICROGLOBULIN, AND CLL CELL LEVELS

S. Harris,[1] J. Ranuio,[1] A. Estrellado,[1] J. Byrd,[2] S. O'Brien,[3] T. Kipps,[4] A. Cesano,[1] H. Mu,[1] S. Tangri[1]

[1]*Biogen Idec, SAN DIEGO;* [2]*The Ohio State University Comprehensive Cancer Center, COLUMBUS;* [3]*The University of Texas/MD Anderson Cancer Center, HOUSTON;* [4]*Moores Cancer Center, University of California San Diego, SAN DIEGO, USA*

Background. Lumiliximab is a monoclonal antibody specific for the human CD23 glycoprotein expressed on the majority of CLL cells, and under investigation for the treatment of patients (pts) with relapsed chronic lymphocytic leukemia (CLL). In the 152-30 clinical study evaluating lumiliximab in combination with fludarabine, cyclophosphamide, and rituximab (L+FCR) in relapsed CLL, a high CR rate of 52% was achieved. High baseline levels of sCD23, β-2 microglobulin and CLL cell counts have all previously been correlated with poor patient outcome. *Aims* In order to determine if L+FCR clinical activity is related to any of these prognostic markers, levels of sCD23, β-2 microglobulin and CLL cell counts were evaluated pre and post treatment in the 152-30 clinical study. *Methods.* 31 pts with relapsed CLL were enrolled to receive up to 6 cycles of L+FCR. sCD23 levels were measured in 22 pts using an enzyme-linked immunosorbant assay. β-2 microglobulin levels were measured in 31 pts using the Immulite system. Peripheral blood samples for CLL cell analysis were measured for 31 pts by flow cytometry; CLL cells were identified as $CD45^+/CD5^+/CD19^+$ lymphocytes. *Results*. Our analyses show that pretreatment levels of sCD23, β-2 microglobulin, and CLL cell counts varied in pts with both favorable and unfavorable outcomes. Also, there was modulation of sCD23, β-2 microglobulin, and CLL cell counts following L+FCR treatment. All pts assessed (22/22) showed induction of sCD23 levels, 21/25 pts showed modest decreases in β-2 microglobulin levels and 31/31 pts had decreases in CLL cell counts after treatment. *Conclusions.* Our analyses show that pretreatment levels of sCD23, β-2 microglobulin, and CLL cell counts are not related to L+FCR efficacy in the 152-30 study. As high baseline levels of all 3 of these factors correlate with poor prognosis, L+FCR may provide clinical benefit to pts with unfavorable clinical outcomes. These findings will be further evaluated in future clinical trials.

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OXIDATIVE STRESS AFFECTS DNA AND CELL MEMBRANES OF B-CELLS FROM B-CLL PATIENTS

R. Collado,[1] I. Oliver,[2] R. Collado,[2] C. Tormos,[3] M. Orero,[2] A. Miguel-Sosa,[2] M. Egea,[2] M. Fandos,[4] G.T. Saez,[4] F. Carbonell[2]

[1]*Hospital General Universitario, VALENCIA;* [2]*Serv. Hematologia, Consorcio Hospital General Universitario, VALENCIA;* [3]*Dept. Bioquímica, Facultad de Medicina (Universidad de Valencia), VALENCIA;* [4]*Medicina (Universidad de Valencia), VALENCIA, Spain*

Background. Accumulation of reactive oxygen species (ROS) and their by-products plays an important role in the pathogenesis of certain human diseases. B-cell chronic lymphocytic leukemia (B-CLL) is a neoplastic disease susceptible of oxidative damage. On the other hand, chromosomal abnormalities are considered important prognostic factors in B-CLL. *Aims.* To determine the degree of DNA damage and cell membrane peroxidation in patients with B-CLL; and, to relate it to the most frequent genetic abnormalities in this disease. *Material and Methods.* 78 untreated B-CLL patients (M/F 43/35; mean age 71 years; Binet stage A: 68 pts, stage B+C: 10 pts; typical/atypical morphology 66/12 pts), and 33 normal controls were enrolled in the present study. The DNA damage was measured assessing the levels of 8-oxo-2'-deoxiguanosine (8-oxo-dG) in lymphocytes and urine by HPLC-EC detection. Lipid peroxidation was studied measuring the levels of malonaldehyde (MDA) in B-cells, and 8-isoprostanes in urine by HPLC-EC and enzyme immunoassay (EIA), respectively. Genetic abnormalities were analyzed by fluorescence *in situ* hybridization (FISH) technique using the followed DNA probes: LSI D13S19 (13q14), LSI P53 (17p13), LSI ATM (11q22-23) and CEP12 (Vysis). *Results.* B-CLL patients show the levels of 8-oxo-dG (lymphocytes p<0.001 and urine p<0.001), MDA (p<0.001) and 8-isoprostanes (p<0.005) significantly increased as compared with control subjects. Regarding chromosomal abnormalities, 25 cases out of 78 (32%) presented del(13q14), 14 cases (18%) trisomy 12, 11 cases (14%) deletion of ATM gene, and 4 cases (5%) alterations of TP53. The relationship among oxidative stress levels and cytogenetic groups are showed on Table 1. *Conclusions.* 1) Oxidative stress induces an important damage on DNA and cell membranes in B-CLL patients. 2) Levels of 8-oxo-dG, MDA, and 8-isoprostane are significantly increased respected to the control group. 3) The obtained results suggest that oxidative stress is higher in those patients with poor prognostic genetic alterations affecting ATM and TP53 genes.

Table 1.

	Controls (n=33)	Del(13q) (n=25)	+12 (n=14)	Del ATM (n=11)	Del TP53 (n=4)
8-oxo-dG lymphocytes	3.7±0.7	41.3±12.8*	43.1±14.4*	44.9±17.8*	61.8±20.7*
MDA lymphocytes	0.2±0.1	1.0±0.6*	0.9±0.6*	1.3±1.1*	2.2±0.7*
8-oxo-dG urine	7.4±2.6	17.8±13.4*	20.7±14.1*	40.4±33.4*	no data
8-Isoprostane urine	64.2±42.7	97.9±48.1	106.8±67.8	102.9±55.8	no data

* p<0.05 respect the normal controls

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IS THE PLATELET FUNCTION ANALYZER (PFA)-100 A RELIABLE METHOD IN MONITORING ASPIRIN TREATMENT IN PATIENTS WITH THROMBOCYTOSIS?

E. Tsantes,[1] G. Mantzios,[2] V. Giannopoulou,[3] P. Tsirigotis,[3] S. Bonovas,[4] E. Mygiaki,[2] E. Rapti,[2] A. Kardoulaki,[2] Z. Kartasis,[5] N. Sitaras,[4] J. Dervenoulas,[3] A. Travlou[6]

[1]Attikon' General Hospital, School of Medicine, University of Athens, Greece, ATHENS; [2]Laboratory of Haematology & Blood Bank Unit, Attikon General Hospital, ATHENS; [32]nd Department of Internal Medicine, Attikon General Hospital, ATHENS; [4]Department of Pharmacology, School of Medicine, University of Athens, ATHENS; [5]Department of Haematology, General Hospital of Chalkida, CHALKIDA; [6]Laboratory of Haematology & Blood Bank Unit, Attikon General Hospital,, ATHENS, Greece

Background. Aspirin provides satisfactory protection against thrombotic episodes in essential thrombocythemia (ET), but at higher platelet counts has been less effective. *Aims.* To compare the platelet function analyzer (PFA)-100 with optical aggregometry, which is often perceived as reference method for the platelet function tests, in order to determine a reliable method in monitoring aspirin's influence on platelet function in patients with thrombocytosis. *Methods.* We studied 36 patients with thrombocytosis. Group A consisted of 10 patients with ET and 6 with reactive thrombocytosis (RT) all receiving aspirin, in order to examine the effect of elevated platelet count on PFA-100 measurements. Group B, serving as control, included 12 patients with ET and 8 with RT who had not taken aspirin or other antiplatelet drugs for at least 7 days before their testing. In all patients, we compared the platelet function measured by classic optical aggregation tests with closure times (CT) obtained by the PFA-100. *Results.* A statistically significant difference was found between aspirin users and non-users regarding PFA-100 collagen and/or epinephrine (CEPI) CTs (p<0.001), and aggregometry with epinephrine (EPI) (p=0.002), arachidonic acid (AA) (p<0.001) and ADP (p=0.01). The definition of platelet responses as normal or pathological showed that PFA-100 CEPI and epinephrine-induced aggregometry is the pair of *Methods.*with the higher agreement in monitoring of platelet dysfunction due to ASA treatment (a=94%). Satisfactory results were also obtained for group B (a=81%). The comparison between PFA-100 CEPI CTs and AA-induced aggregometry exhibited moderate agreement both in the total number of patients and in group A (a=79% and 94%, respectively). PFA-100 collagen and/or ADP (CADP) CTs and ADP-induced aggregometry were not concordant. *Conclusions.* The PFA-100 system appears to be a reliable and rapid method in the assessment of aspirin's antiplatelet effect in patients with thrombocytosis. Regarding aggregometry, the selection of the inducer, its concentration and cut-off points is crucial in defining the response to antiaggregating agents. It still remains to determine whether there is any relevance between the measurements obtained by these *Methods.*and clinical outcome in thrombocythemic patients.

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CURRENT HEMATOLOGICAL FINDINGS IN COBALAMIN DEFICIENCY. A MONO STUDY OF 201 CONSECUTIVE PATIENTS WITH DOCUMENTED COBALAMIN DEFICIENCY

E. Andres, L. Federici, T. Vogel, C. Fohrer

University Hospital, STRASBOURG, France

Background. With the introduction of automated assays for measuring serum cobalamin levels over the last decades, the hematological manifestations related to cobalamin deficiency have been changed from the description reported in *old* studies or textbooks. *Patients and Methods.* We studied the hematological manifestations or abnormalities in 201 patients (median age: 67±6 years) with well-documented cobalamin deficiency (mean serum vitamin B12 levels 125±47 pg/mL) extracted from an observational cohort study (1995-2003). Assessment included clinical features, blood count and morphologic review. *Results.* Hematological abnormalities were reported in at least two-third of the patients: anemia (37%), leukopenia (13.9%), thrombopenia (9.9%), macrocytosis (54%) and hypegmented neutrophils (32%). The mean hemoglobin level was 10.3±0.4 g/dL and the mean erythrocyte cell volume 98.9±25.6 fL. Around 10% of the patients have life-threatening hematological manifestations with documented symptomatic pancytopenia (5%), *pseudo* thrombotic microangiopathy (Moschkowitz) (2.5%), severe anemia (defined as Hb levels <6 g/dL) (2.5%) and hemolytic anemia (1.5%). Correction of the hematological abnormalities was achieved in at least two-third of the patients, equally well in patients treated with either intramuscular or oral crystalline cyanocobalamin. *Conclusions.* This study, based on real data from a single institution with a large number of consecutive patients with well documented cobalamin deficiency, confirms several *old* findings that have been previously reported before 1990's in several studies and in textbooks.

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EPOETIN BETA IS EFFECTIVE FOR 'EVER ANAEMIC' PATIENTS WITH MALIGNANCY RECEIVING CHEMOTHERAPY

M. Podolak-Dawidziak,[1] M.G Garbaczonek[2]

[1]Wroclaw Medical University, WROCLAW; [2]Roche Polska, WARSAW, Poland

Background. Patients with cancer often experience moderate to severe anaemia as a result of the haematopoietic blunting effects of either their malignancy or the subsequent chemotherapy. Though prevailing 2006 guidelines recommended initiating erythropoiesis stimulating agents (ESAs) for chemotherapy induced anaemia (CIA) at Hb levels <11 g/dL, maintaining near 12 g/dL, and withholding at 13 g/dL, but in Poland due to economical condition of the National Health Found it was allowed to start ESAs at Hb concentration ≤9 g/dL. *Aims.* The current study was designed to observe an extent to which treatment with epoetin β will increase the Hb concentration and decrease the need for RBC transfusion in *ever anaemic* (Hb ≤9 g/dL) cancer patients on chemotherapy. *Methods.* Non-interventional observational prospective study has been conducted (2005-2006) in 54 haematology/oncology centers in Poland during routine clinical practice. On five visits (four scheduled visits in median 7 days interval, and the fifth about 4 weeks after stopping epoetin β) the Hb concentration, RBC transfusions, iron supplementation, and adverse events were noticed. The study was approved by a local Ethics Committee. *Materials.* Overall, 382 anaemic patients (206 F, 176 M) median age 57.3±13.5 years (range 20-85) were included into the study. There were 157 patients with lymphoproliferative disorders: multiple myeloma - 101 (26.23%), low grade non-Hodgkin lymphoma - 29 (7.53%), chronic lymphocytic leukaemia - 27 (7.01%) and 225 with different solid tumours e.g. 71 with lung cancer: non-small cell lung cancer - 57 (14.81%) and small cell lung cancer - 14 (3.64%), ovarian cancer - 38 (9.87%), testicular cancer - 21 (5.45%), and others. 152 out of 382 patients (39.48%) were transfusion depended. All patients were scheduled to receive SC epoetin β 30 000 U/wk. Percentage of patients receiving iron supplementation varied in different period of the study from 54% to 32%, and was predominantly oral. *Results.* Treatment with epoetin β resulted in successive increase of Hb concentration from 8,36±0,76 g/dL (visit 1 prior to the study; n=382) to 9.39±1.28 g/dL (visit 2; n=348), 9.85±1.47 g/dL (visit 3; n=335), 10.2±1.39 g/dL (visit 4; n=289), 10.53±1.54 g/dL (visit 5; n=249). RBC transfusion requirements was gradually, but significantly reduced in these patients from 39.48% to 3.38%. Four weeks after stopping epoetin β (control visit; n=345) mean Hb concentration was 10.8±1.63 g/dL and the need for RBC transfusions elevated to 6.75%. Discontinuation of treatment with epoetin β in 93 patients was due to different reasons e.g. complete response (Hb ≥12 g/dL) in 61, lack of success in 14, end of chemotherapy in 11, disease progression in 4, side effects in 2 (cardiac arrhythmia, hypertension) and death of 1 patient. *Conclusions.* Treatment with epoetin β was well tolerated with few side effects and a high response rate in *ever anaemic* cancer patients on chemotherapy, implying an increase in the Hb concentration and a significant decrease in the need for red blood cell transfusions.

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EPIDEMIOLOGY OF THE HEMOGLOBINOPATHIES IN INPATIENTS AND OUTPATIENTS OF A SECONDARY CARE HOSPITAL IN GREECE

I. Passalidou, P. Karapavlidou, M. Chasios, A. Outas, O. Sotiriou

General Hospital Kastoria, KASTORIA, Greece

Background. Hemoglobinopathies are genetic (inherited) disorders of haemoglobin, the oxygen-carrying protein of the red blood cells. In general, hemoglobinopathies are divided into those in which the gene abnormality results in a qualitative change in the haemoglobin molecule and those in which the change is quantitative. Sickle cell anaemia (sickle cell disease) is the prime example of the former, and the group of disorders known as the thalassemias constitute the latter. Sickle cell anaemia (SSA) is an autosomal recessive disorder more common in the Black population.It is caused by a single amino acid mutation (β(6Glu->Val)) of the β-globin chain. Persons with one abnormal gene and one normal gene are said to be carriers of the sickle cell trait. Carriers are unaffected because of the remaining normal copy of the gene. Tha-

lassemias are common in Mediterranean populations as well as in Africa, India, the Mideast, and Southeast Asia. The two main types of thalassemias are alpha-thalassemia and β-thalassemia resulting from a partial or complete lack of synthesis of one of the major alpha- or β-globin chains of haemoglobin A. Mediterranean anaemia or β-thalassemia can range from mild and clinically insignificant (β thalassemia minor) to severe and life-threatening (β thalassemia major, also known as Cooley's anemia) *Aims.* The purpose of this study was to summarize information on the epidemiology of the hemoglobinopathies in inpatients and outpatients of a secondary care hospital in Greece. *Methods.* During a period of 12 years, 9.765 inpatients and outpatients were included in the study. Blood was collected in EDTA tubes from the studied individuals to determine a complete blood count (CBC), haemoglobin electrophoresis and sickle cell test. Electrophoresis results were further investigated with quantitation of Hb A2 in an anion exchange chromatography method (Helena β-thal quik coloumn) and HbF fragment with immunodiffusion. The haematological parameters including Hb, red blood cell (RBC) number, mean corpuscular volume (MCV), and red cell distribution width (RDW) were determined using an automated haematology analyzer (XT-2000 I SYSMEX, Roche). *Results.* -Homozygous β-thalassemia in 5 cases (0.05%). All cases were 5 to 8 years old children with heterozygous β-thalassemic parents -Heterozygous β - thalassemia in 814 cases (8.34%); -Hemoglobin s/ β - thalassemia in 2 cases (0.02%); -Sickle cell disease in 2 cases (0.02%). Both patients were Albanians; -Sickle cell trait in 3 cases (0.03%). All patients were foreigners; -Hereditary persistence of fetal hemoglobin (HPFH) in 2 cases (0.02%): a child and his father. *Conclusions.* The high frequency and clinical severity of the hemoglobinopathies, make them a major public health problem. Carrier detection procedures, genetic counselling, population screening and prenatal diagnosis of the thalassemias and sickle cell anaemia are necessary in order to prevent passage of the abnormal gene to their offspring.

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THE COMPARISON OF HEMATOLOGICAL AND BIOCHEMICAL INDICES OF PLASMODIUM FALCIPARUM-PARASITEMIC AND APARASITEMIC SICKLE CELL PATIENTS

K.N. Nsiah

Kwame Nkrumah University of Science and Technology, KUMASI, Ghana

Background. Sickle cell hemoglobinopathy, a monogenic disorder, is common in tropical countries, where malaria, caused by Plasmodium falciparum is endemic. Even though the hemolytic tendency is a hallmark of SCD, the course and the extent differ, depending on genetic, epigenetic and environmental factors. Homozygote SCD persons are particularly susceptible to P falciparum due to the increased tendency of their red cell destruction by merezoites of the falciparum parasites. *Aims*. This study is premised on a hypothesis that given any population of SCD persons in a malaria-endemic area, Plasmodium parasites would be present in some vulnerable subjects. Secondly, SCD persons with a positive blood smear of malarial parasites should be more anemic, based on the level of hemoglobin, and some other hematological and biochemical markers of hemolysis. Then, using a cut-off hemoglobin level <7 g/dL for severe anemia, a poor prognosis to mortality, the proportion of suh subjects with this severity index of anemia would be found, relative to age-, sex- and genotype-matched steady state SCD persons. The results of the study should give an insight into the challenges posed by malaria infection in a typical tropical environment, on SCD persons. *Methods.* From October 2006 to November 2007, some SCD patients, aged between 5-20 years, attending the Sickle Cell Clinic at the Komfo Anokye Teaching Hospital (KATH) were drafted into this study, approved by the Ethics Committee of the KATH. Blood samples from these subjects were used to test for falciparum parasites. Additionally, hemoglobin, white cells and reticulocytes were measured; likewise biochemical indices, like total bilurubin, the aminotransferases and lactate dehydrogenase. *Results*. Of 330 SCD persons, 69, representing 20.91% were parasitemic; 23 SS females, 17 SS males, 11 SC females, 12 SC males, and three each of unspecified genotype of both sexes. The mean levels (±standard deviation) of Hb, total bilirubin, total WBC, reticulocytes, HbF, AST, ALT and LDH for all the parasitemic were 8.2 (±1.83) g/dL, 29.90 (±26.00) μmole/L, 11.0×10^9 (±5.00), 12.3% (±12.23), 52.5 (±37.25) U/L, 29.7 (±29.37) U/L and 1066 (±325.5) U/L respectively. For sixty six age-, gender- and genotype-matched non-parasitemic subjects, the mean levels of the parameters were as follows; Hb, 8.5 (±1.80), bilirubin, 22.8 (±14.35) μmole/L, total WBC, 10.3×10^9/L(±5.14), reticulocytes, 23.6 (±17.67)%, AST, 42.7 (15.70) U/L, ALT, 23.1 (±7.56) U/L, and LDH, 1009 (±360.9) U/L. Thus, when the SCD patients were not classified into genotypes, it was only the reticulocyte count, for which statistical difference existed between the parasitemic and non parasitemic. However, when patients were grouped into genotypes, the differences in hemoglobin, WBC and reticulocytes reached statistical significance ($p<0.05$) in some cases. SS male steady state had Hb level of 8.1 g/dL, while the parasitemic, had 8.0; SS female steady state, 7.7 g/dL, crises state, 7.5; SC male steady state, 9.6 g/dL, crisis, 8.7 g/dL and SC female steady state, 9.7 g/dL, and crisis, 10.0 g/dL. Parameters, such as LDH and total bilirubin did not show any statistical difference. Twenty of the parasitemic (28.98%) were severely anemic as against twelve (18.18%) of the non-parasitemic, the difference presumably due to the infection, ignoring confounding factors. *Conclusions*. The study has shown 69 (20.91%) of 330 SCD patients had falciparum parasites in their blood film, while 20 of the parasitemic (28.98%), as against 12 (18.18%) of the non-parasitemic were severely anemic, a pointer to debility posed by the infection. Reticulocytosis is a common feature in SCD, reflecting in the brisk erythropoietic activity, which is depressed in the presence of malaria infection.

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UNUSUAL PRESENTATION OF CARNITINE MEMBRANE TRANSPORTER DEFICIENCY: BICYTOPENIA

Y. Aydinok,[1] S. Ucar Kalkan,[2] Y. Aydinok,[3] M. Coker,[2] O. Koroglu,[4] A. Akaslan,[4] R. Ozyurek[5]

[1]Ege University Hospital, IZMIR; [2]Ege University Hospital, Department of Pediatric Metabolism, IZMIR; [3]Ege University Hospital, Department of Pediatric Hematology, IZMIR; [4]Ege University Hospital, Department of Pediatrics, IZMIR; [5]Ege University Hospital, Department of Pediatric Cardiology, IZMIR, Turkey

Carnitine transporter defect is an autosomal recessive disorder caused by lack of functional OCTN2 carnitine transporter. The disease spectrum includes cardiomyopathy, metabolic crisis of Reye-like syndrome and recurrent hypoglycaemic hypoketotic encephalopathy. Here we describe siblings of a consanguineous marriage with two distinct presentations of disease. Eleven year old boy presented with persistent, non-progressive moderate normocytic anemia (haemoglobin; 9.0 g/dl, mean corpuscular volume; 85fl, RDW;13.4%) and neutropenia (absolute neutrophil count; 0.8×10^3/L, white cell count; 3.37×10^3/L) since early childhood with normal platelet count. He suffered from generalized weakness. Growth and development were normal and no pathological clinical findings were detected. Echocardiography showed normal cardiac functions. Bone marrow smear revealed erythroid dysplasia in a relatively hypocellular marrow. Bone marrow biopsy confirmed hypo cellularity (40%) and dyserythropoiesis. Reticulin grade;0. Serum B12 and folate levels were normal while serum ferritin decreased (8 μg/L). Although, serum ferritin increased to normal with oral iron therapy, haemoglobin achieved only to 10 g/dL while neutropenia persisted. In the history, it was informed that his older brother manifested cardiomyopathy with unknown aetiology. Therefore, both patients were put on advanced metabolic screening. Plasma amino acids levels and urinary organic acid profile were normal. Plasma carnitine was severely reduced (in various samples: free carnitine: 0.74-2.28 μmol/L, (N: 7-80 μmol/L)). A carnitine transporter defect was suspected and confirmed by the study of L-[methyl-3H] carnitine uptake by fibroblasts 0.01 pmol/min.mg (patient)/ 0.03 pmol/min.mg (his brother) (diagnosing pathological values: 1.01±0.26 pmol/min.mg). Chronic treatment of oral carnitine was initiated for both siblings. Although, carnitine is well known to have a role in red cell metabolism and anemia was noted in patients of primary carnitine transporter deficiency presented with cardiomyopathy, there is no report of bicytopenia related with this disorder. Further, it is interesting that the same defect would cause two distinct manifestations of disease (etc; cardiomyopathy in one and bicytopenia in the other sibling).

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STUDY OF EC CELLS IN PATIENTS WITH PERNICIOUS ANEMIA

L. Macukanovic-Golubovic,[1] M. Vucic,[2] T. Vukicevic,[2] E. Simonovic,[3] M. Mladenovic,[3] V. Nikolic,[2] I. Cojbasic,[2] O. Simonovic[2]

[1]Clinical Centre Nis, NIS; [2]Clinic of Haematology, Clinical Centre, NIS; [3]General Hospital, LESKOVAC, Serbia

Enterochromathophine (EC) cells are the members of diffuse neuroendocrine system and are classified according to ultrastructure characteristics as EC1-intestinal type; EC2-duodenal type and ECn-gastric

type. Gastric type EC cells are argirophylic and argentaphylic in 10-20%. They are localized in antral nad corporal gastric mucosa. In pathologic conditions EC cells can change their number, morphology and cytochemical characteristics, so as a functional properties. The aim of this study is to investigate presence, type and granularity EC cells in patient with pernicious anemia (PA). During the period from 1997 to 2006 (Clinic of Haematology-Nis), 96 patients with PA were examined as well as 30 patients with dyspeptic syndrome (control group). Classical (HE) histological method was used for pathological evaluation of gastric mucosa. Grimelius (argyrophilic) nad Massons (argentaphilic) *Methods.*were used for the identification of EC cells. Hyperplasia, metaplasia and hypergranularity of EC cells were found in corporal mucosa of PA patients. The number of EC cells was increased proportionally to the changing of gastric mucosa and the grade of intestinal metaplasia (22 to 43 cells/per field). Sometimes, EC cells hyperplasia is of so high grade that resemble intramucous carcinoidosis. EC cells were transformed from close type to the open type: they become triangle shape and high amount of serotonin in them. These results can confirm that new formed, intestinal type of EC cells secrete high quantity of serotonin increasing permeability and inducing the retrograde diffusion of H ions in gastric mucosa. In the way, H ions induce acidosis and inhibit cellular metabolism, leading to citolysis of all attached cells. Serotonin stimulates synthesis of acid mucins and has an inhibition effect on HCL secretion. Hyperplasia and metaplasia of EC cells could be important for the cancer genesis through serotonine effects.

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ANEMIA IN HOSPITALIZED CONGESTIVE HEART FAILURE (CHF) PATIENTS

D. Puente, M. Descalzo, R. Forastiero, F. Perea, R. Ratto, C. Colorio, A. Rossi, M. Tabares, L. Favaloro, G. Pombo, M. Diez, L. Feldman

Fundacion Favaloro, BUENOS AIRES, Argentina

Background. anemia frequently coexists with heart failure and results in a worse prognosis. Reduced hemoglobin levels (Hb) are associated with increased adverse events and mortality. *Aims.* to determine the impact of anemia in patients admitted for CHF at our hospital. *Methods.* we retrospectively analyzed data from 501 consecutive patients with a first CHF episode admitted from May 2004 to September 2005 whose Hb were measured at the time of admission, with a median follow up of 365 days (1-1241). HF and onset dyspnea were classified according to Framingham and NYHA functional class (FC) respectively. Hb <12 g/dLfor women and <13 g/dL for men were considered mild anemia while severe anemia was defined as a level <8 g/dL. Previous history of arterial hypertension, hipothyroidism, renal insufficiency (RI), menopause and treatment with aspirin (ASA), ACE inhibitors and oral anticoagulation (OAC) were recorded. Patients were assigned to 3 groups: I (no anemia), II (mild) and III (severe). Statistical analysis was performed with Pearson's Chi square, one-way ANOVA and log-rank Kaplan-Meier survival function.

Table 1.

	G-I (n=284)	G-II (n=205)	G-III (n=12)	p
male (%)	60.6	65.4	50.0	ns
median Hb (g/dl)	14.1	11.0	7.3	
% dyspnea FC 3/4 at admission	88.4	86.8	100	0.178
% creatinine ≥ 2 mg/dl	9.3	18.4	16.7	0.015
% chronic RI	8.5	21.9	33.3	0.000
% LVEF<30%	41.4	32.4	12.5	0.057
hospitalization (days)	7.3	10.6	11.7	0.002
% patients with transfusions >1 RCU	6.4	18.0	58.3	0.000
% readmissions	20.0	8.8	16.7	0.000
median survival (days)	1090	858	360	0.077
global mortality (%)	34.2	40.5	50.0	

Results. there were 501 patients, 312 (62.3%) men, median age 73 years (16-98). The entire group showed an Hb median of 12.9 g/dL (6.9-20.9). Group I included 284 (56.7%) patients, and 205 (40.9%) and 12 (2.4%) were classified as Group II and III respectively. No difference was found among ASA, ACE inhibitors and/or OAC previous therapy in the 3 groups. Demographic, clinical and outcome features are shown in Table 1. Median survival was longer in G-I according to Kaplan Meier analysis. *Conclusions.* In our experience, anemia was frequent among CHF patients. RI and left ventricle dysfunction were significantly associated to both anemia groups. Low Hb levels were associated to worse symptoms, longer hospital stays, a greater number of subsequent readmissions and a shorter survival.

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THE MOLECULAR SPECTRUM OF BETA-THALASSEMIA IN ROMANIAN POPULATION

R. Talmaci,[1] D. Coriu,[1] L. Dan,[2] L. Cherry,[2] M. Dogaru,[3] S. Badelita,[1] A. Colita,[3] D. Ostroveanu,[1] L. Gavrila[2]

[1]University of Medicine Carol Davila, BUCHAREST; [2]Human Genetics Department, Genetics Institute of Bucharest University, BUCHAREST; [3]Department of Pediatrics, Fundeni Clinical Institute, BUCHAREST, Romania

Beta-Thalassaemia is a worldwide inherited disorder characterized by a reduction or complete absence of a beta-globin expression. This study was designed to identify the beta-thalassaemia mutations in Romanian population. Hematological data were collected with automated cell counters (Coulter). Quantization of hemoglobin was done by cation exchange HPLC and by agarose gel electrophoresis. Analysis of the mutation in the beta-globin gene has been performed using the PCR based *Methods.* Amplification Refractory Mutation System (ARMS), Denaturing Gradient Gel Electrophoresis (DGGE), Real Time PCR Genotyping and direct sequencing. One hundred and twenty four patients with beta-thalassaemia were included: one hundred and two cases with heterozygous beta-thalassaemia; sixteen cases with homozygous beta-thalassaemia; and six patients with unknown patterns in heterozygous state. In addition, four patients were identified with Hb Lepore. Molecular analysis of 134 alleles revealed 13 different mutations: IVS I-110 (33,58%), CD 39 (12,68%), IVS I-6 (12,68%), IVS II-745 (11,2%), IVS I-1 (8,2%), CD 8 (3,73%), CD 5 (2,98%), -87 (2,23%), Hb Knossos (2,23%), CD 6 (2,23%), -30 (0,74%), CD 8/9 (0,74%), CD 51 (0,74%), +22 (0,74%), poly A (0,74%) and unknown mutations (4,47%). A new polymorphism +3 (A-T) associated with IVS I-1 mutation was found in a young patient. The occurrence of this new polymorphism has an impact on the phenotype because when associated with the IVS I-1 mutation it results in a beta-thalassaemia intermediate status. These results show, as we anticipated, that beta-thalassaemia in Romania is of Mediterranean origin. This information on the spectrum of mutations has implications for the control of β thalassaemia in Romanian population and also on the genotype-phenotype correlation of the disease.

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THE FREQUENCY AND MOLECULAR GENETICS OF G6PD DEFICIENCY IN NORTHWEST AND SOUTHEAST OF IRAN

Y.M. Mortazavi,[1] M.S. Soleimani,[2] A.N. Ahmadbeigy lahijani,[2] A.O. Omidkhoda,[2] A. Ghavamzadeh[3]

[1]Zanjan Medical School, ZANJAN; [2]Tarbiat Modares University, Hematology Department, TEHRAN; [3]Shariati, Hematology-Oncology and BMT Reasearch Centre, TEHRAN, Iran

Background. Glucose-6-phosphate dehydrogenase (G6PD) is an X-linked enzymopathy affecting about 400 million people worldwide. Neonatal jaundice, drug induced haemolysis and infection-induced haemolysis may happen in some deficient individuals and lead to considerable morbidity and mortality. The distribution of G6PD deficiency and the molecular genetics of this enzyme vary widely among the different ethnic groups. *Aims.* To find out the frequency of G6PD deficiency and characterize the molecular type of G6PD in deficient individuals in Turk and Balouch ethnic groups of Zanjan and Iranshahr cities. *Subjects and Methods.* One thousand and five hundred unrelated normal male individuals from Zanjan and 305 unrelated normal male students from Iranshahr were screened for G6PD deficiency by fluorescent spot test. DNA was extracted from peripheral white blood cells of individuals who were deficient for G6PD. Polymerase chain reaction (PCR) was used to amplify flanking regions of exons six and seven of this gene using a set of primers. The PCR products were digested by the enzyme

Mboll and electrophoresed on 2.5% agarose gel. *Results.* Thirty-three out of 1500 (2.2%) individuals were shown to be deficient for G6PD enzyme from Zanjan population. Twenty-four out of 33 (75.3%) showed a mutation at nt 563 of G6PD gene which is characteristic of Mediterranean type of mutation. Nine individuals (24.7%) were negative for this mutation. Fifty nine out of 305 (19.3%) individuals of Iranshahr were shown to be deficient for G6PD enzyme. Eighty eight percent had a severe enzyme deficiency and 12% had partial deficiency. At the molecular level, using PCR technique 50/59(85%) showed Mediterranean type of mutation and 9/59 (15%) showed non Mediterranean type of mutation. *Conclusions.* Despite different frequencies exist for deficiency of G6PD enzyme in Turk and Balochi and other ethnic groups in different regions of Iran (central, north, northwest and Southeast) the results of this study and other studies have shown that the incidence of Mediterranean type of G6PD mutation in all the above regions are approximately the same. Therefore, it can be concluded that the gene frequency of G6PD deficiency has been distributed equally among different populations of Iran. This may indicate that the predominant G6PD mutation in Iran is of Mediterranean type.

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RAPID IRON LOADING AFTER DISCONTINUATUON OF CHELATION THERAPY FOR A YEAR - THE CASE OF A PREGNANT ?-THALASSAEMIC WOMAN

F.K. Farmaki,[1] I. Tzoumari,[1] Ch. Pappa,[1] E. Gotsis,[2] V. Verdoukas[3]

[1]General Hospital of Corinth, CORINTH; [2]Euromedica/Enkephalos, ATHENS; [3]White Cross Clinic, ATHENS, Greece

Background. The marked improvement in survival and reduced morbidity in thalassaemia major (TM) allows many patients to parent children In general, in women with transfusion dependent thalassaemia, during pregnancy, iron chelation therapy is ceased. *Aims.* To determine the impact of withholding chelation therapy for an interval of 12 months. *Patients.* A transfusion dependent thalassaemic woman of 38 years old, with primary amenorrhea, splenectomised, which was an excellent complier with chelation therapy. She had pursued combined chelation for 5 years with Deferoxamine (DFO) 35 mg/kg/day and Deferiprone (DFP) 75 mg/kg/day. She showed no evidence of iron overload before embarking on a pregnancy, with normal cardiac, thyroid function and glucose metabolism. She became pregnant by assisted reproduction *Methods.*(IVF). During pregnancy the mean pretransfusion Hb level was maintained at 10.8 g/dL. She was transfused with 33 units of filtered packed red blood cells, a total of 11417ml but her red cell consumption remained unchanged because she had gained 10 kg weight. She had delivered normally and breast fed for 2 months. She had ceased chelation therapy for an interval of 12 months. *Methods.* Iron overload was evaluated: - Mean serum ferritin levels by (MEIA); -Non-invasive heart & hepatic iron quantification, by annual Signa-MRI 1.5 Tesla, multi-echo T2* sequences; - LIC (Liver iron concentration) was calculated by FerriscanTM based on repeated MRI measurements. *Results.* - Before pregnancy: mean serum ferritin=46 μg/L, T2*Heart=34,1msec, T2*Liver=34,2msec and LIC=1,0 mg/g/dwt, all regarded as normal. - After pregnancy: mean serum ferritin=1583μg/L, T2*Heart=27,3msec. The most striking feature was T2*Liver=2,25msec and LIC=11,3 mg/g/dwt, regarded as moderate to high iron loading, as it was 5 years ago, before starting combined chelation therapy. Thus the liver during the 12-month chelation-free period accumulated 10.3 mg/g dry weight, i.e., 0.85 mg/g dry weight per month. *Conclusions.* This case report demonstrates clearly that pregnant women with TM should be monitored carefully for iron loading before they embark upon a pregnancy and afterwards. The main message is that iron overload can develop rapidly and physicians caring for patients with transfusion dependent Thalassaemia should be particularly alert to any discontinuation of chelation therapy over time. Patients should also be made aware of this risk in order to maintain their chelation therapy consistently.

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GENETIC HETEROGENEITY OF BETA THALASSAEMIA IN CATALONIA: MOLECULAR CHARACTERIZATION OF 74 CASES

M.M. Mañú Pereira,[1] A. Cabot Dalmau,[2] N. Radó Trilla,[3] M.T. Coll Sibina,[4] J.L. Vives Corrons[5]

[1]Hospital Clínic, BARCELONA; [2]Hospital de Mataro, MATARO; [3]Hospital Clínic i provincial, BARCELONA; [4]Hospital de Granollers, GRANOLLERS, Spain

Background. Antenatal and prenatal screening programs have been undertaken in order to prevent such major thalassemic disorders. Mutations leading to β-thalassaemia follow a geographic pattern. The 8 most prevalent mutations accounting for more than >90% of all alleles in the Mediterranean region are: CD39 (C>T), IVS1:110 (G>A), IVS1:6 (T>C), IVS1:1 (G>A), IVS2:745 (C>G), IVS2:1 (G>A), -87 (C>G) and CD6 (-A). However, a study performed in Catalonia in 1988 showed that only 8 mutations, including two different ones; CD8 (-AA) and IVSI:5 (G>C), were responsible for the total of cases. *Aims.*The main objective of this study is to investigate the β-thalassaemia underlying mutations to ensure proper genetic counseling and prenatal diagnosis. *Materials and Methods.*74 patients referred for β-thalassaemia diagnosis were studied at molecular level. DNA was extracted from peripherical leukocytes and basic hematological parameters were obtained. Patient's geographical origin was also requested. The 8 most prevalent mutations of β-globin gene in the Mediterranean: CD39 (C>T), IVS1:110 (G>A), IVS1:6 (T>C), IVS1:1 (G>A), IVS2:745 (C>G), IVS2:1 (G>A), -87 (C>G), CD6 (-A) were screened by ASO methodology. Those samples with a negative result were direct sequenced for the β-globin gene from position -384 upstream of the Cap site to position 42 of the IVS-II and from position 776 of IVS-II to 194 bp after the termination codon. *Results*. Screening of the most prevalent mutations allowed us to characterize 55 out of the 74 cases here studied. 11 different β-gene mutations were identified by direct sequence. Distribution of the identified β-gene mutations are shown in Table 1, where comparison with the data of 1988 is also provided. *Conclusions.* Characterization of β-gene alleles leading to β-thalassaemia has decreased from more than 90% to 74.33% by analyzing the most prevalent mutations from the Mediterranean area. The 4 most prevalent mutations in our study, CD39(C>T), IVS1:110(G>A), IVS1:6(T>C) and IVS1:1(G>A), are common in the West Mediterranean countries. However, IVSII:745 C>G, IVSII:1(G>A), -87(C>G) and CD6(-A) are only responsible for 2.70% of our cases. In comparison with the 1988 study in Catalonia, the analysis of the 8 mutations then identified is responsible for 82.6% in the recent study. The relative percentage of each mutation has also changed. In 1988 CD39 mutation was responsible for 64.0% of the cases, nowadays it is responsible for only 28.4% of the cases. Molecular heterogeneity has increased: 19 different mutations, including Chinese and sub-Saharan variants are now responsible for all cases. Since immigration flows and increased rates of rare variants make genetic diagnosis more difficult, this shows the high importance of identifying mutations leading to β-thalassaemia in order to provide appropriate genetic counseling and prenatal diagnosis.

Table 1.

MUTATION	ORIGIN	% 2007	% 1988
PREVALENT MUTATIONS CD39 C>T, IVSI:110 G>A, IVSI:6 T>C, IVSI:1 G>A IVSII:745 C>G, IVSII:1 G>A, -87 C>G, CD6 –A	43 SPAIN 5 EGYPT 3 MOROCCO 2 RUSSIA 1 ITALY 1 SUBSAHARIAN	74.33	96.8
CD8 –AA	1 SPAIN 3 MOROCCO	5.42	1.7
IVSI:5 G>C	1 SPAIN 1 MOROCCO	2.70	1.5
CD8/9 +G	1 SPAIN 1 MOROCCO	2.70	-
CD41/42 –TTCT	2 CHINA	2.70	-
CD24 T>A	2 SUBSAHARIAN	2.70	-
CD5 -CT	2 SPAIN	2.70	-
CD37 G>A	1 SPAIN	1.35	-
IVSI:1 G>T	1 INDIA	1.35	-
IVSII:849 A>C	1 SUBSAHARIAN	1.35	-
-29 A>G	1 SUBSAHARIAN	1.35	-
-88 C>T	1 SPAIN	1.35	-

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PHARMACOKINETIC CASE STUDY: DEFERIPRONE IN A TRANSFUSION DEPENDENT-THALASSEMIA PATIENT WITH RENAL FAILURE UNDERGOING HAEMODIALYSIS TREATMENT

E. Schaart,[1] T. Lombardo,[2] D. Cole,[1] F. Tricta,[1] C. Tsang[1]

[1]ApoPharma Inc., TORONTO, Canada; [2]Servizio Talassemia Ospedale S. Bambino., CATANIA, Italy

Background. There are no published data on the pharmacokinetic (PK) profile of the oral iron chelator deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one) in patients undergoing haemodialysis. We examined the PK profile of deferiprone, in a 48 year old male with transfusion dependent-thalassemia with diabetes mellitus and renal impairment undergoing haemodialysis and with severe allergic reactions to deferoxamine. Patient informed consent was obtained prior to starting this case study. A single oral dose of 1,250 mg of deferiprone, equivalent to 25 mg/kg [standard dose: 25 mg/kg tid]) was administered with 240 mL of water approximately 30 minutes after a standardized breakfast. Blood samples were taken 5-45 minutes prior to dosing (time 0) followed by sampling at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 hours. Haemodialysis was set for the initial 4-hour period after dosing and dialysate was sampled. Deferiprone was analyzed in all fluids using a high-performance liquid chromatography (HPLC) method. *Results*. The plasma PK profile in this case study shows the following results, a Cmax of 5.99 mcg/mL and AUC 17.5 mcg/h/mL of deferiprone. In this patient, deferiprone absorption and serum concentrations attained a maximum (Tmax) at 2 hours. In comparison with published data of deferiprone in thalassemia patients, it appears that the Cmax and AUC was about a half of that seen in normal patients with normal renal function, but the $T^1/_2$ was about the same. *Summary and Conclusions.*If this subject is typical of dialysis patients, it would suggest that deferiprone does not accumulate in patients with renal failure undergoing dialysis. It would have been ideal to compare Tmax, Cmax and AUC to thalassaemia patients with normal renal function, but we were unable to have a direct comparison due to different methodologies used by other study populations. While one patient is insufficient to draw conclusions, this first report of PK in a dialysis patient indicates they may exhibit lower serum concentrations of deferiprone. Possible explanations include a larger apparent volume of distribution and/or decreased absorption. Based on deferiprone dialysate concentrations, clearance via the dialysate does not explain the low serum concentrations. Additional studies are warranted to determine the disposition of deferiprone in such patients and then establish the best therapeutic practice for renal impaired iron overloaded patients requiring chelation therapy with deferiprone.

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A CASE OF MYELODYSPLASTIC SYNDROME ASSOCIATED WITH CD14$^+$CD56$^+$ MONOCYTOSIS, EXPANSION OF NK LYMPHOCYTES AND DEFECT OF HLA-E EXPRESSION

F. Alfinito, L. Luciano, R. Della Pepa, C. Palladino, G. Ruggiero, G. Terrazzano

University of Naples, NAPOLI, Italy

Myelodysplastic syndromes (MDS) are characterized by ineffective hematopoiesis with potential progression to acute myeloid leukemia (AML). Immune-mediated mechanisms have been suggested in the pathogenesis of MDS and the expression of CD94/NKG2 receptors has been correlated to Natural Killer (NK) lymphocytosis and LGL disorders. Alteration of HLA-E, a non classical Human Leukocyte Antigen class I (HLA-I) molecule, has been proposed in hematopoietic diseases. Here, we describe an MDS patient showing peculiar CD14$^+$CD56$^+$FasL/CD178$^+$ monocytosis, expansion of polyclonal CD56$^+$CD3$^-$ NK cells and defective HLA-E expression on monocytes and polymorphonuclear cells (PMN). Patient NK exerted *in vitro* autologous killing of PMN and expressed increased level of the activating (CD94/NKG2C) HLA-E binding receptor. We propose that the polyclonal NK lymphocytosis, the defective HLA-E expression and the presence of FasL/CD178 on CD14$^+$CD56$^+$ monocytes could be relevant for the pathogenesis of the myelodysplastic disorder in the patient.

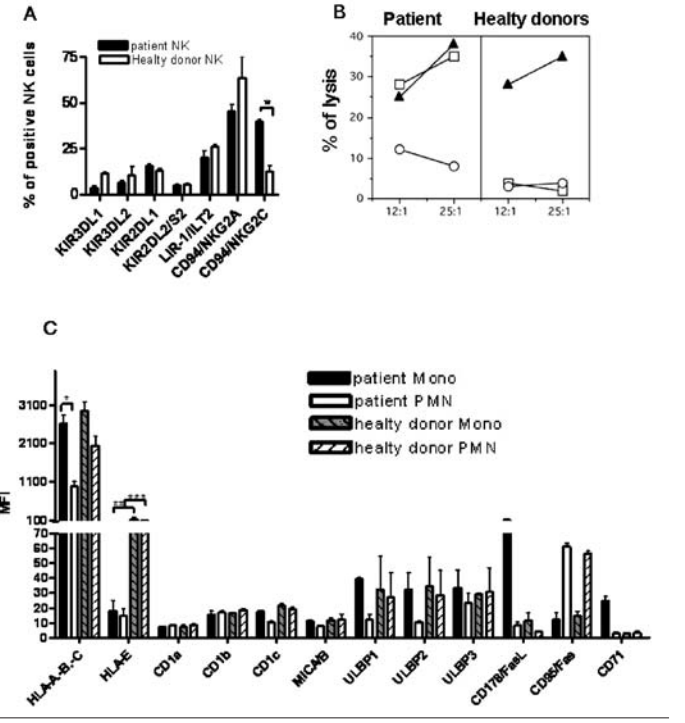

Figure 1.

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CLINICAL AND PROGNOSTIC SIGNIFICANCE OF CD34 EXPRESSION IN BONE MARROW BIOPSIES IN MYELODYSPLASTIC SYNDROMES

A. Savic,[1] V. Cemerikovic-Martinovic,[2] V. Dinic-Uzurov,[1] I. Savic,[1] N. Rajic,[1] I. Milosevic,[1] B. Zeravica,[1] I. Urosevic,[1] D. Agic,[1] N. Vlaisavljevic,[1] S. Popovic[1]

[1]Clinical Centre of Vojvodina, NOVI SAD; [2]Patohistology laboratory Histolab, BELGRADE, Serbia

Background. CD34 antigen is expressed on hematopoietic and endotel cells in bone marrow. Expression of CD34 is increased in substantial portion of MDS patients, particularly in high risk patients, what was associated with unfavorable survival in some studies. *Aims.* Aim of this study was to determine the CD34 expression in bone marrow biopsies in MDS patients and to analyze it in the context of different clinical, laboratory and prognostic parameters, as well as to define its prognostic significance. *Methods.* The study was conducted in 53 MDS patients and 20 control patients who have different diseases predominantly lymphomas but with histological normal bone marrow and no evidence of bone marrow involvement. CD34 expression was determined by CD34 monoclonal antibody and labeled streptovidin biotin peroxidase method. The positivity was determined counting the 500 cells and expressed as percentage. *Results.* Among the 53 MDS patients there were 37 males and 16 females with average age of 62 years. In control group were 11 males and 9 females with average age of 52.7 years. The distribution of MDS patients according to FAB classification was as follows: RA 18 (34%), RARS 2 (4%), RAEB 21 (40%), RAEB-T 2 (4%), and CMML 10 (19%). Average CD34 expression in MDS group was 1,37%, range was 0-8,8%, and in control group 0,78%, range 0-1,60%. The difference was statistically significant (student T test, Welch correction, p<0,05). CD34 expression average and range in MDS subgroups was: RA 0,81% 0-4,00%, RARS 0,45% 0,2-0,70%, RAEB 1,52% 0-8,8%, RAEB-T 2,30% 0.8-3,3%, CMML 2,06% 0-5,2%. CD34 expression average and range in low risk MDS (20 Pts) was 0,75% 0-4% and in high risk MDS (33 Pts.) 1,7235% 0-8,8%. There was a statistically significant difference in CD34 expression comparing RA and CMML group and high risk and low risk MDS (Mann-Whitney U test p<0,02). Median survival for all MDS patients was 20,33 month. Median survival in group with CD34 expres-

sion less than 2% was 22 month, comparing with 6 month in patients with CD34 expression over 2%. This difference was statistically significant (log rank test $p<0.05$). In multivariate analysis CD34 expression together with karyotype and transfusion dependence has statistical significance (Cox hazard method $p<0,05$). IPS and percentage of blasts have no statistical significance in the multivariate analysis. *Conclusions.* CD34 expression in bone marrow biopsies is higher in MDS patients comparing with control, as well as in high risk comparing with low risk patients. The cutoff 2% seems to have prognostic significance.

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IMPACT OF MEAN PLATELET VOLUME ON SURVIVAL OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES

E. Kapsali, Ch. Tsaousi, E. Hatzimichael, L. Benetatos, K. Bourantas

University Hospital, IOANNINA, Greece

Background. Survival of patients with myelodysplastic synndromes (MDS) varies considerably and prognostic models are important for therapeutic approach. Identifying simple laboratory markers that can predict survival can be very helpful. *Aims.* Thrombocytopenia, along with other cytopenias has been incorporated in scoring systems and classifications of MDS. There are conflincting reports for Mean Platelet Volume (MPV) and Mean Platelet Mass as prognostic markers.This is a study of the impact of these parameters on survival of patients with MDS. *Methods.* Peripheral blood samples of MDS patients were analysed in automated analyser SYSMEX SE 9500 and MPV was calculated (range 7.5-12.5 fl). Mean Platelet Mass was calculated as MPV × Platelet count(mL/L).Data concerning patients' age at diagnosis, type of MDS (FAB-WHO classification), karyotype, treatment and overall survival were recorded. *Results.* Thirty- eight patients with MDS were included in the study. There were 15 men (40%) and 23 women (60%). The median age at diagnosis was 71.9 years (range 17-92). According to the FAB classification 36 (94.8%) had refractory anemia (RA), 1(2.6%) patient had refractory anemia with ringed sideroblasts (RAS) and 1 (2.6%) patient had refractory anemia with excess of blast (RAEB). According to the WHO classification 27(71%) patients had RA, 8 (21%) patients had refractory anemia with multilineage dysplasia (RCMD), 1 (2.6%) patient had MDS 5q-, 1 (2.6%) patient had RAS and 1(2.6%) patient had refractory anemia with excess blasts-2 (RAEB-2). Two groups of patients were formed. Group A with MPV<10.5 fl and Group B with MPV≥10.5 fl. Group A included 11(29%) patients with median survival of 40 months and Group B included 27(71%) patients with median survival 35 months. There was no difference in transfusion requirements between the two groups. Platelet mass was over 1.2 mL/L (high platelet mass) in 30 (78%) of patients. Only 2 (5%) patients had low platelet mass (<0.60 mL/L) and 6 (16%) patients had intermediate platelet mass (0.60-1.2 mL/L). *Summary and Conclusions.* Mean Platelet Volume and Mean Platelet Mass seem to provide no additional information as far as overall survival and severity of disease is concerned. Platelet number is of importance along with other mechanisms of dysplasia and dysfunction of bone marrow elements in patients with MDS.

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INTRAVENUOS (IV) 5-AZACITIDINE (5-AZA) AS SINGLE-AGENT IN THE TREATMENT OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS) OR ACUTE MYELOID LEUKEMIA (AML) INELIGIBLE FOR MORE INTENSIVE THERAPY: PRELIMINARY DATA OF OPEN-LABEL, SINGLE-ARM, SINGLE-CENTER PHASE II STUDY

N. Di Renzo,[1] M. Dargenio,[2] M.R. De Paolis,[2] P. Forese,[2] R. Matera,[2] E. Pennese,[2] G. Pugliese,[2] G. Reddiconto,[2] A. Valacca,[2] C. Vergine[2]

[1]Vito Fazzi Hospital, LECCE; [2]Hematology Division Vito Fazzi Hospital, LECCE, Italy

Background. 5-azacitidine, a ring analog of the pyrimidine nucleoside cytidine, induces reexpression of silenced genes in cancer cells through inhibition of DNA methyltransferase with effect on cell differentiation, gene expression and DNA synthesis. Recently, 2 clinical trials involving high-risk MDS patients have shown a survival benefit for patients treated with 5-azacitidine administered subcutaneously (SC) compared with those receiving supportive/conventional care regimens. However, when 5-Aza is given SC, erythema and pain in the site of injection are not uncommon adverse events both reducing patients' compliance for the treatment. Moreover, 5-Aza given IV has been show to be safe and efficacious in several pharmacokinetic studies. *Aims.* In order to assess efficacy and toxicity profile of 5-Aza given as short intravenous (IV) infusion, we started an open-label, single-arm, single-center phase II study including patients with MDS or Acute Myeloid Leukemia (AML), either *de novo* or secondary according to FAB classification, not eligible for more intensive treatment or no more responding to hematopoietic growth factors. *Methods.*Patients previously treated with 5-Aza SC were ineligible. Treatment consisted of 5-Aza 75 mg/m^2 given as 1-hour IV infusion once daily on days 1-7 every four weeks for at least six courses. Patients responding continued the treatment until progression or loss of response, while patients failing to achieve at least a hematologic improvement after six courses or showing any grade 3-4 persistent toxicity (more than 8 weeks) were excluded from study. Primary study endpoints were both complete response (CR) and partial response (PR) rates, while secondary study endpoints were the hematological improvement (HI) rate and frequency and grading of toxicity. Response and HI were evaluated according to IWG response criteria and toxicity by NCI-Common Toxicity Criteria. *Results.*Up to date, 12 patients, median age 73 years (range 46-84 yo) have been enrolled; median time from diagnosis to treatment was 21 months. Diagnosis at the study entry was: AML n=4, RAEB n=2, RAEB-t n=3, CMMoL n=2 and RARS n=1. According to IPSS risk 1 patient was defined as low-risk, 3 as Int-1, 3 as int-2 and 5 as high-risk. All patients had clinically relevant co-morbidity: previous cardiac infarction (n=2), hypertensive cardiomyopathy (n=5), chronic renal failure (n=2) and insulin-dependent diabetes (n=3). Most of patients were RBC transfusion-dependent (75%) and 16% both RBC and PLTs transfusion-dependent. Each patients received in median six courses of 5-Aza (range: 2-11). Ten patients result evaluable for response and toxicity. One patients achieved CR (10%) and 5 (50%) had an HI with an ORR of 60%. Three patients (30%) had major erythroid response with two of them becoming RBC transfusion independent and two had a minor erythroid response. ANC increased was observed in two patients. The median time to response was 120 days. Two patients failed to achieve at least an HI and additional two had progressive disease. No death may be related to 5-Aza. Two patients died of progressive disease after 2 cycles, 1 of cardiac re-infarction after 4 courses and 1 of acute pulmonary edema after 5 cycles. The toxicity profile of 5-Aza given IV seems to be similar to that of 5-Aza SC. Forty percent of patients developed grade 3-4 neutropenia mainly during the first two courses of therapy and lasting less than two weeks. Two patients had febrile neutropenia resolved following broad spectrum antibiotics therapy. Transient grade 3-4 thrombocytopenia was observed in 40% of patients requiring treatment delay (median 4 weeks) but no dose reduction. Non hematologic toxicity was uncommon and only of 1-2 grade. No grade 3-4 hypokalemia, weakness, rigor or petechiae were observed. Only one patient had grade 3 costipation. *Conclusions.* These very preliminary data show that 5-Aza IV is safe and effective as well as 5-Aza SC in the treatment of elderly patients with MDS or AML and clinically relevant comorbidities, improving also the patients' compliance.

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EXPRESSION OF THE HUMAN PIM-2 (HPIM-2) GENE IN PATIENTS WITH ACUTE MYELOBLASTIC LEUKAEMIA (AML) AND ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)

K. Kapelko-Slowik, D. Urbaniak-Kujda, B. Jazwiec, J. Dybko, M. Slowik, D. Wolowiec, S. Potoczek, I. Frydecka, K. Kuliczkowski

Wroclaw Medical University, WROCLAW, Poland

Background. A human PIM-2 is proto-oncogene that encodes serine/threonine kinase which interacts with various signalling molecules. HPim-2 is highly expressed in haematopoietic tissues and in leukaemic and lymphoma cell lines which is consistent with a role during haematopoiesis and during oncogenic transformation. *Aims.* The aim of the study was to investigate whether the human PIM-2 is altered in acute myeloblastic leukaemia (AML) and acute lymphoblastic leukaemia (ALL). *Patients.* Thirty-seven patients were included: 22 with AML and 15 with ALL (10 with T and 5 with B subtype), aged 21-70 (x=41). Twenty-two patients reached complete remission (CR). Bone marrow samples were collected at the time of diagnosis. Control samples were obtained from 8 healthy donors. *Methods.*We analyzed hPIM-2 expression by quantitative RT-PCR analysis. RNA was isolated from 3mlns mononuclear cells by using standard *Methods.*(TriPure Reagent, Roche Diagnostics). cDNA was synthesized by AMV reverse transcriptase (Finnzyme). Sequence for primers was forward, 5'-CTTTCCTTCCAATACCCCA-3' and reverse, 5'-CCATCTTCCATTCCTTCCC-3'. As a n internal control c-ABL was used with primers c-ABL: 5'-CCCAACCTTTTCGTTG-CACTGT-3' and 5'-TGA CTGGCGTGATGTAGTTGCTT-3'. The intensity of each band was compared densitometrically. *Results*. Expression of the hPIM-2 in all leukemic patients (n=37) and both subgroups: AML (n=22) and ALL (n=15) was significantly higher than in normals

(p=0,0001; p=0,0009; p=0.0002, respectively). Patients who reached CR expressed hPIM-2 significantly lower than patients with primary resistance to chemotherapy (with no CR) (p= 0,01). Moreover we have found the correlation between hPim-2 expression and patients age (r=0,45, p=0,005) and WBC in peripheral blood (r=0,34, p=0,04). *Summary.* PIM-2 expression is increased in patients with AML and ALL Our data indicate that expression of hPIM-2 could be considered as an additional prognostic marker in patients with AML and ALL.

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CHEMOKINE RECEPTORS EXPRESSION IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

M. Ramírez,[1] C. Martínez,[1] A. Gómez,[1] A. Lassaletta,[1] M. Guibelalde,[2] J.L. Fuster,[3] C. Esquembre,[4] J. Molina,[5] C. Calvo,[6] P. Gómez,[7] J.L. Vivanco,[8] E. Bureo,[9] L. Madero[1]

[1]Hospital Universitario Niño Jesús, MADRID; [2]Hospital Son Dureta, PALMA DE MALLORCA; [3]Hospital Virgen de la Arrixaca, MURCIA; [4]Hospital General, ALICANTE; [5]Hospital Virgen del Camino, PAMPLONA; [6]Hospital Miguel Servet, ZARAGOZA; [7]Hospital Reina Sofía, CÓRDOBA; [8]Hospital Doce de Octubre, MADRID; [9]Hospital Marqués de Valdecilla, SANTANDER, Spain

Background. B-cell acute lymphoblastic leukemia (B-ALL) in children is a bone marrow disease, while most T-ALL originates from the thymus. Detection of ALL cells in other organs implies the circulation of lymphoblasts through the blood stream followed by homing into tissues. Extramedullary organs may act as sanctuaries for lymphoblasts, preventing the exposure to adequate levels of chemotherapeutic drugs. Typical extramedullary relapses are seen in testes and in central nervous system (CNS). Homing of leukocytes into the different organs is a complex process in which several molecules have been involved (selectins, chemokines, integrins). *Aims.* We are currently studying the role of the axis chemokine receptors - chemokine ligands in CNS leukemic infiltration of children with B- or T-ALL. In the present abstract we show our initial experience. *Methods.* Marrow aspirates were obtained from 35 children in 11 Spanish pediatric oncology units. We detected the expression levels of 9 CCR and 6 CXCR molecules in ALL blasts at diagnosis by flow cytometry. Levels of chemokine ligands will be quantitated by Cytometric Bead Array in samples of cerebrospinal fluid obtained at the moment of diagnosis. Clinical data were recovered from the clinical records. *Results.* We found that chemokine receptors expression differed between B-ALL and T-ALL blasts. T-ALL expressed significantly higher levels of CCR4 while B-ALL expressed more CXCR5. We also compared the levels of CCRs and CXCRs expression between high- and standard-risk leukemias, and found that high-risk ALL tend to express higher levels of the receptors. This difference was more pronounced among T-ALL. When we analysed only B-ALL (n=24) samples, we found a different pattern of expression depending on the maturation state of the leukemia: pro-B, pre-B and common. CCR4, CCR5, CCR6, CXCR4 and CXCR5 levels were particularly higher among pre-B leukemias compared to those among the other 2 subtypes. Levels of chemokine ligands will be quantitated by Cytometric Bead Array in samples of cerebrospinal fluid obtained at the moment of diagnosis, searching for the ligands of the highly expressed chemokine receptors by lymphoblasts. *Conclusions.* This preliminary analysis of the ongoing study shows that chemokine receptors expression in childhood ALL differs depending upon the lineage and the maturation status of the leukemia. Further analysis will show whether chemokines have a role in CNS leukemic disease in these patients.

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IN VIVO EXPRESSION OF WILD-TYPE NUCLEAR SURVIVIN AND ITS SPLICE VARIANTS 2B AND DELTA-EX3 IN ACUTE MYELOID LEUKEMIA

J. Serrano-Lopez, V. Martin-Palanco, J. Serrano, J. Sanchez-Garcia, R. Rojas, C. Martin, S. Tabares, J. Roman-Gomez, C. Herrera, A. Torres-Gomez

Reina Sofia Hospital, CORDOBA, Spain

Background and Aims. The regulation of apoptotic cells death may have a profound effec on the pathogenesis and progression of Acute Myeloid Leukemia (AML). Survivin (Wild type, 142aa) is a member of the inhibitor of apoptosis protein family (IAP) with a low or even undetectable level in differentiated tissues and cells. By contrast, Survivin expression is detected in most cancer-transformed cells and this finding is associated with chemotherapy resistance, increased tumor recurrence and shortened overall survival, making anti-survivin therapy an attractive modality of cancer treatment. Recently, three different splice variants have been described for human survivin such as survivin-2B with 165 aa and survivin-Delta-Ex3 with 137aa. These variants have different subcelullar location and different funstion. Thus, 2B variant promotes cellular apoptotis, whereas WT and delta-Ex3 exert anti-apoptotic funtion. In this study we analyse the survivin WT expression and its splice variants 2B and DeltaEX3- in nuclear extracts from AML cells. *Methods.* A total of 28 patients diagnosed with AML (19 *de novo* and 9 secondary) were included in this study. Median age was 61.5 years ranging from 12 to 85. Median leukocyte count was 13.15×10^9/L (range: 0.93-285). FAB subtypes were: M0=2; M1=6; M2=8; M3=5; M4=3; M5=4; with the following cytogenetic findings: t(15;17) (5), t(8;21) (2), Complex cariotype (4), del7 (1) and normal (8). Mononuclear marrow cells were obtained by Ficoll/Histopaque gradient centrifugation. Cell lysates were harvested with Q-Proteome cell compartment (Qiagen) and protein concentration assayed using Protein Assay Kit (Bio-Rad). Protein samples (50 μg/sample) were separated on SDS-PAGE (Criterion XT Bis-Tris gels 12%, Bio-Rad). Proteins were transferred to nitrocellulose membrane (Pall Life Science) and block with 5% non-fat dry milk. Primary antibody was anti-survivin and anti-beta-actin (Cell Signalling Technology) were incubated overnight at 4° in blocking buffer. This was followed by incubation with horseradish peroxidase conjugated secondary antibody. Proteins were visualized by enhanced chemiluminescence (ECL-Plus Western blotting detection system) in Chemigenius-2 and quantified using Gene-Tools software. Specificity was assessed by blocking-peptide experiments *Results.* Survivin WT was undetectable in 35.7% samples but its splice variants 2B and Delta-Ex3 were absent in 14.3% and 28.6% respectively. The remaining samples were either low level positive (less than normal bone marrow) or high levels (67.5%, 52.6% and 45% for WT, DeltaEx3 and 2B respectively. There was a statistical correlation between the levels of Survivin WT and the levels its splice variant 2B (p<0.01, R:0,87) and also DeltaEx3 (p=0.07). Neither survivin WT nor any splice variants expression correlated with other biological or clinical data as FAB subtypes, age, White blood count at diagnosis, cytogenetic/molecular risk or secondary-AML. There was no difference in the levels of pro-apoptotic splice variant 2B comparing relapsing and no-relapsing patients. However, the levels of anti-apoptotic survivin WT or the splice variant Delta-Ex3 were lower in relapsing patients (0.7 *vs* 1.4 and 1.2 *vs* 3.0) although these figures did not reach statistical significance. *Conclusions.* Wild-type Survivin and splice variants Delta-Ex3 and 2B are detectable in approximately two thirds of AML samples at diagnosis and half of them display high levels. There is a good correlation between the level of expression of WT and its splice variants. However, the clinical importance of this expression remains to be determined.

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ACQUISITION OF IL-3 INDEPENDENT GROWTH OF BA/F3 CELLS BY A SECONDARY LEUKEMIA ASSOCIATED FUSION GENE, MLL-MAML2

N. Nemoto, Y. Katsura, K. Suzakawa

University of tsukuba, TSUKUBA, Japan

Background. MLL-MAML2 is a newly identified fusion gene in secondary AML, MDS and ALL with inv(11)(q21q23). MLL-MAML2 has been shown to cause aberrant NOTCH signaling. However, the mechanism of leukemogenesis by MLL-MAML2 is largely unknown. *Aims.* To investigate the mechanism, we transfected MLL-MAML2 expression vector into Ba/F3, an interleukin-3(IL-3) dependent cell line. *Methods.* Ba/F3 cells were maintained in IL-3 Medium (RPMI1640medium with 10% Fetal Bovin Selum, 10% conditioned medium from Il-3 producing WEHI cell line). 2×10^6 Ba/F3 cell and 2 μg plasmid DNA were transfected with electroporation. Transfected cells were selected with G418. IL-3 was deprived from culture media. Activation of STAT5, ERK and MEK in Ba/F3 cells were examined by Western blot analysis. The expression of IL-3 was examined with RT-PCR. *Results*. MLL-MAML2 transfected Ba/F3 cells began to proliferate on the 9th day of culturing in IL-3 deprived media. Activation of STAT5, ERK and MEK was detected in IL-3 independent cells but not in MLL-MAML2 transfected cells before IL-3 deprivation. The expression of IL-3 was detected in IL-3 independent cells. *Conclusions.* These data suggested that MLL-MAML2 can transform hematopoietic cells by autocrine of IL-3.

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INVESTIGATION OF 4 MOLECULAR MARKERS IN 65 PATIENTS WITH *DE NOVO* ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE - A SINGLE CENTER EXPERIENCE

B. Katrincsakova, T. Szotkowski, M. Divoka, M. Holzerova, J. Hubacek, K. Indrak, M. Jarosova

University Hospital, OLOMOUC, Czech Republic

Background. A number of leukemia specific cytogenetic abnormalities play a role of independent prognostic factors in acute myeloid leukemia (AML) and are used for risk adopted treatment strategies. However, approximately 50% of newly diagnosed AML have a normal karyotype (NK). *Aims.* Our study was aimed at characterization of AML with NK based on alterations in molecular markers of prognostic significance, such as FLT3, NPM1, MLL and BAALC genes. *Patients and Methods.* Bone marrow or peripheral blood RNA samples obtained at diagnosis from 65 adult AML patients all with NK were analyzed for the presence of mutations in the NPM1 gene (NPM1mut), for internal tandem duplication (ITD) of the FLT3 gene, partial tandem duplication (PTD) of the MLL gene and for expression of the BAALC gene. A method developed by Noguera *et al.* (2005) for simultaneous detection of NPM1mut and FLT3/ITD by capillary electrophoresis (performed on ABI Prism 310) and a commercially available product for BAALC gene expression quantification (Ipsogen, France), performed at LightCycler instrument, were established for screening of the above mentioned molecular changes. Direct sequencing of NPM1 cDNA as well as a previously established conventional PCR specific for FLT3/ITD followed by agarose gel electrophoresis were carried out to verify results of the capillary electrophoresis screening. The presence of MLL/PTD was assessed based on a method of Jamal *et al.* (2001). *Results.* In our cohort, 52% (34/65) of NK AML carry mutations (all heterozygous) in the NPM1 gene, in 42% (27/65) FLT3/ITD is present and high BAALC expression was detected in 31% (20 of 65) of AML samples analyzed; all but 1 of 65 cases are negative for MLL/PTD. Out of 54 curatively treated *de novo* AML patients (30 female; 24 male); median age 55 years /range 22 to 73/, median follow-up time 155 weeks; median overall survival /OS/ 50 weeks), 87% of patients (47/54) achieved complete remission /CR/. In the latter group of curatively treated *de novo* AML patients, the evaluated molecular markers were distributed as follows: 50% (27/54) NPM1mut, 39% (21/54) FLT3/ITD, 33% (18/54) high BAALC, 2% (1/54) MLL/PTD. The coincidence of NPM1mut with FLT3/ITD was observed in 56% (15/27) NPM1mut cases. The statistical correlation of the above mentioned molecular data with the disease course will be summarized and presented. *Conclusions.* Assessment of NPM1 mutations, FLT3/ITD and BAALC expression provided us a tool to uncover at least 1 molecular change in 88% (57/65) of *de novo* AML without any cytogenetic abnormality included in our study. While in the remaining 12% (8/65) of our AML patients with NK none of the molecular markers examined were detected, we will continue in evaluation of additional gene/s alterations, like CEBPA and RAS gene mutations. In summary, our preliminary data provided us an insight into the molecular heterogeneity of AML patients with NK monitored in our center and will guide us in stratification of these patients into prognostic subgroups. However, continual follow-up as well as additional patient enrollment are required to prove the prognostic significance of these findings.

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CORE BINDING FACTOR ACUTE MIELOID LEUKEMIAS (AML): PROGNOSTIC FACTORS

E. Luño,[1] C. Sanzo,[1] M. Xandri,[2] I. Granada,[2] E. Puigdecanet,[3] B. Espinet,[3] M. González,[1] J. Bergua,[4] F. Ramos,[5] N. De las Heras,[5] R. Collado[6]

[1]Hospital Universitario Central de Asturias, OVIEDO; [2]H.Germans Trias i Pujol, BARCELONA; [3]Hospital del Mar, BARCELONA; [4]H. San Pedro de Alcántara, CÁCERES; [5]H Virgen Blanca, LEÓN; [6]H General Universitario, VALENCIA, Spain

Introduction. Acute myeloid leukemia with t(8;21)(q22;q22) and inv(16)(p13q22)/t(16;16)(p13;q22) are included in the same good prognosis category. Most patients achieve a complete remission after standard induction chemotherapy and long-term remission rates have been reported. Despite these results, 20-30% of patients relapse. The aim of the study was to determine the prognostic factors in core binding factor (CBF) *de novo* AML. *Patients and Methods.* 72 patients with CBF leukemia, 48 t(8;21)(q22;q22), 14,6% of them variants translocation, and 24 inv(16) (p13q22) were included. For descriptive statistics, median, range and mean or percentage were calculated. Categorical variables were evaluated with Chi-square analysis and Fisher's test. t-Student was employed to compare continuous variables. Overall survival (OS) and leukaemia free survival (LFS) were plotted by the Kaplan-Meier method and differences between curves were analyzed by the log-rank test. *Results.* 42 males and 30 females. Three t(8;21) were AML-M4 and 45 AML-M2; all inv(16) were AML-M4Eo. Only five t(8;21) and one inv(16) had multilineage dysplasia (p=0,39). In 32 (66,7%) patients a t(8;21) were the sole cytogenetic abnormality, 9 had a secondary change with a sex chromosome lost in seven (14,6%), and a complex karyotype in other seven. 17 (70,8%) had single inv(16), six a associated abnormality and only one multiple changes. Anyone had t(16;16). The median (SD) age was 44 years (18,3) (range 6-82). The mean of age, hemoglobin, platelets, percentage of blasts in the bone marrow didn't showed significant differences, but leukocytes counts was higher for inv(16) (54,6×10^9/L *vs* 15,7 ×10^9/L)(p<0,001), in fact, 10/24 inv(16) and only one t(8;21) had leukocyte counts >50×10^9/L. 66 patient were treated intensively and 25 of them were consolidated with STC (19 Auto and 6 Alo), 37,8% in the t(8;21) group and 38,1% in inv(16) group (p=1). 91% t(8;21) and 86% inv(16) (p=0,6) achieved complete remission (CR) but 24,4% and 33,3% respectively relapsed. The median LFS of patients who achieved CR was not reached with a relapse risk of 33% at ten years (anyone relapsed after twenty month). Patients >50 years (p=0,03) or >65 years (p=0,01) or leukocyte counts >50 ×10^9/L (p=0,009) had shorter LFS. The median OS was 66,2 months. 39 (59%) patients are alive at the end of follow-up. Patients under 50 years (p=0,003) or <65 years (p=0,001) or platelets >50 ×10^9/L (p=0,04) or leukocyte <50×10^9/L (p=0,0007) had a longer overall survival. Other variables including primary cytogenetic abnormality, associated changes or SCT consolidation don't show significant differences in univariate analysis. After multivariate analysis only leukocyte count showed a independent prognostic value for both LFS (OR=3,78, CI 95% 1,24-11,5, p=0,01) and OS (OR=1,37, CI 95% 0,45-9,16, p=0,002), in core binding factor *de novo* AML. *Conclusions.* According our results, patients with inv(16)(p13q22) have frequently a high leukocyte count and this in core binding factor *de novo* AML is a bad prognostic factor. It seems appropriate to design a post- remission strategy for this patient subgroup.

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MICRORNA PROFILING IN ACUTE MYELOID LEUKEMIA

A. Santoro,[1] G. Cammarata,[1] C. Agueli,[1] F. Fabbiano,[1] M. La Rosa,[1] D. Salemi,[1] L. Dagnino,[1] A. Marfia,[1] M.G. Bica,[1] L. Cascio,[1] F. Messana,[1] M. Pagano,[1] S. Mirto[1]

[1]A.O. V.Cervello, PALERMO, Italy

Introduction. Naturally occurring microRNA (miRs) are an abundant class of 19-25 nucleotide non-coding RNAs that play important roles in cell proliferation and differentiation by acting as inhibithors of specific target genes at post-transcriptional level (Ambros, 2003). MicroRNAs may be important regulators of mammalian hematopoiesis (Chen, 2005; Fazi, 2005; Garzon, 2006; Debernardi, 2007) and their involvement in the pathogenesis of chronic lymphocytic leukemia has been recently demostrated (Calin ,2002; Cimmino, 2005). Nearby 50% of known mirNAs are found in clusters, moreover, plus than 50% of miRNA genes are located in cancer-associated genomic regions or in fragile sites, suggesting that miRNAs may play an important role in the pathogenesis of cancer (Calin, 2004). Specific miR genes may contribute for oncogenesis working as classical tumor suppressor genes (TGS) or as classical oncogenes (OG), they may be important regulators of mammalian hematopoiesis and may cause deregulation of target genes involved in cellular processes critical for leukemia development. Several lines of evidence support the role of miRNA in biological processes that are aberrant in cancer, such as differentiation and apoptosis. Actually we have very few data available on expression profile of miRs in acute leukemia. To analyze the role of miRs in leukemogenesis we performed a quantitative expression study of set of 380 human miRNA genes in AML blasts and in normal bone marrow cells. *Methods.* Total RNA samples for the expression analysis were isolated from leukemic blast cells and from total bone marrow cells of healthy subjects.To detect miRNA expression level, we used the specific assay available for human microRNA (TaqMan Low Density Array - Human MIRNA, 4342265 Applied Biosystems) that contains 380 miRs selected and spotted on a MicroFluidic Card (MFC)(Applied Biosystems). TaqMan Micro RNA Assays are designed to detect and accurately quantitate mature miRNAs using Applied Biosystems real-time PCR instruments. The Low Density Array

was performed in accordance to manufacturer's protocols. PCR data were quantified using the SDS 2.3 software and normalized using the RNU48 as endogenous control. The cycle threshold (Ct) value, calculated relatively to the endogenous control was used for our analysis (ΔCt).To calculate relative changes in gene expression between different sample we used the 2-ΔΔCT method. We analyzed gene expression by 3 different statistical methods, namely the Significance Analysis of Microarrays (SAM), the Empirical Bayes Analysis of Microarrays (EBAM), and Multiple Testing Procedure (MTP). *Results and Discussion.* In view to confirm the feasibility of miRNA gene expression studies and to validate the TaqMan Low Density Array - Human MIRNA platform we performed 22 MCF assay using cDNA from 21 AML patients (1 FAB-M0, 4 M1, 4 M2, 3 M4, 4 M5, 1 M6 and 4 secondary leukemia) and one pool of 4 bone marrow healthy donor. All the experiment were done successfully allowing us to choose the RNU48 as the more stable endogenous control. Our preliminary data suggests a strong correlation of expression levels of some miRNA such as miR-181, miR-155, miR-221 and miR-222 , with morphological and genetic sub-type.

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HEALTHY BLOOD DONOR WITH UPPER-LIMIT HEMATOCRIT AND PLATELET VALUES, CARRYING V617F MUTATION IN JAK2 GENE

P. Bianchi, E. Fermo, F. Mozzi, M. Marconi, A. Zanella

Fondazione IRCCS OMPMARE, MILANO, Italy

Background. The somatic mutation V617F of JAK2 gene has been identified as a pathogenic factor in typical chronic myeloproliferative diseases (MPDs), in particular PV, TE, and myelofibrosis with myeloid metaplasia. JAK2V617F mutation may occur at low level also in apparently healthy subjects: 37/3935 subjects (Xu *et al.,* 2006) and 5/52 healthy blood donors (Sidon *et al.,* 2006); however the mutation has not been detected in other studies on normal subjects (Passamonti et al 2006). JAK2V617F has been recently found in 0.2% of haematologically normal young women primigravides, associated with an increased risk of foetal loss (Mercier, 2007). These data suggest that JAK2V617F mutation may occur in the absence of MPD phenotype or that is not sufficient per se to induce MPDs. *Aims.* We look for the presence of JAK2V617F mutation in healthy blood donors with confirmed upper-limit Hct and/or Plts values. Actually, previous studies indicated that some upper-limit Hct donors had early stages polycythemia vera (Zanella *et al.*, 1987). *Methods.* We studied 177 consecutive repeat blood donors (92 M, 85 F; median age 45 years, range 19-66) displaying Hct and/or Plts values higher than the 95° percentile of the normal reference distribution (Hct >0.47 for M and >0.42 for F; Plts >300×10^9/L), confirmed on at least two different occasions in the last 12 months. All subjects had been admitted to blood donation on the basis of negative clinical history and normal results on both physical examination and routine laboratory testing. 83 of them (55 M and 28 F) had upper-limit Hct levels (median 0.48, range 0.47-0.51 for M; 0.43, range 0.42-0.47 for F); 85 had Plts >300×10^9/L (median 338×10^9/L, range 300-454), and 9 donors had both upper-limit Hct and Plts. DNA was extracted from whole blood; all samples were analyzed by allele-specific PCR according to Baxter et al (2005), and by fluorescent allele specific PCR (McClure *et al.,* 2006) on ABI PRISM 310 Genetic Analyzer. *Results.* One blood donor was found to be positive for JAK2V617F mutation by fluorescent PCR, showing a positive signal when compared to a positive control corresponding to 2% of mutated allele. An eight years haematological follow-up showed haemoglobin levels ranging from 14.5 to 16.9 g/dL, Hct from 0.40 to 0.49, Plts from 360 to 531×10^9/L and WBC from 6.3 to 9.4×10^9 /L. The presence of mutation was confirmed a second time on purified granulocytes 6 month after the first examination. To search for a latent form of polycytemia vera the patient underwent red blood cell mass determination which gave normal results (24.8 mL/kg; ref. values <36 mL/kg). Also serum EPO levels (5.8 mUI/mL n.v. 3,7-29,5) and LAP (50; n.v. 11-94) were normal. *Conclusions.* Our data support the hypothesis that low levels of JAK2V617F mutation may be present in healthy individuals; only a long follow-up will allow to conclude if the finding of JAK2V617F mutation in normal subjects with upper-limit hematocrit and platelets values may be predictive of pre-clinical state of MPD.

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ASPIRIN NON-RESPONSIVENESS AS MEASURED BY PFA-100 IN PATIENTS WITH CORONARY ARTERY DISEASE

Z.S. Stanojkovic

Blood Transfusion Institute Nis, NIS, Serbia

Background. Aspirin treatment in patients with acute coronary disease has been shown to reduce the risk of recurrent vascular event and death by 25%. The use of aspirin is not uniform for all patients and recurrent events may be due to aspirin resistance. *Aims.* Monitoring the effects of the aspirin treatment in patients with coronary artery disease. *Methods.* Platelet activation was measured in 90 patients with coronary artery disease with PFA-100 (Dade Behring). All patients were receiving daily aspirin dose of 100 mg. The samples were taken with citrate (3,2%). The normal range closure times (CT) with PFA-100 were 55-112 (cartridge with collagen and ADP-CADP) and 79-164 (cartridge with collagen and epinephrine-CEPI). *Results.* CEPI values under 180s are marked as resistant. 12 patients (13%) of 90 were identified as aspirin resistant group who had CEPI value 160±17s. The same group of patients had lower values CADP 72±9s. In the group of 78 patients who were receiving daily aspirin dose, too, CEPI value 285 ±15s and CAPD median 88±10s. *Conclusions.* PFA-100 provides a relatively simple test to potentially identify aspirin-resistant patients.

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THE INFLUENCE OF CIGARETTE SMOKING ON THROMBOEMBOLIC EVENTS IN A COHORT OF HEREDITARY THROMBOPHILIC PATIENTS

M. Pacurar,[1] D. Lungeanu,[2] D. Nicola,[1] F. Cheveresan,[3] M. Ionita[3]

[1]Municipal Hospital, TIMISOARA; [2]University of Medicine and Pharmacy of Timisoara, Department of Medical Informat, TIMISOARA; [3]University of Medicine and Pharmacy of Timisoara, Hematology Department, TIMISOARA, Romania

Background. Even if the precise mechanisms are unclear, numerous studies have found a clear and significant association between cigarette smoking and cardiovascular-related mortality and morbidity. *Aims.* To determine the influence of cigarette smoking on the number and incidence of thromboembolic events in a cohort of thrombophilic patients with an established prothrombotic risk factor. *Methods.* Individuals with a suspicion of hereditary thrombophilia were enrolled between November 2000 and January 2006 as part of a larger thrombophilia study. Those with determined thrombophilic risk factors (resistance to activated protein C, deficiency of antithrombin, protein C or S, high activity of factor VIII, hyperhomocysteinemia or a combination of these defects) formed the study group. The thromboembolic events were recorded. *Results.* Eighty seven patients aged over 15 (41 women and 46 men) were found with at least one thrombophilic risk factor and 19 of them were cigarette smokers. The mean number and the incidence of the thromboembolic events were higher among the cigarette smokers (2.316 and 1.686, respectively) compared to those encountered among the non-smokers (1.765 and 1.26, respectively). *Conclusions.* We concluded that smoking may indeed increase the number of thromboembolic events in thrombophilic patients, although the statistical significance could not be achieved in our study due to the small number of subjects. Further data should be obtained for conclusive results.

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AMENDABLE FACTORS FOR BLEEDING DIATHESIS IN CASES BLEEDING ASSOCIATED WITH WARFARIN USAGE

E. Esin,[1] C. Beyan[2]

[1]Ankara Numune Education and Research Hospital, ANKARA; [2]Gulhane Military Medical Academy, ANKARA, Turkey

Background. Warfarin is a widely used oral anticoagulant for various indications. *Aims.* The purpose of our study is to determine amendable factors for bleeding diathesis in cases who bled due to warfarin. *Methods.* This study was performed on 85 cases, 44 was women, who admitted to emergency service for bleeding and whose ages range between 32-89 years. *Results.* On admission, there were melena in 35 cases (41,2%), hematuria in 23 (27,0%), ecchymosis in 22 (25,9%), epistaxis in 18 (21,2%), hematemesis in 10 (11,8%), hemoptysis in 7, hematom in 3, vaginal bleeding in 3, hematochesia in 1, delayed bleeding after tooth extraction in 1 and bleeding from lesion in 1 case. There was bleeding from multiple sites in 33 cases (38,8%). Time from bleeding to hospital admission was mean 2,7±3,2 days (0-15 days, median 1 day). Mean

weekly warfarin dose on hospital admission was 31,5±10,6 mg/week (8,7-57,5 mg; median 35,0 mg). Time from the start of warfarin use to bleeding was 30,7±48,1 months (1-229 months; median 10 months). Bleeding was observed in the first month of warfarin use in 25 cases (29,4%). Twentynine cases (34,1%) had previous bleeding episodes. Ind›cations for warfarin treatment were chronic atrial fibrillation in 28 (32,9%), cardiac valve operation in 20 (23,5%), cerebrovascular accident in 17 (20,0%), venous and/or pulmonary embolism in 16 (18,8%) and peripheral arterial events in 4 cases. Treatment was started by cardiology in 33 (38,8%), cardiothoracic surgery in 29 (34,1%), neurology in 12 (14,1%), pulmonary medicine department in 4, hematology in 3, internal medicine department in 2 cases and neurosurgery and thoracic surgey in 1 cases each. Eighteen cases (21,2%) were followed by a regular physician. Only 26 cases (30,6%) were on routine follow-up. Number of drugs taken by patients other than warfarin was mean 5,0±2,6 (0-14; median 5 drugs). Forty-two cases (49,4%) were using aspirin, 29 cases (34,1%) were using nonsteroidal anti-inflammatory drugs other than aspirin and 16 cases (18,8%) were using antibiotics on admission to emergency service for bleeding due to warfarin. Meanwhile, 23 cases were using various unprescribed drugs as analgesics without physician order (5 cases were using aspirin and 18 cases were using nonsteroidal anti-inflammatory drugs). Thirty-six cases (42,3%) were knowing that warfarin use necessiated regular control. The number of cases knowing that a laboratory test is needed for follow-up of warfarin use were 32 (37,6%). Only 28 cases (32,9%) were knowing that this drug could cause bleeding. Seven cases (8,2%) were aware that warfarin might be influenced by other drugs and only 1 case (1,2%) was aware that foods might influence warfarin treatment. *Summary and Conclusions.* As a result, in the majority of cases admitted to emercency service for bleeding due to warfarin use, there were great lack of knowledge which might cause bleeding diathesis. Educational programs directed to patients using warfarin and physicians following these patients should be evolved to reduce morbidity and mortality caused by bleeding due to warfarin usage.

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STUDY TO ASSESS THE CORRELATION OF PREDICTIVE COUNT OF CD34 CELLS IN THE BLOOD TO THE YIELD IN PERIPHERAL BLOOD STEM CELL HARVEST

S. Choudhuri, A. Bloor

Christie Hospital NHS Trust, MANCHESTER, UK

In the UK , transplant centres routinely perform CD 34 cell count by flow cytometry in peripheral blood . However, controversy exists as to whether the number of CD 34 cell count in the peripheral blood correlates with CD 34 cell count in the apheresis component. In our survey we aimed to analyse the correlation between the predictive count and yield of CD34 cells and thereby its utility in ensuring correct timing , maximum yield , cost effectiveness and the confounding factors which could have possibly contributed to the poor correlation. Retrospective case note and data base review was performed on patients undergoing apheresis from June 2002 to June 2007 in a large UK Bone Marrow Transplant Centre. CD 34 predictive and yield was charted and correlation calculated by Spearman's Rho 2 tailed method. A total of 417 patients comprising of Myeloma (49.4%), Non Hodgkin's lymphoma (NHL) (21.1%), Hodgkin's lymphoma (HL) (15.1%), others (14.4%) was identified. Though clinically significant, a poorer correlation coefficient of 0.70 was found in lymphoma patients in comparison to myeloma patients (0.73). Further analysis of the lymphoma patients in the 5 year and the latest 1 year (June06 to June 07) period revealed correlation coefficient between the predictive and yield in NHL patients of 0.75 (5 years) and 0.62 (1 year) and in HL patients of 0.78 (5 years) and 0.69 (1year). 7 patients (6 males) ,average age of 43.2 years , with high predictive and subsequent poor collect was identified in the 1 year period. They had a diagnosis of HL (42.8%) , Follicular lymphoma (42.8%) and T cell ALL (14.2%). 42.8% patients had received 4 and 28.5% had received 3 previous lines of treatment. 3 of 7 patients encountered problems with intra venous access during the procedure. 6 used filgrastim and 1 used lenograstim. 3 patients with weights of 114 ,110 and 146 kilogram had required double dose Gcsf. 6 of 7 (85.7%) procedures were performed by operators who at the time were trainees with limited experience. This study concludes that a good correlation exists between the predictive and yield CD 34 cell counts and therefore should be continued to be performed prior to the harvest. However, elderly male patients and low grade lymphomas seem to fare less well in terms of correlation . Extensive pre-treatment should be avoided if feasible. No definite association was detected between a poor correlation and the type and dose of gcsf used. We recommend attention to thorough examination of the patient prior to the procedure with a view to identify access problems and insert femoral line if need be to save multiple change of access sites and adequate training and supervision of trainee staff by more experienced operator.

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AUTOLOGOUS BONE MARROW-DERIVED PROGENITOR CELL TRANSPLANTATION FOR MYOCARDIAL REGENERATION AFTER ACUTE MYOCARDIAL INFARCTION-CASE REPORT (FIRST EXPERIENCE)

A. Pivkova,[1] L. Cevreska,[1] Z. Stojanoski,[1] S. Genadieva Stavrik,[1] O. Karanfilski,[1] H. Pejkov,[2] B. Georgievski[1]

[1]Universirty Clinic of Hematology, SKOPJE; [2]University Clinic for cardiovascular diseases, SKOPJE, Macedonia

Background. Recent clinical data suggest that the transplantation of bone marrow derived, or circulating blood progenitor cells, may beneficially affect postinfarction remodelling processes after acute myocardial infarction. We present our first experience with infusion of autologous BM derived stem cells in patients with AMI. Two male patients (age range 33-41 years) with the first extensive anterior, ST elevation, acute myocardial infarction (AMI), were treated by primary angioplasty. Bone marrow derrived stem cells were administered by intracoronary infusion whitin 30 days after the infarction. Bone marrow was harvested in the amount of 1000 ml by multiple aspirations from posterior iliac crest under general anesthesia, and aseptic conditions. Stem cells were separated from bone marrow with Cobe Spectra cell separator, stored over night on +4°C and infused through the catheter into the infarct-related artery in 8 equal boluses of 20 mL with median number CD34 cells of $1,6\times10^6$/mL. Myocardial viability in the infarcted area was confirmed by dobutamine stress echocardiography testing 10-14 days after infarction. Early findings in two patients showed significant improvement of left ventricular systolic function 3 months after the infarction. There were no major cardiac events after the transplantation during further follow-up period (30-120 days after infarction). Significant improvement in myocardial perfusion in the patients 4 months after the infarction was also detected. *Conclusions.* Preliminary results of the study showed that the transplantation of bone marrow-derived progenitor cells into the infarcted area was safe, and feasible, and might improve myocardial function. Further follow-up will show if this treatment is effective in preventing negative remodeling of the left ventricle and reveal potential late adverse events (arrhythmogenicity) and propensity for restenosis.

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AUTO-IMMUNE CYTOPENIA AND HAEMOPOIETIC STEM CELL TRANSPLANTATION

M. Benakli, R. Ahmed Nacer, R. Belhadj, F. Mehdid, N. Rahmoune, M. Baazizi, A. Talbi, R.M. Hamladji

Pierre Marie Curie Center, ALGIERS, Algeria

Introduction. contrary to the autograft , allograft of haematopoietic stem cells (HSC) presents immunological complications were graft *vs* host disease (GVHD) and late immunologic recovery after graft are factors of comorbidities and autoimmune dysfunctions. We report in this study the incidence of auto - immune cytopénias observed in 858 patients underwented grafts of HSC. *Material and Methods.* from April 1998 to December 2006, 916 grafts of HSC underwented for malignant and no malignant disorders. For the study, 858 patients(pts) are appraisable (Autografts: 223, Allografts: 635 of whom 458 myéloablative and 177 non myéloablative conditioning). The intensification and the conditioning regimens included chemotherapy alone. For allografts, GVHD prophylaxis associated ciclosporine with methotrexate or cellcept. During the follow-up, the diagnosis of auto - immune cytopénia is assessed after the exclusion of another aetiology responsible of cytopenia, the bone marrow examination, the direct Coombs test and the immunosuppression treatment response. At Dember 2006 maximal follow up is 118 months and minimal 12 months. *Results.* among the 223 pts with autograft, 2 auto-immune thrombocytopenic purpura (AITP) preceding relapse were observed (1 NHL, 1 Hodgkin disease) in a median delay of 415 days, one response to steroids and the other no response to steroids but responsive to Rituximab. Among the 635 pts with allograft, 7 (1,1%) presented an auto - immune cytopenia (4 AITP, 2 Evans syndroms and 1 auto - immune hémolytic anemia) occurring in a median delay of 349 days (42 - 1110). For 4 pts (1 AIHA, 2 AITP, 1 Evans), a GVHD preceded the auto immune cytopenia. From a therapeutic view, corticotherapy permitted a response for 3 pts (1 AITP, 1 AIHA, 1 Evans). Two pts no

response to steroids (1 AITP, 1 Evans) evolved well under Rituximab. One patient (AITP) corrected his rate of platelets spontaneously. One patient (AITP) died in a chronic GVHD. *Conclusions.* auto - immune cytopenia occurring after autograft seem bound to the disease, inversly after allograft an immunological conflict bound to the graft. The Rituximab seems to be an efficient alternative therapeutic for pts with no response to steroids.

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ALLOTRANSPLANTATION FOR CHRONIC LYMPHOCYTIC LEUKAEMIA A FOLLOW-UP OF 12 PATIENTS

J.H. Holowiecki, M. Krawczyk-Kulis, B. Stella-Holowiecka, T. Czerw, J. Wojnar, M. Markiewicz, S. Giebel

Silesian Medical University, KATOWICE, Poland

The role of allogenic hematopoietic stem cell transplantation (alloHSCT) in CLL patients continues to be defined in clinical trials. It seems to offer the only curative option but the relatively high transplant related mortality (TRM) is the major obstacle. Thus it should be considered for younger patients who fail to respond to purine analogues-based therapy, relapse <12 months after such therapy or have adverse cytogenetic features. *Materials.* Twelve patients (F/M=6/6), median age 44y (36-53), time from diagnosis to alloHSCT 1,8y (0,5-7,5) were analyzed. Before alloHSCT patients were treated using 1-5 different chemotherapy regimens, all but one obtained purine analogues-based therapy. Other treatments included radiotherapy (n=2), anti-CD20 MoAb (n=2), anti-CD52 MoAb (n=2) and repeated 2 autologous HCT (n=1). Disease status at alloHCT was: CR n=4, PR n=4, NR n=4. The indication for alloHSCT was purine analogues refractory/relapsed disease or high risk disease (for patients in CR). AlloHCT characteristics: HLA matched Sibling Donor HSCT (n=10), HLA single allele mismatched SibDHSCT (n=1), matched Unrelated Donor HSCT (n=1). Stem cell source for SibD transplant: bone marrow -3, peripheral blood -7 (two using positive selection of $CD34^+$ cells and CD3 cell add back), BM+PB -1, for URD-HCT: bone marrow in 1 pt. Conditioning: myeloablative Ctx+TBI (n=2); Ctx+TBI+alemtuzumab (n=1) and potentially myeloablative TreoFlu: treosulfan (14 g/m^2 ×3), fludarabine (30 mg/m×5) (n=2); reduced intensity (RIC): MelFluCam: alemtuzumab (20 mg ×5)+fludarabine (30 mg/m^2 x5)+melphalan (140 mg/m^2) (n=7). The number of transplanted cells: nucleated cells $4,25\times10^8$ (0,043-12) ; CD34(+) cells $4,34\times10^6$ (1,1-9,6) ; $CD3^+$ cells 35×10^6 (12,7-411) / kg recipient body weight. All transplantations were performed in intensive care, sterile air units. GVHD prophylaxis consisted of cyclosporine A and methotrexate. *Results.* All of 12 patients engrafted, but one after second transplantation. Hematopoietic recovery was as follows: granulocytes to 0,5 G/l -21 d (13-36) ; PLT to 50 G/L -20d (13-40). Detailed results are summarized in Table 1.

Table 1.

Conditioning regimen	Myeloablative (n=5 pts.)	Reduced intensity RIC (n=7 pts.)
Primary engraftment	5	6
Donor lymphocyte infusion (DLI)	-	3
Progression after alloHSCT	-	2
Alive in CR	3	4
Alive in PR	-	1
Death	2	2

The probability of OS and DFS for the whole group at 6,4 years equals 49% and 42%, respectively with median observation time of 2,7 y (0,8 -6,4). This observation compares well with recent other CLL alloHSCT data and suggests that allotransplantation offers an effective treatment with curative potential for high risk subgroup of CLL patients. Use of RIC may reduce TRM but it seems to be paid by impaired engraftment and higher probability of progression.

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THE ROLE FOR AUTOLOGOUS STEM CELL TRANSPLANTATION AS INTENSIVE THERAPY IN HEMATOLOGICAL DISEASES: SINGLE INSTITUTION REPORT

J.A. Lopez Lopez,[1] P.A. Gonzalez Sierra,[1] M.M. Anguita Arance,[1] D. Gutierrez-Meca Maestre,[2] J.M. Ramirez Huerta,[1] F. Gamez Contreras,[1] S. Esteban Muñoz,[1] M. Nieto Hernandez,[1] M. Trujillo Perez,[1] A. Dominguez Arranz,[1] C. De Santis Scoccia,[1] M.L. Escudero Piedra,[1] S. Duran Nieto,[1] A. Carrero Gonzalez,[1] A. Alcala Muñoz[1]

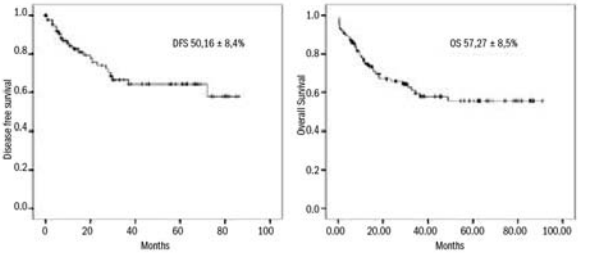
[1]Complejo Hospitalario Jaén, JAÉN; [2]Hospital de Torrevieja, TORREVIEJA, Spain

Background. Transplantation of autologous haematopoietic stem cells (ASCT) is an established therapy today applied for many severe haematologic malignancies. Compared to allogenic, autologous bone marrow transplantation has a lower regimen-related mortality due to absence of graft *vs* host disease, but relapse rates are higher. *Aims.* To assess the characteristics, outcome, results, disease free survival (DFS), overall survival (OS), transplant related mortality (TRM), in our patients with an hematological disease which ASCT was performed in. *Patients and Methods.* We retrospectively analized our transplant activity since 1999 until january 2008. A hundred patients received chemotherapy regimen adapted to hematological disease and after that, high dose chemotherapy followed by ASCT wich were obtained from peripheral blood. Median age 41,2 years (range 9 to 66). 58 of them were male and 42 female. Diagnoses distribution are shown in Table 1. All patients were mobilizated in the recovery phase post-chemotherapy with G-CSF.Prophylactic antibiotic, aciclovir and granulocite-colony stimulation factor were administred to all patients. Regimen related toxicity (RRT) was graded according to NCI-CTC 2.0. Kaplan-Meier OS, DFS and TRM were calculated. *Results.* No adverse event was observed during stem cell infusion. Engraftment day occurrs at day 9 (range 6-12). Digestive toxicity (mucositis) was the main RTT: grade III-IV in 20 patients. Only one patient had a severe hepatic veno-occlusive disease. Early TRM occurred in 6% of patients. No late TRM was observed. Microbiological isolations were demonstrated in 35 patients (S. Haemoliticus in a high percentage). At the time of analysis 63 patients were alive and 37 had died. Cause of death was TRM in 6%, relapse or progresion after transplant in 25 patients. DFS and OS rates were: 60,16±8,4% and 57,27±8.5%, respectively after a median time of 37,6 months (range 2.57-91).Survival by disease groups are shown in Table 1.

Table 1. OS and DFS in our experience.

Hematological disease	Patients (male/female)	Pre-transplant stage (type of response to prior chemotherapy regimen)	Conditioning regimen	OS by disease (CI 95%)
Acute myeloid leukemia (AML)	11 (5/6)	11 CR	BEA (from Gondo et al.)	66,58±23,8%
Acute lymphoblastic leukemia (ALL)	10 (6/4)	10 CR (9 CR1, 1CR2)	BuCy	29,4±10,8%
Hodgkin's disease (HD)	16 (10/6)	10 CR, 6PR	CBV	49,65±17,9%
Non-Hodgkin's lymphoma (NHL)	45 (24/21)	9 Low grade lymphoma (all in CR2/CR3) 36 High grade lymphoma (31 CR1/CR2 and 5 PR)	BEAM	60,8±11,4%
Multiple myeloma (MM)	18 (12/6)	10 CR, 5 PR, 3 VGPR	MEL-200	39,9±17,8%
				OS 57,27±8,5%

Note. Cr: complete remission; PR: partial remission; VSPR very good partial response



Conclusions. 1) Autologous stem cell transplantation in acute leukemias is controversial but it is less effective at treating ALL than AML, as we

can see in this study. 2) The current results suggest that ASCT is a good proposal for consolidation in a group of lymphoma patients sharing adverse prognostic factors with a high risk of relapse. 3) Our statistics in OS, DFS and TRM are similar to others published. We are still working on improve our results and the outcome of patients that undergo ASCT. 4) Further studies should be done to investigate new approaches to prevent relapse post-ASCT.

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ALLOGENEIC BONE MARROW TRANSPLANTATION WITH NON-MYELOABLATIVE CONDITIONING IN PRIMARY SEVERE IMMUNODEFICIENCIES - THE REPORT OF TWO CASES

A. Krasowska-Kwiecien, J. Gozdzik, Sz. Skoczen, A. Wedrychowicz, O. Wiecha, W. Czogala, M. Majka

Jagiellonian University Institute of Pediatrics, KRAKOW, Poland

Stem cell transplantations (SCT) in severe primary immunodeficiency diseases are performed at the marked risk of complications due to serious clinical conditions prior to the transplant, mostly infections, i.e. recurrent pneumonia with respiratory insufficiency or sepsis. In some cases SCT must be carried out in active viral or fungal infection, if resistant to antimicrobial agents. A consensus about conditioning in these cases still remains to be established. Considering the risk of the procedure and that full chimerism is not an absolute objective, a reduced intensity conditioning (RIC) transplantation might be an optimal approach. *Aims.* We present two cases of primary severe immunodeficiences treated with HLA identical sibling donor transplantation preceded by RIC. *Methods.* The first patient was diagnosed with Omenn syndrome (5-month-old at the time of SCT), complicated by cytomegalovirus infection refractory to anti-viral agents and the other child with chronic granulomatous disease (2-year-old at SCT) was transplanted despite strong suspicion of pulmonary aspergillosis, as complete resolution of pulmonary infiltration could not have been achieved with multiple anti-fungal treatment. Because of the active infectious state and the severe general condition of both patients, RIC consisting of fludarabin in a total dose of 150 mg/m^2 and melphalan of 140 mg/m^2 was introduced. The children received 7×10^6 CD34$^+$ cells/kg, and 4×10^6 CD34$^+$ cells/kg respectively. For GvHD prophylaxis CsA was employed. *Results.* Both patients developed absolute neutropenia and thrombocytopenia after RIC SCT. Recovery of neutrophils of more than 1.0×10^9/L on the day +9 and platelets above 20.0×10^9/L on the day +19 were observed in the first infant and both neutrophils and platelets recovery on the day +22 in the other child. The early clinical post-transplant course was unremarkable, the first child presented transient systemic skin rash as an acute graft *vs* host disease (GvHD), the other patient showed no GvHD. No signs or symptoms of other transplant-related organ toxicity or chronic GvHD were observed in both children. In the first patient a persistent haematopoietic full donor chimerism was demonstrated in the blood cells from the day +14, which has maintained since then. In the second child full donor chimerism was detected on the day +24, but it was gradually decreasing in following examinations despite cyclosporine withdrawal. The chimerism reached the value 25% when adoptive therapy with donor lymphocyte infusions (DLI) was administered. The patient received 2,3×10^6 donor CD3$^+$ cells /kg body weight in three DLI procedures. Then the chimerism accounted for 53% of donor cells in recipient bone marrow and 44% in peripheral blood. Both patients' clinical state is good, with the immune system gradually recovering. The first infant has been showing the negative molecular test for CMV since the day +64, and the second child presents no signs of mycosis. *Conclusions.* We suggest that in primary severe immunodeficiency syndromes in the presence of serious clinical conditions the RIC allogeneic transplantation could be recommended as an effective therapy with a reduced risk of complications related to both SCT and the underlying disease.

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HISTORY OF RENAL FUNCTION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES BEFORE AND AFTER BONE MARROW TRANSPLANTATION

L. Zubkiewicz,[1] T. Burycz,[2] A. Wiela-Hojenska,[3] K. Glowacka,[4] K. Kuliczkowski,[5] K. Orzechowska-Juzwenko[6]

[1]Wroclaw Medical University, WROCLAW; [2]Herbapol Inc. Experimental Laboratory, WROCLAW; [3]Department of Clinical Pharmacology, Wroclaw Medical University, WROCLAW; [4]Departmen of Clinical Pharmacology, Wroclaw Medical University, WROCLAW; [5]Department of Hematology, Wroclaw Medical University, WROCLAW; [6]Department of Clinical Pharmacology, Wroclaw Medical Univesity, WROCLAW, Poland

Aims. The aim of our study was to evaluate the renal function in patients with hematological malignances treated with busulfan p.o. and cyclophosphamide i.v. (BuCy) as conditioning regiment prior allogeneic bone marrow transplantation. *Methods.* The observations were carried out in 22 patients (10 - with acute myeloblastic leukemia, 3 - with acute lymphoblastic leukemia, 8 - with chronic myelogenous leukemia and 1 - with osteomyelofibrosis) before the treatment and after 1st, 3rd and 4th day of busulfan administration, and 1 week after bone marrow transplantation. The glomerular function was monitored by estimation of the creatinine concentration in urine, and creatinine, urea, uric acid and electrolytes concentrations in plasma. The tubular function was studied by the estimation of the lysosomal enzyme N-acetyl-beta-D-glucosaminidase (NAG) activity in urine and the concentration of low molecular weight protein alfa-1-microglobulin (α1m), as early markers of renal tubular dysfunction. *Results.* The mean values of glomerular function parameters were in normal range but the plasma concentration of urea after 4 days of busulfan administration, creatinine - 1 week after transplantation differed significantly regarding the values obtained before the beginning of cytostatic therapy. Among the parameters of tubular function changes in the urinary NAG activity were not significant but after 3rd (3.92±3.04 U/g creatinine) and 4th (4.88±5.77 U/g creatinine) day of busulfan its activity has slowly increased (before busulfan - 2.86±2.47 U/g creatinine, after 1st day of therapy - 2.99±1.61 U/g creatinine). We observed statistically significant increase in α1m concentration after 3rd (2.61±2.60 mg/g creatinine) and 4th (4±4.07 mg/g creatinine) day of myeoablative treatment (before busulfan - 1.04±0.71 mg/g creatinine, after 1st day of therapy - 1.39±0.91 mg/g creatinine). *Summary*. Our results lead to conclusion that in patients with hematological malignancies administration of busulfan high doses may damage renal tubules. This should be considered during the individualization of the therapy.

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CORRELATION OF INFUSED PERIPHERAL BLOOD AUTOGRAFT ABSOLUTE LYMPHOCYTE COUNT AND SURVIVAL AFTER AUTOLOGUS STEM CELL TRASPLANTATION IN PATIENTS WITH HODGKIN AND NON-HODGKIN LYMPHOMA

E. Eduardo,[1] M.J. Majado,[1] A. Minguela,[2] M.I. Macizo,[2] F. García-Candel,[2] M.V. Sanchez-Martinez,[2] M.V. Sanchez-Martinez,[2] C. García-Insausti,[1] A. Morales,[1] J.M. Moraleda[1]

[1]Hospital Virgen de la Arrixaca, MURCIA; [2]Hospital Virgen Arrixaca, MURCIA, Spain

Background. Early lymphocyte recovery predict better progression-free survival (PFS) and overall survival (OS) after autologus stem cell transplantation (ASCT) for lymphoma patients and is an independent prognostic factor for PFS. Factors affecting early lymphocyte recovery are poor known but it seems to be an important factor the dose of infused lymphocytes in the peripheral blood autograft (A-ALC) demonstrated to be an independent prognostic factor for OS and PFS. In addition there is a strong correlation between infused A-ALC and absolute lymphocyte count on day 15 (ALC-15). *Aims.* To show this results in our patients and investigate the correlation between A-ALC, infused CD34, the counting of colony-forming units granulocyte-macrophage (CFU-GM) and infused bone marrow mononuclear cells (MNCs). *Methods.* We analyzed 20 patients (males 17 and females 3; median age 46, range 13-64) with non-Hodgkin's lymphoma (17 patients) and Hodgkin´s disease (3 patients) underwent ASCT. Data analyzed were PFS, OS, A-ALC, infused CD34 cell, autograft CFU-GM , infused MNCs, ALC-15, ALC on day 30 (ALC-30), ALC at 3 months (ALC-3 m), ALC at 6 months (ALC-6 m) and ALC at first year of ASCT (ALC-1y). *Results.* Data are shown in the Table 1. We have not found correlation between A-ALC and ALC-15, and with PFS or OS as in previous reports. In these, the A-ALC cut-off was 0,5×10^9/Kg and after ASCT the day of measurement of ALC was the day 15. We found statistically significant differences between ALC-15 and ALC in the other different moments of measurement. There is strong correlation between infused A-ALC and infused cell CD34, and with MNCs, as well as between CD34 and MNCs, and CFU-GM; we also observed strong correlation between infused MNCs and CFU-GM formed. *Conclusions.* Lack of correlation between A-ALC and ALC-15, PFS and OS is probably due to the limited number of patients studied and the limited number of infused A-ALC (0,23×10^{9}Kg *vs* 0,5×10^9/Kg). ALC-30 could be studied and investigate correlation with A-ALC, PFS and OS in most comprehensive patients series. Like pre-

vious reports our findings show no correlation between CD34 cell count and ALC but there is a correlation between CD34 cell count and infused A-ALC, both being two important autograft cellular lineages, the first involved in hematopoietic recovery and the second involved in immune recovery and in the effect graft *vs* lymphoma.

Table 1.

A-ALC X10^3/Kg	CD34 x10^6/Kg	MNCs x10^8/Kg	CFU-GM x10^6/Kg	ALC-15 x10^3/mL	ALC-30 x10^3/mL	ALC-3m x10^3/mL	ALC-6m x10^3/mL	ALC-1y x10^3/mL
0,235	7,3	12,34	10,26	**790**	**1450**	1450	1300	1300

Data mean

T-STUDENT	ALC-15/ALC-30	ALC-30/ALC-3m	ALC-3m/ALC 6m	ALC 6m-ALC-1y
P	**0.007**	NS	NS	NS

T-STUDENT	ALC-15/ALC-3m	ALC-15/ALC-6m	ALC-15/ALC 1a
P	**0,003**	0,008	0,008

T-student analysis between ALC in different moments of inmune reconstitution

	CD34 x10^6/Kg	MNCs x10^8/Kg	CFU-GM x10^6/K	ALC-15 x10^3/mL	ALC-30 x10^3/mL	ALC-3m x10^3/mL	ALC-6m x10^3/mL	ALC-1y x10^3/mL
A-ALC/Kg	0,03	0,0001	NS	NS	NS	NS	NS	NS
CD34x10^6/Kg		0,0001	0,007	NS	NS	NS	NS	NS
CMN x10^8/Kg			0,005	NS	NS	NS	NS	NS
CFU-GMx10^6/K				NS	NS	NS	NS	NS

Spearman correlation rho factor; NS: no statistically significant differences

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IN VITRO INVESTIGATION OF THE APOPTOTIC EFFECT OF HEPARIN ON LYMPHOBLASTS BY USING FLOW CYTOMETRIC DNA ANALYSIS AND FLUOROMETRIC CASPASE-3 AND -8 ACTIVITIES

E. Erduran,[1] O. Deger,[1] D. Albayrak,[2] Y. Tekelioglu,[1] T. Ozdemir[1]

[1]Karadeniz Technical University, TRABZON; [2]OndokuzMayis University, School of Medicine, Department of Pediatric hematology, SAMSUN, Turkey

Background. In addition to its antihypertensive, antiinflammatory, antiproliferative and anticoagulant effects,›t was shown in various studies that low dose heparin caused apoptosis on lymphoblasts. *Aims.* The aim of this study was to indicate whether the apoptotic effect of heparin *in vitro* on lymphoblasts developed due to the extrinsic pathway of apoptosis via the caspase-3 and caspase-8 activation in newly diagnosed acute lymphoblastic leukemiz (ALL) patients *Methods.* Twelve newly diagnosed children with ALL was included in the study.The lymphoblasts were incubated with 0, 10, and 20 U/mL heparin concentrations at 0, 1, and 2 h. The percentages of the apoptotic lymphoblasts were calculated by flow cytometry (FCM), and activities of caspase-3 and -8 were simultaneously measured by fluorometric protease activity method. *Results.*The apoptotic effect of heparin on the lymphoblasts was determined in 10 and 20 U/mL heparin concentrations (p<0.005 and p<0.001, respectively) while no apoptosis was detected in 0 U/mL heparin concentration at 0, 1, and 2 h. The apoptotic percentages of the lymphoblasts were higher at the first hour than those at 0 and 2 h in 10 and 20 U/mL heparin levels (p<0.001). The highest apoptosis was found at first hour in 20 U/mL heparin concentration. Increased concentrations of heparin had an increasing effect on the percentages of the apoptotic lymphoblasts. Significantly higher caspase-3 and -8 activities were determined in 10 and 20 U/mL heparin concentrations than those in 0 U/mL heparin concentration at 0, 1, and 2 h (p<0.001). There were no significant differences between the caspase-3 and -8 activities in 10 and 20 U/mL heparin concentrations at 1 and 2 h (p>0.05), while statistically significant differences were simultaneously detected in the apoptotic rates of the lymphoblasts (p<0.001). This may be due to that the study included the limited patients, or measurement of the caspase activities is a more sensitive method than the FCM analysis for determination of apoptosis because the activation time of the caspases takes a long time period. *Conclusions.* It was thought that the apoptotic effect of heparin *in vitro* on lymphoblasts developed due to the extrinsic pathway of apoptosis via the caspase-3 and -8 activations in newly diagnosed ALL patients.

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BORTEZOMIB COMBINED WITH HISTONE DEACETYLASE INHIBITOR ITF2357 OR ARSENIC TRIOXIDE EXERTS SYNERGISTIC ANTI-PROLIFERATIVE AND PRO-APOPTOTIC EFFECTS IN P39 CELL LINE: A POSSIBLE NEW APPROACH TO HIGH-RISK MYELODYSPLASTIC SYNDROMES

S. Galimberti,[1] M. Canestraro,[1] H. Savli,[2] G. A. Palumbo,[3] B. Nagy,[4] F. Di Raimondo,[3] M. Petrini[1]

[1]Hematology, PISA, Italy; [2]Biology department - Kocaeli University, KOCAELI, Turkey; [3]Hematology Univeristy of Catania, CATANIA, Italy; 41st Department of Obstetrics and Gynecology, Semmelweis University, Budapest, BUDAPEST, Hungary

Background. NF-kB, one of the fundamental transcription factors, able to control the expression of genes involved in apoptosis, cell cycle progression, inflammation, and angiogenesis, is constitutively activated in the P39 cell line and its activity levels are higher in advanced myelodysplastic syndromes (MDS), with a higher risk of transformation into acute leukemia. As previously reported by other Authors, in this model we confirmed that bortezomib was able to inactivate NF-kB, to exert an antiproliferative and pro-apoptotic effect, and to block cell cycle in the G2 phase. Moreover, we found that bortezomib significantly increased the release of reactive oxygen species (ROS), down-regulated WT1 gene expression, without any differentiating effect. Because others Authors reported a synergistic anti-proliferative effect exerted by the histone deacetylase inhibitor vorinostat plus bortezomib in leukemia and myeloma cells, we started to co-incubate P39 with increasing concentrations of bortezomib and ITF2357, at constant ratio. This association offered synergistic anti-proliferative and pro-apoptotic effects. Moreover, a synergistic increase of ROS production and down-regulation of the WT1 were observed. Interestingly, when bortezomib was associated to ITF2357, EMSA assays showed that NF-kB was not yet inactivated, thus supporting the idea of a very complex control of proliferation and apoptosis in our MDS model. After that, because arsenic trioxide (ATO) was reported to inactivate NF-kB, to offer about 30% of hematological improvement in patients affected by MDS and to be a valid alternative approach in multiple myeloma, we decided to combine bortezomib with ATO. As previously observed for the combination with ITF2357, when combined with arsenic bortezomib exerted also synergistic anti-proliferative and pro-apoptotic effects, and a synergistic down-regulation of WT1 expression. *Methods.* Cell viability was assessed by MTT; apoptosis by the Annexin V/ propidium cytofluorimetric analysis. ROS production was evaluated by dihydrorhodamine 123 (DHR) and flow-cytometry assay. For gene expression studies, samples were evaluated by real-time PCR by using the TaqMan® Low Density Array Human Apoptosis Panel (Applera). WT1 mRNA levels were assessed according to Galimberti *et al.* Samples were hybridized on Whole Human Genome Microarray (Agilent). The genes identified as de-regulated by bortezomib were then analyzed for network and gene ontology by Ingenuity Pathway Analysis software. Results have been confirmed by using the TaqMan® Low Density Array Human Apoptosis Panel. *Results.* These experiments showed that several NF-kB inhibitors, such as IKB and BCL3, were down-regulated by the combination of bortezomib and ITF2357. Moreover, when P39 were co-incubated with bortezomib and arsenic trioxide, the PPAR, P53, IL6, IL2, hypoxia, Huntington's disease, TLR and cell cycle were the pathways more significantly modified. Interestingly, 15 genes were de-regulated by the association only: SHC1, MLL, ITGAV; BCRA2 were down-regulated, while SPP1 (osteopontin, target for P53, pro-apoptotic) resulted up-regulated. Moreover, only when combined with ATO, bortezomib increased the down-regulation of HMOX1, ICAM1, JUN, and PMAP1, so explaining the synergistic anti-proliferative and pro-apoptotic effects observed in our model. *Conclusions.* In summary, biological results and gene expression assays suggest the possible use of bortezomib combined with proteasome inhibitors or arsenic trioxide in the treatment of high-risk MDS. In vivo trials will be useful to confirm this hypothesis coming from our *in vitro* studies.

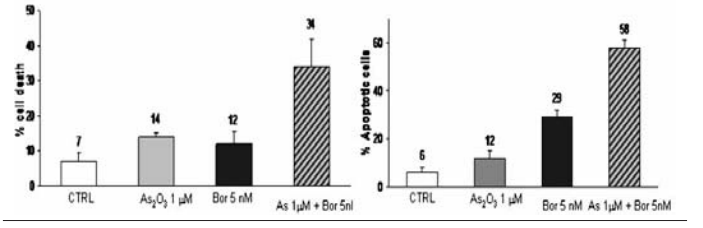

Figure 1.

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DEFERASIROX FOR THE TREATMENT OF THALASSAEMIC CHILDREN AND ADOLESCENTS WITH TRANSFUSIONAL HAEMOSIDEROSIS

E. Zevgaridou, I. Tsatra, M. Economou, M. Athanassiou-Metaxa

Hippokration General Hospital, THESSALONIKI, Greece

Deferasirox is a novel oral chelation agent that has recently been commercially available for the treatment of patients with haemosiderosis. The Aim of this study was to evaluate the effectiveness of deferasirox and register any side effects during a period of almost 2 years that has been used in our clinic for the treatment of thalassaemic children with transfusional haemosiderosis. *Methods.* 22 young patients aged 3-20 (mean 8±6.2) years with β thalassaemia major-transfusional haemosiderosis were assigned to receive an initial dose of 20 or 30 mg/kg/day according to their blood consumption one year prior to initiation of treatment. Efficacy of deferasirox was evaluated by changes in serum ferritin levels which were tested every 3 months and the dose was adjusted accordingly (max dose 40 mg/kg/d). Any side effects were registered. *Results.* Following deferasirox therapy average ferritin levels were significantly reduced (p:0.018). The reduction was between 7-79% (mean 33%±24.4). Ferritin levels were increased in 3/22 patients. Adverse events occurred in 8/22 (36%) patients. 6/22 (27%) patients developed transaminasaemia. 4/6 responded well to treatment interruption for 1-2 weeks and gradual escalation to the original dose. Deferasirox was discontinued on 2/6 patients as transaminasaemia reoccurred after reinitiating treatment. 2/22 (0.09%) patients developed allergic rash. In 1 case the rash was severe and required interruption of treatment and concomitant administration of antihistamines. The rash did not reappear after reinitiating and gradual increasing the dose. Creatinine was mildly increased on 1 patient and responded well to dose reduction by 5 mg/kg/day. One patient decided to discontinue treatment, although it was effective at reducing his serum ferritin, because he was experiencing changes in mood, fatigue and nightmares. After treatment discontinuation these symptoms settled. *Conclusions.* Deferasirox at doses 20-40 mg/kg/d was well tolerated and effective in reducing serum ferritin levels on most thalassaemic children and adolescents with transfusional haemosiderosis. Side effects were mild and reversible when treatment was interrupted. There was no obvious reason for its failure to reduce ferritin levels in 3/22 (14%) of our patients. There was also no clear relationship between blood consumption, dose of deferrasirox and change in ferritin levels which led as think that individual factors (possibly genetic?) may influence the response to treatment. As the number of patients in our study is small further studies are required in order to assess the efficacy and safety of this novel chelation agent.

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RECOMBINANT FVIIA EFFECTIVELY CONTROL BLEEDINGS WHEN PATIENTS ARE PROPERLY SELECTED. RETROSPECTIVE ANALYSIS

J. Kristensen, H. Alizadeh, V. Shammas, S. Sajwani, H. Narayanan, J. Altaf

Tawam Hospital, AL AIN, United Arab Emirates

Background. Recombinant activated factor VII is approved for promoting hemostasis in hemophilia A and B patients with inhibitors and in Europe as well as for treatment of congenital factor VII deficiency and Glanzmann's thrombasthenia. Recombinant FVIIa has generated a great deal of interest and controversy as a potential *general hemostatic agent* to enhance hemostasis in patients with bleeding who may or may not have an underlying hemostatic defect. In spite of the significant lack of data in many areas, rFVIIa is being used for a variety of off-label indications. We report 3 years off-label use. Aims and *Methods.* Retrospective analysis of patients that received rFVIIa over a 3 years period (2005-2007). The indication, dose, total number of doses given as well as response to the treatment were evaluated. Seventy eight patient's files were reviewed. Pharmacoeconomic calculations weren't possible to do in this retrospective analysis. *Results.* In the majority of the cases the patients received between 90-110 µg/kg BW of rFVIIa. The number of doses given varied from 1 to 21 (majority received 1-3 doses). The patient that received 21 doses of rFVIIa was a case of Glanzmann thrombastenia that underwent emergency caesarian section. TheTable 1 summarizes the different indications for the treatment as well as the outcome of the treatment. rFVIIa was effective in controlling bleeding in 37 of 78 cases. In 22 of 78 cases the effect was difficult to evaluate due to other interventions, uncontrolled underlying disease or if given as prophylactic treatment. In 8 cases no proper response evaluation was done, in 7 cases rFVIIa was judged not to be indicated and in 1 case contraindicated (DIC). In 3 cases no response to rFVIIa was observed. No thromboembolic events were observed. Eight children below the age of 15 received rFVIIa. Their age was between 8 month and 13 years and the dose used was in most cases around 90 µg/kg BW. In three of the seven cases rFVIIa was given prophylactic. *Summary.* In our institution a clear hemostatic response to rFVIIa was documented in 47% of the cases. In 8 cases appropriate evaluation before and after the rFVIIa as well as corrections of other hemostatic abnormalities wasn't done. It's known that children have a higher clearance rate of rFVIIa than adults and they often require higher doses of rFVIIa to secure hemostasis. However, all the children that were actively bleeding received standard doses of rFVIIa and everyone except for one child were there was some slight oozing after the injection achieved complete hemostasis. It is important that patients that could benefit from rFVIIa are properly selected and that an underlying hemostatic defect is corrected. For that reason the prescription has now been restricted to hematologist and a flow chart been developed to identify patients that could benefit from rFVIIa.

Table 1.

Indication	Number	Outcome	Number
Multi trauma	14	Effect certain	37
Obstetric and Gynecology	3	Effect uncertain*	22
Bleeding due to anticoagulation	2	No proper evaluation	8
Medical conditions associated with bleeding	15	Not indicated	7
Hematological malignancies	24	Contraindicated	1
Other malignancies	2	No response	3
Thrombocytopenia/Platelet dysfunction	11	Total	78
Factor VII deficiency	4	*Cases were the effect on hemostasis could have been due to additional treatment modalities or if given prophylactic	
Uncontrolled bleeding	3		
Total	78		

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DASATINIB IS EFFECTIVE AND SAFE IN SYSTEMIC MASTOCYTOSIS WITH D816V MUTATION: CASE REPORT OF TWO PATIENTS

P. Pregno,[1] M. Rondoni,[2] A. Chiappella,[1] S. Paolini,[3] L. Griso,[1] G. Martinelli,[3] U. Vitolo[1]

[1]Hematology, San Giovanni Battista Hospital and University, TORINO; [2]Hematology, Santa Maria Delle Croci Hospital, RAVENNA; [3]Hematology/Oncology, L and a Seragnoli, BOLOGNA, Italy

Background. Systemic Mastocytosis (SM) is a clonal disease due to an abnormal accumulation of tissue mast cells in one or more organ systems. Clinical features are heterogeneous ranging from an indolent to very aggressive course. In absence of curative options, therapy for aggressive SM was chemotherapy, corticosteroids, alfa-IFN. Imatinib is active in wild-type c-kit SM, but patients carrying the c-kit D816V mutant tyrosine-kinase are resistant to it. In this report we tested the use of dasatinib in two patient with D816V mutation, referred to our Hematology Dept. *Patient 1.* In June 2006 a 55-yrs old woman was hospitalized because of dyspnea and fever. CT-scan demonstrated pleuric effusion, ascitis, hepato-splenomegaly, abdominal lymph-nodes enlargement. A diagnosis of SM was done by a lymph-node biopsy and 60% of bone marrow (BM) involvement with the D816V mutation was detected. There were mild leucocytosis, anemia and thrombocytopenia; LDH was normal; tryptase level was 663 µg/L (<13,5). Skeletal X-ray was normal; L-VEF was 70%. After a brief treatment with corticosteroids, Dasatinib was started with slowly increasing doses from 20 to 70 mg/day. The therapy was well tolerated, complicated by diarrhoea treated with symptomatic drugs. Clinical features rapidly improved with resolution of fever, dyspnea and pleuric/ascitis effusions disappereance. Follow-up CT-scan demonstrated progressive reduction of lymph-nodes enlargement, stable hepatomegaly and normalization of spleen size. BM aspirate performed on December 2007 demonstrated residual involvement of the disease (50%) with disappearance of D816V point mutation. Blood counts demonstrated a mild leucocytosis and a resolution of anemia and thrombocytopenia; tryptase level was always elevated. At now,

the patient is still on treatment with Dasatinib 70 mg/day and pulsed low doses of corticosteroids when necessary. *Patient 2.* In October 2007 a 55-yrs old man went to us because of muscolar-skeletal pain, fever, dysphnea and relevant weight loss. CT-scan demonstrated pleuric effusion, ascitis, hepato-splenomegaly and abdominal lymph-nodes enlargement. Lymph-node biopsy demonstrated the diagnosis of SM and 20% of BM involvement with D816V mutation positivity was confirmed. There were leucocytosis, anemia and thrombocytopenia; LDH was 314 U/l; tryptase level was 213 µg/L. Skeletal X-ray was normal; L-VEF was 68%. After a brief treatment with corticosteroids, Dasatinib was started with slowly increasing doses from 20 to 70 mg/day. Dasatinib was very well tolerated without side effects. Clinical picture rapidly improved with resolution of fever, dyspnea and pain; both pleuric effusion and ascitis disappeared. CT-scan performed after 3 months of treatment demonstrated resolution of lymph-nodes enlargement and hepatic/spleen size normalization. BM aspirate with D816V detection is ongoing. Blood count is normal, but Tryptase level is always elevated. At now, the patient is fine and still on treatment with Dasatinib 70 mg/day with rapid de-escalation of corticosteroids. *Conclusions.* Management of patients affected by SM request careful monitoring of side effects, complications and worsening of the disease. Dasatinib may be an effective therapeutical approach in patients with D816V point mutation. In our hands this drug was well tolerated, with promising results in terms of efficacy and safety: large studies are needed to confirm it.

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AGE DEPENDANT PHARMACOKINETICS OF ROSCOVITINE ANT ITS EFFECT ON BRAIN CDK5 AND ERK 1/2 IN RAT PUPS

H. Sallam,[1] P. Jimenez,[2] M. Vita,[1] A. Cedazo-Minguez,[1] H. Moustapha[1]

[1]Karolinska Institutet, STOCKHOLM; [2]Astra Zeneca, SÖDERTÄLJE, Sweden

Background. Roscovitine is a selective cyclin dependant kinases (Cdks) 1, 2, 5, 7 and 9 inhibitor that is passing phase II clinical trials to evaluate its therapeutic efficacy in a number of tumors in adults including leukemia, breast- and lung cancers. It is well known that pharmacokinetics are critical when the anti-tumor properties are translated from *in vitro* into therapeutic activity, and subsequently for the selection of candidate drugs for clinical trials. The pharmacokinetic parameters of roscovitine in mice, adult rats and adult humans have shown that the drug is rapidly absorbed and distributed into peripheral tissues, extensively metabolized and a rapidly cleared with elimination half-life range between 30 min in adult rats to 3 hours in man. Recently, we have shown that the brain exposure to roscovitine is 30% of that found in plasma in adult rats. *Aims.* 1) To study the the pharmacokinetics of roscovitine in plasma and in different brain regions in infant rats. 2) To study the effect of roscovitine on Cdk5 and Erk1/2 in different brain regions. *Methods.* 14 days old Sprague-Dawley rat pups injected i.p. with a single dose (25 mg/kg) of roscovitine.Control pups were injected the vehicle alone. Animals were sacrificed at different time points up to 48 hours. Blood was collected and the brains were then dissected into frontal cortex (FC), hippocampus (HC) and cerebellum (CR). The concentrations of roscovitine in the plasma and brain were determined using HPLC-UV. The effect of roscovitine on Cdk5 and Erk1/2 was studied by measuring the protein levels of p35 and pErk1/2 in the brain using western blotting. The pharmacokinetic parameters were calculated using WinNonlin. Results were compared to our previous report about pharmacokinetics of roscovitine in adult sprague-dawley rats. *Results.* High exposure (AUC= 4200 µg min/g) to roscovitine in both plasma and brain of rat pups after a dose of 25mg/kg compared to that reported in adult rats (AUC=42 and 170 µg.min/g in barin and plasma respectively). The elimination half-life was 7 hours compared to 30 min observed in adult rats. Compared to adults, the exposure in young rats was 25- and 100-fold higher in plasma and brain, respectively. These differences could be explained by the immature blood brain barrier as well as the immaturity of CYP450 enzymes. Cdk5 was inhibited transiently until 2hr indicating activity of the drug and Erk1/2 was activated at the same time. *Conclusions.* In the present study, we have demonstrated that a single administration of roscovitine induced a transient inhibition of Cdk5 and a parallel transient activation of Erk1/2 in the pups brain. Thus together with the fact that roscovitine is currently on phase II trials for the treatment of different cancers in adults, roscovitine may be a promising candidate for the treatment of primary and secondary brain tumors in pediatric patients.

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RESCUE WITH PERIPHERAL BLOOD STEM CELLS IN TWO CASES OF CHEMOTHERAPY-REFRACTORY ACUTE LYMPHOID LEUKAEMIA: REPORT OF TWO CASES

S. Rocco,[1] L. Pezzullo,[1] O. Finizio,[2] L. Bene,[2] M.G. Ferrara,[2] G.R. Nunziata,[2] C. De Rosa,[2] L. Mettivier,[2] V. Mettivier[2]

[1]AORN Cardarelli, NAPLES; [2]AORN Cardarelli, Divisione di Eamtologia, NAPLES, Italy

Relapse is a common feature in ALL. Refractoriness to chemotherapy leads in prolonged aplasia, infections, transfusion requirement and poor general conditions. Here we report 2 consecutive cases in which infusion of previously collected peripheral stem blood cells (PBSC) during aplastic phase post-chemotherapy and the presence of leukemic infiltration of bone marrow was followed by haematological reconstitution and morphological and immunophenotypic remission of the disease and the chance to undergo to allogeneic bone marrow transplantation. *Case n.1.* Patient aged 22 years, male. Diagnosis of common-ALL without molecular markers in june 2004. Initial treatment was according GIMEMA LAL2000 protocol. No compatible sibling were found. Patient relapsed in February 2007 and was treated with high dose cytosine arabinoside and mitoxantrone. On day 35 there were no haematological recovery, daily transfusion requirement and fever of unknown origin. Bone marrow examination showed poor cellularity and about 30% of leukemic blasts. On day 40 previously collected after induction therapy PBSC were infused with a cellularity of 6×10^6/kg. On day 55 complete recovery of aplasia was present and bone marrow examination showed a rich normal population confirmed by immunophenotypic assay. *Case n. 2.* Patient aged 36 years, male. Diagnosis of common-ALL without molecular markers in february 2005. Induction treatment was according GIMEMA LAL0904 protocol. No compatible sibling were found. Relapse occurred in February 2007, reinduction treatment was with high dose of idarubicin and cytosine arabinoside. On day 35 patient conditions were seriuosly compromised. There were no haematological recovery with daily transfusion requirement. Candidemia and reactivation of cytomegalovirus were also present. Bone marrow examination showed a leukemic infiltration of about 30% in the contest of a very poor cellularity. On day 40 PBSC previously collected after induction therapy were infused with a cellularity of 5×10^6/kg. On day 55 complete recovery of aplasia, better general conditions with recovery from fever were present and bone marrow examination showed a rich normal population confirmed by immunophenotypic assay. Search of unrelated donor for both patients was began at the time of relapse, and 60 days after infusion patients were in good clinical conditions, with normal hemocrome and were undergoing to allogeneic bone marrow transplantation. Exact mechanism of remission of the disease is unclear. An occupation of space simply covering blasts is possible and so immunological control of blasts could be hypotesizied. Anyway, infusion of PBSC as rescue from a prolonged aplastic phase resulted in recovery of normal hemopoiesis and allowed patients to go over a life threatening period and to obtain complete remission of the disease. It was possible to carry on planning TMO. Because ALL patients are good mobiliziers probably a good policy could be to collect PBSC in this disease and to use them in order to short aplastic phase due to intensive chemotherapy after relapse.

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ANGIOGENIN SERUM CONCETRATION IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA AND ACUTE MYELOID LEUKEMIA

K. Kapelko-Slowik, D. Urbaniak-Kujda, J. Dybko, B. Jazwiec, M. Kielbinski, M. Slowik, D. Wolowiec, S. Potoczek, I. Frydecka, K. Kuliczkowski

Wroclaw Medical University, WROCLAW, Poland

Background. Angiogenesis is critical for the clinical progression of haematopoietic malignances and depends on angiogenic factors. Angiogenin is a powerful factor produced by neoplastic cells and host microenvironment. High levels of angiogenin correlate with a poor prognosis in patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). No data are available on angiogenin in acute lymphoblastic leukemia (ALL). Therefore, in this study we investigated the clinical significance of the angiogenin in sera of patients with ALL and AML and correlated them with other prognostic factors. *Patients.* Fifty -eight previously untreated patients were enrolled in the study: 29 with ALL and 29 AML, aged 22-79 (median=47.5). Females became 26 patients and males 32 patients. Thirty-three patients reached complete remission (CR). Serum samples were collected at the time of diagnosis. Con-

trol samples were obtained from 8 healthy donors. *Results.* Angiogenin levels were significantly higher in ALL and AML patients than in healthy individuals (p=0.007, p=0.008). No difference between angiogenin level in AML, ALL patients was found. Higher levels of angiogenin correlated with prolonged survival periods in both ALL and AML patients (p=0.004, p=0.004). Moreover we found correlation between angiogenin level with white blood cell counts (WBC) in leukemic patients (p=0.003). No correlation between angiogenin level and various patients features including: age, hemoglobin, platelet counts and poor prognosis cytogenetics were found. There was no significant correlation between angiogenin level and complete remission rate in ALL and AML. *Conclusions.* Our study demonstrated elevated serum angiogenin levels in patients with ALL and AML. High angiogenin levels were associated with increase survival of patients with ALL and AML, which the mechanism remains not clear. Moreover we show for the first time the elevated serum level of angiogenin in patients with ALL what can suggest a role of angiogenin in the pathogenesis this of disease.

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EFFECT OF HYPOXIA AND OCT1 INHIBITION ON THE ANTIPROLIFERATIVE EFFECT INDUCED BY IMATINIB, DASATINIB, AND NILOTINIB IN CML CELL LINES

P. La Cava, A. Chiarenza, D. Tibullo, M. Cavalli, P. Guagliardo, G. Palumbo, F. Di Raimondo

Division of Hematology, University of Catania, CATANIA, Italy

Background. treatment of Chronic Myeloid Leukemia (CML) is at present based on imatinib which is able to induce molecular remission in most patients. However, it has been demonstrated that some patients became resistant to treatment. In addition, imatinib does not induce apoptosis in CD34+ leukemic cells and this could be responsible for reappraisal of disease when therapy is stopped. Since the stem cell niches in bone marrow are mainly localised in the zones with the lower oxygen gradient, we evaluated if an hypoxic environment could reduce the apoptotic effect of imatinib and other TK inhibitors like nilotinib and dasatinib. We also know that Imatinib uptake is an active process mediated by a group of transporters that includes the organic cation transporters (hOCT) and it has been shown that different expression of OCT1 may play a critical role on intracellular drug levels and, hence, resistance to imatinib. However, the importance of OCT1 for the transport of nilotinib and dasatinib is not known. *Methods.* CML cell lines K562 and LAMA84 were plated into 96 wells plates and incubated with imatinib, dasatinib and nilotinib±prazosin (a selective OCT1 inhibitor) in 10% serum RPMI medium under hypoxic (3% O_2) or normoxic (21% O_2) conditions. After 24 hours viable cells were counted by XTT or trypan blue. *Results.* Cells treated with imatinib cultured under hypoxic conditions demonstrated decreased antiproliferative effect compared to normoxic conditions. However, the protective effect of hypoxia was not present when we tested dasatinib and nilotinib. The addition of prazosin almost abrogated imatinib and dasatinib activity while did not modify the effect of nilotinib either in normoxic and hypoxic conditions. *Conclusions.* Our data indicate that an hypoxic environment may reduce the efficacy of imatinib but does have any effect on the other two new TK inhibitors, dasatinib and nilotinib. In addition, we confirm that OCT1 is necessary for imatinib entrance into the cells and provide the first evidence that this could be true also for dasatinib while nilotinib is not transported by OCT1.

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STUDY OF ASCORBATE (VITAMIN C) STATUS IN CHRONIC MYELOID LEUKEMIA PATIENTS

I. Asfour,[1] M. Ghozlan,[2] S. El-Kourashy,[2] N. Arafa,[2] G. El-Gohary,[2] R. Afifi[2]

[1]Ain Shams University, CAIRO; [2]Ain Shams, CAIRO, Egypt

Carcinogenesis is a multistage process.Vitamin C *L-ascorbic acid*, a natural antioxidant, can prevent cell damage and subsequent development of cancer by neutralizing free radicles and oxidants. The goal of this study is to estimate the serum ascorbic acid level in newly diagnosed chronic myeloid leukemia patients at presentation and after receiving cytoreductive therapy as a trial to evaluate the impact on survival of those patients comparing with other control group. Serum ascorbic acid (AA) level has been quantitatively estimated using a specific high performance liquid chromatography (HPLC) with ultraviolet *UV* detection method in 20 control healthy subjects and 25 patients with chronic myeloid leukemia (CML) at presentation and after 1 month of cytoreductive therapy. vitamin C level was estimated using two measuring units (µg/L) and (µmol/L). Pre-treatment serum AA levels (7.0677 µg/L or 40.208 µmol/L were highly significantly lower than post treatment (7.5922 µg/l or 43.1856 µmol/L), in the patient group with(p<0.01) and both levels were significantly lower the AA levels in normal healthy individuals (7.9907 µg/L or 45.3687 µmol/L) with (p<0.001 *vs* p<0.05). Using the Wilcoxons Rank Sum test , there was highly significant statistical difference between the presence of the anaemic symptoms at presentation with (p<0.01) and the splenic size at presentation and post therapy, in the patient group with their lower serum ascorbic acid level with (p<0.001 *vs* p<0.05). Besides, there was high significant difference between the presence of higher total leucocytic count (TLC)and lower vitamin C levels whether pre or post-therapy (p±0.33) (r-0.34 *vs* -0.515) respectively.Besides there was high significant difference showing positive correlation between the presence of higher platelet count (p±0.33) (r 0.35) and higher serum creatinine level(p±0.39) (r 0.39) together with high vitamin C levels at presentation. Our results are important as recent studies suggest introducing ascorbic acid as an attractive molecular target agent in CML patients through changing in the redox state caused by its antioxidant activity.

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RESULT OF IMATINIB USE IN CHINESE PATIENTS WITH CHRONIC MYELOID LEUKAEMIA IN CHRONIC PHASE

V. Li,[1] H.S.Y. Liu,[1] F.H.Y. Chan,[1] T.K.H. Lau,[1] B.C.S. Kho,[1] S.F. Yip,[2] Y.S. Liang,[1] R. Chu,[1] J.C.W. Chan[1]

[1]Pamela Youde Nethersole Eastern Hospital, HONG KONG SAR; [2]Queen Mary Hospital, HONG KONG SAR, China

Background. Imatinib revolutionalized the management of CML. Whether its use in Chinese patients produces similar results as the Caucasians was uncertain. *Aims.* To review the efficacy and safety of imatinib in Chinese CML patients in chronic phase. *Methods.* A retrospective review of Chinese patients in a hospital in Hong Kong with a diagnosis of Ph/BCR-ABL positive CML and received imatinib. Criteria for treatment responses and failure were according to the recommendations from the expert panel of European LeukemiaNet. *Results.* From 1/5/2002 to 31/12/2007, 27 CML-CP patients received imatinib were found. *Clinical parameters.* The median age was 48 years old (range 23 to 79), M:F ratio of 2:1. The mean presenting haemoglobin 10.2 g/dL (range: 6.6-15.6), platelet count 564 (178-2175)×10^9/L, WCC 216.0 (23.5-527.2) ×10^9/L, PB blasts 2.1%(0-7.7), BM blast 2.9% (0.5-6), Sokal score 1.35 (0.58-4.67), Hasford score 1071 (315-2014). 88.9% had Philadelphia chromosome demonstrated, 11.1% had BCR-ABL fusion by FISH with negative karyotyping. Treatment and outcome: 55.6% received upfront imatinib, 40.7% had initial interferon α then switched to imatinib, 3.7% received long term hydroxyurea then switched to imatinib. All received a starting dose of 400mg daily. Clinical assessment, laboratory investigations including FISH and Q-PCR studies were performed at regular intervals. BCR-ABL kinase domain study was performed where indicated. Response rates: After the commencement of imatinib, 92.5% achieved CHR at 3 month. 66.7% achieved at least minor cytogenetic response (miCyR) at 6 months, 66.7% achieved major CyR at 12 months, 66.7% achieved complete CyR at 18 months. (Table 1).

Table 1. Response rates at various time intervals.

	3 months (post imatinib)	6 months	12 months	18 months
CHR	**92.6%**	92.6%	92.6%	92.6%
miCyR		**12.5%**	20.8%	16.7%
PCyR		**12.5%**	**12.5%**	12.5%
CCyR		**41.7%**	**54.2%**	**66.7%**
Meeting cytogenetic goal		66.7%	66.7%	66.7%

Overall, 96.3% achieved CHR, 70.8% achieved CCyR, 2 patients had achieved MMR, which is an underestimated figure due to recent availability of the test and lack of baseline Q-PCR data for calculation of log-reduction. Resistance rates: 6.7% developed primary resistance, 3.3% secondary resistance. All resistant patients were analysed for BCR-ABL kinase domain mutation. 1 patient had F317L mutation. All could not achieve cytogenetic response despite increasing the dose of imatinib and were switched to newer generation of TKI. Survival data: At 31/12/07, 92.6% had progression-free survival(remaining in CP), 77.8% had event-free survival (without progression or loss of response). Over-

all survival is 92.6%. Two(6.7%) succumbed. One due to an event unrelated to CML, 1 died of blastic transformation 38 months after the initial diagnosis despite being treated with various TKIs. Safety profile: 55.6% experienced grade 3 or 4 haematological toxicity (CTCAEv.3) requiring temporary suspension of imatinib. Majority of them tolerated well upon recommencement. Frequencies of grade 4 neutropenia, thrombocytopenia and anemia were encountered in 7.4%, 18.5% and 0% respectively. Skin rash, muscle cramps, periorbital edema and hypophosphataemia were common. *Conclusions.* Imatinib is an effective and well-tolerated drug for the majority of Chinese CML-CP patients. A significant proportion of our cohort received other treatments before imatinib was available might explain the slightly lower RR, EFS, PFS and OS than expected. A minority of patients had primary or secondary resistance to imatinib.

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QUALITY OF LIFE (QOL) IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA (CML) ON IMATINIB. A PILOT STUDY.

L. Kalvodova,[1] M. Doubek,[1] D. Zackova,[1] L. Dusek,[2] M. Jurova,[1] J. Mayer[1]

[1]University Hospital, BRNO; [2]Institute of Biostatistics and Analyses, Faculty of Medicine, BRNO, Czech Republic

Background. Imatinib mesylate is an oral targeted therapy for CML. In clinical trials, imatinib has demonstrated a high level of efficacy in CML patients and is associated with significantly less toxicity than previously used therapies. However, the QoL has not been still thoroughly investigated in imatinib treated patients. *Aims.* The objective of this study was to assess the QoL of patients receiving imatinib and to verify our clinical observation that there are differences in QoL in the groups of short time and long time imatinib treated CML patients. We report here the pilot comparison of two groups of patients: patients 15 months from the beginning of imatinib treatment (group A; n=10), and patients 50 months from the beginning of imatinib treatment (group B; n=12). *Methods.* SF-36 and EORTC QLQ-C30 (version 2) questionnaires were used. Arithmetic means and its 95% confidence limits were used to describe the primary data in both cohorts of patients. Two-sample t-test and one-way ANOVA test were used to compare the groups, and to search for influential cofactors of QoL measures. *Results.* EORTC QLQ-C30 transformed scores: global health status (without invalidity and rheumatic diseases): 66.7 (group A) *vs* 88.9 (group B); p=0.024. Symptomatology, problems in total in group A, and in group B: 11.9 *vs* 3.5 (p=0.035). SF-36 transformed scores for physical components without invalidity and rheumatic diseases: 50.6 (group A) *vs* 86.4 (group B); p=0.013. SF-36 transformed scores for mental components: 58.8 (group A) *vs* 89.5 (group B), p=0.041. *Conclusions.* The CML patients treated with imatinib for 50 months have better QoL then the patients treated for 15 months.

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UNMET NEEDS IN CML IN CLINICAL PRACTICE IN ITALY: RESULTS OF THE ITALIAN COHORT OF THE UNIC STUDY

E. Morra,[1] F. Di Raimondo,[2] A.M. Liberati,[3] G. Alimena,[4] N. Cantore,[5] S. De Matteis,[6] F. Ferrara,[7] M. Intorcia,[8] C. Paga,[8] E. Pungolino[1]

[1]Ospedale di Niguarda Ca' Granda, MILAN; [2]Institute of Hematology, University of Catania, CATANIA; [3]Policlinico Monteluce, PERUGIA; [4]University La Sapienza, ROME; [5]A.O.R.N. San G.Moscati, AVELLINO; [6]Policlinico Gemelli, ROMA; [7]Ospedale A Cardarelli, NAPOLI; [8]Bristol-Myers Squibb, ROMA, Italy

Background. Imatinib is widely used for the treatment of chronic myeloid leukaemia (CML) and is associated with improved response rates in these patients. However, few data exist that can help quantify the number of imatinib resistant/intolerant CML patients and provide an overview of their current management in everyday practice. *Aims.* The Unmet Needs in CML (UNIC) study, conducted across eight European countries (Austria, Belgium, France, Italy, The Netherlands, Spain, Sweden, UK), aimed to address these knowledge gaps. Here, we present the results of the cohort of patients from Italy. *Methods.* UNIC was a cross-sectional study, with retrospective chart review of patients currently treated for CML or Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph+ALL). Patients were recruited September 2006 to March 2007. The study was designed to estimate the proportion of (i) patients ever treated with imatinib and (ii) imatinib-treated patients who have experienced imatinib resistance and/or intolerance (primary objectives), as well as disease management patterns. A registry was collected of potentially eligible patients - those aged ≥18 years and treated for CML/Ph+ALL at the participating centres (academic, non-academic, private clinic or other). Case Report Forms (CRFs) were completed for eligible patients until the recruitment target was reached. Data were collected at the most recent visit and retrospectively through clinical chart review. *Results.* Of the 1678 patients in the Italian registry, CRFs were completed for 307 CML patients not included in other clinical trials. Analysable data were available for 288 chronic phase (CP) CML patients, of whom 287 (99.7%) had received imatinib at some point during the follow-up period. By last observation, 40% of imatinib-treated CP CML patients in the Italian cohort needed a change in imatinib dose, compared with 49% in the total European sample (Table 1). In the Italian cohort, 32% imatinib-treated CP CML patients needed a dose increase and 29% needed a dose decrease. In total, 8% of CP CML patients in the Italian cohort discontinued imatinib therapy, compared with 20% in the total European sample (Table). Patients were defined as imatinib resistant/intolerant if resistance/toxicity led to a change in, or discontinuation of, imatinib use, as reported in their medical chart. Reported rates of imatinib resistance or intolerance in the Italian cohort were similar to those reported in the total European population of patients with CP CML (Table 1). Thirteen (4.5%) percent of CP CML patients discontinued imatinib due to toxicity. With respect to disease monitoring, 9% (26/287) of CP CML patients had not had a PCR analysis to assess molecular response in the last 12 months. Furthermore, 65% (20/31) of imatinib-resistant CP CML patients had not been assessed for mutations since diagnosis. *Summary and Conclusions.* In this large observational study of CML patients in Italy, nearly all patients were exposed to imatinib therapy. Nearly half experienced imatinib resistance and/or intolerance. Compared with the total European sample of CP CML patients, fewer patients in the Italian cohort received imatinib dose modification or discontinued imatinib treatment. Molecular monitoring of disease appeared to be used less often in clinical practice in Italy than according to recommendations.

Table 1.

Imatinib-treated patients, n/N (%)	Italian chronic phase CML cohort	Total European chronic phase CML sample
At least one change in imatinib dose by last observation	108/273 (39.6)	677/1370 (49.4)
Imatinib dose increase	87/275 (31.6)	563/1365 (41.2)
Imatinib dose decrease	78/273 (28.6)	441/1352 (32.6)
Discontinued imatinib during the follow-up period	22/286 (7.7)	289/1441 (20.1)
Imatinib resistant*	32/287 (11.1)	206/1441 (14.3)
Imatinib intolerant*	114/287 (39.7)	552/1441 (38.3)
Imatinib resistant and/or intolerant*	129/287 (44.9)	643/1441 (44.6)
Imatinib resistant and intolerant*	17/287 (5.9)	115/1441 (8.0)

*As reported in the medical chart

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LOSS OF CCYR IN CML CP ON GLIVEC THERAPY IS DEPENDENT ON THE TIME OF ITS ACHIEVEMENT

A. Zaritskey,[1,2] E. Lomaia,[2] E. Machulaitene,[1] N. Turina,[1] E. Romanova,[1] N. Ilyina,[3] T. Sheider,[4] I. Martinkevitch,[5] K. Abdulkadirov[5]

[1]Medical University, ST. PETERSBURG; [2]Centre of Heart, Blood&Endocrinology, ST. PETERSBURG; [3]City Hospital N15 ST. PETERSBURG; [4]Regional Hospital, LENINGRAD REGION; [5]Institute of Hematology, ST. PETERSBURG, Russian Federation

The majority of CML CP patients achieve CCyR while Imatinib therapy. The main predictor of progression free survival is CCyR. It was shown that the progression to AP/BC does not depend on time to CCyR. Commonly progression to AP/BC takes 3-4 years time duration. Thus, the aim of our study is to evaluate more early events after CCyR-ie loss of cytogenetic response. Patient inclusion in the study was population based. Probability of CCyR was 67% by 1 year and 89% by 2 years. The iniatial dose of Glivec was 400 mg. In case of suboptimal response by 12 months the dose was escalated to 600 mg. Patients without of CCyR by 24 months except 2 pts were switched to other types of therapy. 78 patients with CCyR and follow -up more than 6 months after it were

included in the study. Time to CCyR was as follows: less than 6 months 45 pts, 6-12 months 18 pts, more 12 months to 18 months 6 pts and more 18 months 9 pts. Loss of CCyR was considered as appearence at least one Ph-positive cell. *Results.* The probability of CCyR loss depended significantly on the time to CCyR (Figure1). No one of the patients has lost CCyR after 24months. 19/78(24%) pts have lost CCyR and only 3 of them have lost major CyR. 13/19 obtained stable second CCyR after Glivec dose escalation, 3/19 are still in major CyR but without CCyR. 3 patients which had lost major CyR were switched to other therapies. *Conclusions.* Loss of CCyR is probable but rare event during Glivec therapy. The major risk of CCyR loss is late acheivment of CCyR. Events after 2 years of CCyR are uncommon. Second CCyR could be obtained by dose escalation

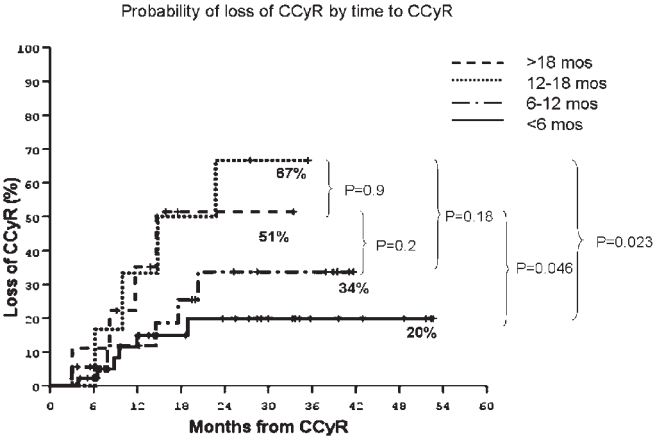

Figure 1.

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EFFICACY AND SAFETY OF BORTEZOMIB PLUS DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

R. Garcia Sanchez, R. Saldaña, I. Caparrós, A. Gallardo, R. García-Delgado, A. Rosell, A. Campos, M.P. Queipo de Llano, I. Pérez, M.J. Moreno, S. Del Castillo, S. De la Torre, G. Ramírez

Hospital Virgen Victoria, MÁLAGA, Spain

Background. Bortezomib is a reversible proteaosome inhibitor with activity in multiple myeloma (MM), obtaining durable responses when given as monotherapy or combination therapy for relapsed or refractory MM, without severe toxicity. The latest studies prove a 43% of response rates with bortezomib alone and 70.7% with bortezomib plus dexamethasone, in patients that had received 1-3 prior treatment schedules. *Aims.* To evaluate the efficacy and safety of bortezomib plus dexamethasone in patients with relapsed or refractory MM that received this drug plus dexamethasone after two or more prior therapies. We evaluated toxicity and overall survival. *Patients and Methods.* We treated 36 patients in our hospital with relapsed or refractory MM treated with standard dose bortezomib (1.3 mg/m^2 IV bolus on days 1, 4, 8 and 11) plus dexamethasone (40 mg/d PO the same days) each 3-week cycle. We included 19 males and 17 females with mean age 62.1 (range 44-80) and the median follow-up period was 37 month (median 13.5 month, range 1-37). The prognostic markers at time of diagnosis were beta2-microglobuline>3 in 52.8% of patients, mean of plasma cells in bone marrow 30.2%, extramedullary involvement 55.5%. The number of prior therapies was 2.2 (range 1-7) and 11.1% of patients had undergone prior autotransplant. Response was evaluated according to the EBMT criteria after 4° cycle and the end of the treatment (with a mean of 7.5 cycles, range 4-12) . Adverse events were graded based on WHO toxicity scale. *Results.* We analysed 29 patients (80.5%) after 4 cycles obtaining an overall response (OR) rate of 82.7%; complete response (CR) 6.9%, near complete response (CnR) 10.3%, partial response (PR) 51.7%, minimal response (MR) 13.8%, stable disease (SD) 10.3%, progression 6.9%. At the end of the treatment 22 patients (66.1%) had been evaluated with the following responses: OR 72.7%, CR 22.7%, CnR 9.1%, PR 27.3%, MR 13.6%, progression 27.3%.The overall survival was 63.8%. Toxicity profile WHO grade 3-4: thrombocytopenia: 16.7%; neuropathy 13.9%; gastrointestinal: 0%;zoster herpex virus infection: 19.4%. In 55.5% of patients a dose reduction of bortezomib was required. CONCLUSI'N: Bortezomib is an effective agent with acceptable toxicity for the treatment of patients with relapsed/refractory MM. The response rates, overall survival and toxicity in our series are similar to data described in previous studies although a longer follow up is needed in order to confirm these results.

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A COHORT ANALYSIS OF 493 CHINESE PATIENTS WITH MULTIPLE MYELOMA FROM FOUR REGIONAL HOSPITALS IN HONG KONG

T.K.H. Lau, C.W. Chan

Pamela Youde Nethersole Eastern Hospital, HONG KONG, Hong Kong

Background Multiple myeloma (MM) is the second most common lymphoid malignancy in Western countries representing 10-15% of all haematological malignancies. There is little information in Chinese. *Aims.* This study attempts to collect data on the demographic characteristics, presenting clinical and laboratory features, treatment and overall survival of Chinese patients with MM encountered at several regional hospitals in Hong Kong. *Methods.*Medical records of Chinese patients in whom MM was diagnosed from 1991 through 2006 at four regional hospitals in Hong Kong were retrospectively reviewed. The diagnosis of MM was based on the Durie and Salmon diagnostic criteria (1975) before 2001, and on either the World Health Organization diagnostic criteria or the International Myeloma Working Group diagnostic criteria after 2001. *Results*. Of the 493 study patients, 55% were men. The median age was 68 years (range 25 - 93) with 3% younger than 40 years and 45% older than 70 years of age. At presentation, anaemia (haemoglobin <10 g/dL) was present in 71% of patients, hypercalcaemia (corrected serum calcium > 2.6 mmol/L) in 35%, and renal impairment (serum creatinine > 110 umol/L) in 54%. Bone marrow aspirate showed more than 30% plasma cells in 77% of patients. Conventional radiograph revealed lytic lesions in 65% of patients. Serum protein electrophoresis revealed a localized band in 78% of patients, and monoclonal light chain was detected in the urine of 78% of patients. The pattern of paraproteins as shown by immunoelectropheresis showed IgG in 56% of patients, IgA in 24%, light chain in 15%, and IgD in 4%. Soft tissue plasmacytoma and amyloidosis were present concomitantly with MM in 20% and 3% of patients respectively. Fifty-five percent of patients received melphalan and prednisolone induction chemotherapy, 26% anthracycline-containing combination chemotherapy, and 9% supportive or palliative treatment alone. Eleven percent of patients received stem cell transplantation - 6% autologous and 5% allogeneic. The median overall survival was 20 months (range 1-156). *Conclusions.* This is a retrospective study of Chinese patients with MM in Hong Kong over a span of over 10 years. The treatment varied from the conventional therapy of melphalan and prednisolone to combination therapy including the use of anthracyclines to haematopoietic stem cell transplantation. The overall survival was shorter than the commonly reported 30 months or above. Individual patient group analysis is difficult due to the retrospective nature of study. Nonetheless it may serve as a historical cohort for comparison of treatment outcome with newer therapeutic agents like thalidomide and bortezomib. The study also proved that the demographic, clinical and laboratory features of Chinese patients with MM resemble those of other races. Better diagnostic approach and disease monitoring, such as the use of serum free light chain assay, and the development of unified and stratified treatment protocols with multicentre clinical trials would definitely improve patient survival in the future.

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PROPHYLAXIS WITH ACYCLOVIR IN BORTEZOMIB-DEXAMETASON BASED THERAPY FOR MULTIPLE MYELOMA REDUCES THE INCIDENCE OF HZV, BUT NOT CMV INFECTION

G. Giulio, G. Farina, S. Piano, T. Grafone, C. Nicci, A. Marcellino, S. Storti

John Paul II Center, CAMPOBASSO, Italy

Background. Bortezomib is used in Multiple myeloma (MM) therapy because it reversibly inhibits proteasome function.This results in an increased level of NF-KB inhibitor, because of its reduced degradation. The direct effect is myeloma cell apoptosis, down-regulation of myeloma cell adhesion molecules and decreased cytokine transcription and secretion in the bone marrow. Recent data reported that the activation of NF-KB pathway causes a cellular antiviral response including the production of alpha/beta interferon , cytokines and other proteins that resctrict viral infection. So inhibition of the NF-KB patway may increase the risk of Herpes Zoster Virus (HZV) and Cytomegalovirus (CMV) reactivation. *Aims.* To evaluate increased incidence of HZV and CMV infec-

tion in patients with MM treated with bortezomib and steroids with or without immunomodulant drugs or chemotherapy, if antiviral prophylaxis is performed or not. *Methods.* This study is a prospectic randomized study. We treated 19 patients with bortezomib and steroids based therapy with or without thalidomide or chemotherapy (Lyposomial Doxorubicin). 10 patients were randomized to receive antiviral prophylaxis with acyclovir. 9 pazients did'nt received antiviral prophylaxis. *Results.* Median number of therapy cycles administered was 4 (range 2-9). M/F ratio was 10/9. Median age was 68 years (range 49-81). All patients showed advanced disease (Salmon and Durie stage III). 4/19 (21%) patients had renal failure. 9/19 patients (47%) showed a monoclonal component IgG/k or lambda type, 3/19(15%) IgA/K and IgA/λtype, 2/19(10%) IgD and micromolecular type. 3/19 (15%) patients presented abnormal cariotype (del13). 8/19 patients (42%) were in first line therapy; 11/19 (57%) were in third and in fourth lines therapy. Their median follow up was 25 mounths (7-43). Patients treated with velcade-dexametason based regimen showed a lymphocyte and a platelet count nadir within a median of two therapy cycles. We observed that 5 on 9 patients without antiviral prophylaxis, developed HZV infection within two therapycycles. On the other hand, none of the 10 patients prophylaxed with acyclovir, developed HZV infection, with a Yates corrected chi square test of 4.9 (p 0.026) and an ideterminated OR and RR. Fisher's exact test showed p 0.01. 2 on 10 patients receiving acyclovir developed CMV infection. Mortality for CMV or HZV infection was absent. Decreased levels of lymphocyte or platelets count after 2 cycles of therapy, association of velcade and dexametason with thalidomide or Caelyx, type of monoclonal component, were not associated with viral infections. *Conclusions.* In our study antiviral prophylaxis with acyclovir appears to be effective in avoiding HZV infection, but it don't protect from CMV infection. Indeed also in patients receiving acyclovir prophylaxis a close control of CMV infection by PCR analysis is suggested.

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ALTERNATION OF SELECTED BIOLOGICAL PARAMETERS AFTER AUTOLOGOUS TRANSPLANTATION IN MULTIPLE MYELOMA PATIENTS

T. Pika,[1] J. Minarik,[1] M. Budikova,[2] J. Bacovsky,[1] V. Scudla[1]

[1]3rd Internal Department, OLOMOUC; [2]Department of clinical biochemistry, Faculty hospital Olomouc, OLOMOUC, Czech Republic

Background. Multiple myeloma (MM) is a malignant hematological disease characterised by unlimited proliferation and the accumulation of neoplastic transformed plasma cells, monoclonal immunoglobulin production detectable in the serum and/or urine and various levels of expressed organ affection. The bone marrow microenvironment in relation to the pathological plasmacellular infiltration is characterised by a very large system of intercellular and cytokine interactions among stromal cells of bone marrow, bone elements and tumour cells themselves, being in a close relation with their own biological qualities of the disease. Some less usual deducable biochemical parameters appear to be very promising as possible future markers not only in the eventual neoplastic transformation of potential malignant monoclonal gammopathy of undetermined significance and its differentiation from the early stage MM but also as the markers of advanced stage, progression and monitoring of treatment response in patients with multiple myeloma. *Aims.* The aim of the study is to compare serum levels of 12 selected biological parameters at time of diagnosis and after high dose melphalan therapy with autologous stem cell transplantation (ASCT). *Methods.* Twenty-nine patients enroled the study, all patients were treated with standart induction therapy with VAD (vincristine, adriablastine, dexamethasone) for 4.-5. cycles, afterwards HD-melphalan (200 mg/m^2) with autologous stem cell support. Treatment response was evaluated according to EBMT criteria (CR - complete response, VGPR - very good partial response, PR - partial response). CR+VGPR treatment responses were assesed in 15 (52%) patients, PR response in 12 (42%) patients and 2 patients had stable disease. Serum levels of selected biological parameters were evaluated in time of diagnosis and after 100 days after ASCT. For evaluation of serum levels of analysed parameters were used following *Methods.* radioenzymatic assay (thymidinekinase), radioimmunoanalysis (β-2microglobulin (β-2m), ICTP, PINP), enzymoimmunoassay (sIL-6R, sVCAM, sICAM-1, sOPG) and quantitative enzymatic immunoassay (sHGF, sVEGF, syndecan-1/CD138 and sFas). Wilcoxonãs test was used for statistical evaluation. *Results.* In whole group (CR+VGPR+PR), significantly lower levels were found in case of β-2m (p= 0,0001) and sHGF (p=0,0003), differences were higher in CR+VGPR group (β-2m p= 0,001 and sHGF p= 0,005) than in PR group β-2m (p= 0,016) and sHGF(p=0,033). In whole group were assesed higher levels of sICAM-1 (p=0,026), sVCAM (p=0,007), ICTP (p=0,001), PINP (p=0,001) and sFas (p=0,00003). Differences were higher in CR+VGPR group sVCAM (p=0,005), ICTP (p=0,002), PINP (p=0,009), sFas (p=0,001) than in PR group PINP (p=0,028) and sFas (p=0,005). *Conclusions.* High-dose therapy with autologous stem cell support is associated with significant change of cytokine network. Decrease of β-2m after succesfull treatment was confirmed in many studies. Decrease of serum levels of hepatocyte growth factor after treatment showed, that sHGF seems to be promising marker for potential evaluation of treatment response. Higher bone turn-over was assesed after stem cell engraftment.

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ISS WAS HIGHLY PREDICTIVE OF PROGNOSIS IN A BRAZILIAN STUDY IN MYELOMA PATIENTS

C.A. De Souza,[1] A. Maiolino,[2] V. Hungria,[3] G. Oliveira,[1] E. Miranda,[1] D. Mercante,[2] E. Rego,[4] L. Oliveira,[4] C. Chiattone,[3] M. Nucci,[2] I. Metze,[1] C. De Souza[1]

[1]State University of Campinas, CAMPINAS; [2]Federal University Clementino Fraga Filho, RIO DE JANEIRO; [3]Santa Casa Medical School, SÃO PAULO; [4]State University of São Paulo, RIBEIRÃO PRETO, Brazil

In 2003 the International Myeloma Foundation (IMF) group proposed a new International Prognostic Index (ISS). MM Brazilian group recently published in a retrospective analysis the utility of ISS in Brazil. This study presents the use of ISS in a multicentric prospective clinical trial performed in Brazil and its impact in survival. From October 2003 to January 2008, 229 untreated patients under 71 years old were enrolled in a prospective study developed in Brazil. All patients signed the informed consent and the protocol was approved by the Ethical committees. 190 patients were analyzed. 11 did not presented data for ISS classification. At the end, 179 patients were included in this analysis, 53 (29.6%) ISS I, 68 (38%) ISS II and 58 (32.4%) ISS III; 93 (51.9%) were male and 86 (48.1%) female; the median age was 55 y (27-70) for the whole group; 38 (21.2%) Durie-Salmon II and 141 (78.8%) DS III. DS I were excluded from this clinical protocol. 132 out 179 (77.6%) had 13q deletion analysis. In all patients were performed hematological (including BM), biochemistry and radiological analysis. The treatment was based on three phases: debulking with 3-6 VAD followed by high dose cyclophosphamide (4 g/m^2) for mobilization plus ASCT and consolidation using dexamethasone with or without thalidomide. The statistical analysis was made by Chi-Square, Kaplan-Meier curves (log-rank test) and ANOVA. ISS I distributed by DS system showed DS II 15/53 (28%) and DS III 38/53 (72%); ISS II had DS II 15/53 (28%) and DSIII 38/53 (72%); ISSII had DSII 19/68 (28%) and DSIII 49/68 (72%) and ISSIII had DSII 4/57 (7%) and DS III 53/57 (93%) (p<0.0001). Concerning the presence of 13q deletion, 12/36 (33%) ISS I, 16/51 (31%) ISS II and 17/45 (37%) ISS III (NS). The median observation time for whole group was 22 months and for alive patients 24 mo (1-62). 44 out 179 (24%) died, most of them in VAD phase due to progression. 135/179 (76%) are alive, ISS I 45/53 (85%), ISS II 57/68 (84%) and ISS III 33/58 (57%) (p<0.001). The OS in 60 mo by ISS was 76%, 75% and 36% for ISS I, II and III, respectively (p<0.0001). The EFS in 60 mo by ISS was 38%, 32% and 10% for ISS I, II and III, respectively (p<0.0001). The ANOVA showed significant difference for plasma cells bone marrow infiltration, creatinine and hemoglobin levels (p< 0.0001). The authors emphasized the importance of ISS at diagnosis due to high capacity to discriminate among groups with low cost.

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MULTIPLE MYELOMA'S (MM) EXTRAMEDULLARY RELAPSES POST HIGH-DOSE-THERAPY (HDT)

V. Bongarzoni, B. Anaclerico, A. Chierichini, S. Fenu, P. Anticoli Borza, M. Cedrone, B. Ronci, P. Iacovino, L. Annino

Azienda Ospedaliera S.Giovanni Addolorata, ROME, Italy

Background. if tandem HDT is retained the golden standard therapy in adult MM patients, this procedure does not prevent from successive disease progression. The new target agents such as proteasome inhibitors and antiangiogenesis factors are able to induce good response in relapsed patients, if these do not seem to control extramedullary relapse (ER). *Aims.* we have retrospectively reviewed our tandem HDT MM series to evaluate: a) the incidence and the site of ER, b) the treatment schedule employed, c) analyse the impact of treatment used on disease outcome. *Methods.* from June 2002 to December 2007, at our Institution 26 MM patients- median age 58 y (min 57- max 63y)-underwent tandem autol-

ogous stem cell transplant (ASCT); 5 (19%) of them developed a ER in a median time from SCT of 26 mos (min12- max 40 mo). As initial characteristics of these 5 cases: 3 IgG-k and 2 micromolecular MM, respectively; we classified 3 as having ISS stage III and 2 stage II; FISH assay showed a chromosome 13 deletion in 2 cases. In 4/5 patients ER + bone marrow (BM) relapse occurred after 2 previous isolated haematologic relapses; were treated with Thalydomide + Bortezomib except in 1 case. The ER sites were: orbita (1), clivus + right thoracic mass (1), pancreas (1), hepatic+ pulmonary + supra-clavicular nodules (1), bulky disease (1). As treatment for ER+BM, a schedule includes Bortezomib + dexamethasone + liposomal doxorubin. After the third cycle:, 1 patient died and 4 achieved a haematologic partial response (PR), and a complete ER disappearance at NMR and/or CT scan. The median response length was 4 mos (min 1.5-max 4), median survival from ER occurrence was 4.5 mos and median OS from MM diagnosis 50 mos (min 21- max 54). *Comments.* if the series reported is limited, the ER occurred in nearly 20% of our cases and represented a catastrophic event. Furthermore to date this type of relapse seem to be poor responsive to both conventional drugs and new agents.

1301

CLINICAL STUDY OF 35 CASES OF PRIMARY SYSTEMIC AMYLOIDOSIS

D. Coriu,[1] R. Talmaci,[1] S. Badelita,[1] C. Dobrea,[1] M. Dogaru,[2] D. Ostroveanu,[1] G. Coriu,[3] E. Galatescu[3]

[1]University of Medicine 'Carol Davila', BUCHAREST; [2]Department of Hematology, Fundeni Clinical Institute, BUCHAREST; [3]'Dr. Constantin Angelescu' Hospital, BUCHAREST, Romania

Background. Primary systemic amyloidosis (AL) is the most aggressive and lethal form of amyloidosis. We wished to study patients with a premortem diagnosis of primary systemic amyloidosis to determine what clinical and laboratory features might assist in recognizing the disease and assessing prognosis. *Patients and Methods.* Retrospective study of 35 cases of AL amyloidosis diagnosed in a university hospital center between 2002-2007. *Results.* The average age at diagnosis was 56 years (between 29 years and 80 years), twenty-one males and fourteen females. The incidence of organ involvement was as follows: heart; congestive heart failure - 31,4%; arrythmias - 17,1%; echographic signs 57,1%; kidney (nephrotic range proteinuria or renal failure - 54,2%); peripheral nerves 37,2%; liver 11,4%; gastrointestinal tract 20%; spleen 11,4%. Twenty-three patients (65,7%) had one or two organ systems involved, whereas twelve (34,2%) had three or more organs involved. Diagnosis was formulated on biopsy of the involved organ (kidney 14,2%, liver 2,8%,), on rectal biopsy (5,7%) and on abdominal fat aspiration (60%). Majority of the patients (60%) had less than 5% plasma cell in the marrow; 14,2% of the patients had between 6-10%; 17,1% of the patients had between 11-20%; 8,5% of the patients had more than 21% plasma cells. Serum free light chains (FLC) assays and serum / urine immunofixation showed a monoclonal protein in 87,5% of the patients. 31,3% of these patients had a monoclonal intact imunoglobulin (IgG 22,8%, IgA 8,5%) and 68,7% of these patients had only a free monoclonal light chain in the serum. Lambda light chains were noted in the serum of 71,4% of the patients with a monoclonal protein. For few patients these techniques fail to demonstrate a monoclonal protein. In these cases, definitive identification of amyloid deposits was done by mass spectrometry (MS/MS) using material extracted from formalin -fixed, amyloid -containing tissue biopsy or subcutaneous fat aspirate. In two cases systemic hereditary amyloidosis lysozyme type was diagnosed, respectively transthyretin type. All the patients with AL amyloidosis received treatment with melphalan -dexamethasone or VAD. Presently, 23 patients (65,7%) are still alive with a median follow-up 24,3 of months (ranging from 1 to 174 months).12 patients died after a median survival of 10 months (ranging from 1 to 36 months). *Conclusions.* Accurate diagnosis and classification are essential for the prognosis and treatment of the disease. The diagnosis of amyloidosis must be supported by bioptic examination, serum and urine immunofixation, serum FLC assays and in special cases by amino acid sequencing and mass spectrometry of fibrillar material.

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1302

REDUCED DOSE OF NON-PEGYLATED LIPOSOMAL DOXORUBICIN WITH CYCLOPHOSPHAMIDE, VINCRISTINE AND PREDNISONE±RITUXIMAB FOR PREVIOUSLY UNTREATED ELDERLY PATIENTS WITH AGGRESSIVE LYMPHOMA NON SUITABLES FOR STANDARD CHEMOTHERAPY

E. Gimeno,[1] A. Alvarez,[2] C. Pedro,[2] E. Abella,[2] M. Gomez,[3] J. Comin,[3] B. Sanchez,[2] T. Gimenez,[2] C. Besses,[2] A. Salar[2]

[1]Hospital del Mar, BARCELONA; [2]Hematology, BARCELONA; [3]Cardiology, BARCELONA, Spain

Background. CHOP±Rituximab (R) is the standard regimen for elderly patients with aggressive lymphoma but many of them are not fitted for it due to severe associated comorbidities. The aim was to evaluate retrospectively the efficacy and safety of a modified-CHOP (with reduced dose non-pegylated liposomal doxorubicin (NPLD)±R in elderly patients with clinically aggressive lymphoma not tributary to anthracycline-containing chemotherapy regimen. *Patients and Methods.* Retrospective analysis of 16 patients with previously untreated aggressive lymphoma. Gender: 9 men/7 women; median age 76 years (62-85); seven patients stage I-II (IPI=1-2) and 9 patients stage IV (IPI=2-5). Median baseline left ventricular ejection fraction (LVEF) was 60.2% (31-80). Comorbidities: active liver disease (2 patients, one patient with hepatocellular carcinoma), severe chronic obstructive pulmonary disease (3 patients), severe cardiomiopathy (4 patients) and others (7 patients). Schedule: NPLD 30 mg/m^2, cyclophosphamide 750 mg/m^2, vincristine 1.4 mg/m^2, prednisone 100 mg/d d1-5±R 375 mg/m^2 d1 every 21 days as a first line therapy. Pegfilgrastim was used on day 2 at standard dose. *Results.* Fifteen patients are evaluable for efficacy (1patients is on active treatment). Median number of cycles was 4 (range 4-6). A complete response (CR/uCR) was achieved in 11 patients (84.6%) (in 1 patient, the CR was achieved after involved field radiotherapy) and partial response in 2 patients (15.4%) after chemoterapy. Two patients died due to infectious complications and 3 patients relapsed during follow-up at 3, 5 and 13 months respectively, all dying with active disease. Overall Survival at 12 months 75% (95%CI 53-97) and Progression-free survival at 12 months 67% (95%CI 43-91), median time of follow-up for surviving patients of 16.6 months (4.9-28.3). Treatment was well tolerated with grade III-IV neutropenia in 16.6% of cycles and 4 hospital admissions for febrile neutropenia. No other relevant toxicities were observed. Therapy was delayed in 8.3% of cycles. LVEF was not significantly different before and after treatment with 1 patient showing a significant improvement in his LVEF. *Conclusions.* This preliminary data indicate that reduced dose NPLD in modified CHOP regimen is an active and well tolerated treatment in patients with severe comorbidities and formal contraindications to receive standard therapy. Further exploration of this regimen administered every 14 days is warranted.

1303

DOSE ATTENUATION OF R-CHOP FOR THE TREATMENT OF ELDERLY PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

Y.J. Choi, J.S. Chung, K.W. Lee, H.J. Shin, G.J. Cho, M.K. Song, Y.M. Seol

Pusan National University Hospital, BUSAN, South-Korea

Background. More than half of the patients with newly diagnosed aggressive lymphomas are older than 60 years. The addition of rituximab to the conventional CHOP regimen, administered every 21 days, has conclusively demonstrated to lead to a significant improvement of the outcome in elderly patients with DLBCL and survival benefit. But treatment related toxicities and delay of regimen are frequent. *Aims.* The aim of this study was to evaluate the efficacy and toxicity of dose attenuated CHOP with rituximab 3 weekly in untreated elderly DLBCL. *Methods.* CHOP regimen consisted of cyclophosphamide(600 mg/m^2), doxorubicin (30 mg/m^2), vincristine (1 mg/m^2) on day 1 , and prednisolone (20 mg bid) given on day 1 to 5 every 3 weeks. Rituximab (375 mg/m^2) was administered the same day as CHOP. *Results.* This study included 37 patients with DLBCL with a median age of 70 years (range:64-81). Seventy six percent had an international prognostic index score>1, 35% had stage IV disease. Toxicity was calculated over 192 cycles administered. Grade 4 neutropenia developed in 2.6% of cycles and no grade 4 non-hematologic toxicity didn't developed except asthenia (1 person). The overall response rate was 83.3% (complete response rate: 72.2%). 2 year overall and event free survival rates were 79.8% and 67%, respectively. *Conclusions.* Dose attenuated R-CHOP is effective and tolerable for elderly patients with DLBCL.

1304

THERAPEUTIC MANAGEMENT OF CENTRAL NERVOUS SYSTEM LYMPHOMAS IN A SINGLE HAEMATOLOGICAL CENTRE

L. Iványi, E. Marton, M. Plander, G. Gyánó, L. Czumbil

Univ Teach Hosp Markusovszky, SZOMBATHELY, Hungary

Primary central nervous system lymphoma (PCNSL) is defined as an extranodal lymphoma arising in the central nervous system in the absence of systemic disease. Because of their rare occurence among lymphomas, the optimal treatment could hardly be established.AIMS of the STUDY.In this retrospective survey we analyzed the results of combined treatment (sytemic and intrathecal chemotherapy followed by consolidation radiotherapy) in patients (pts)with primary or relapsed central nervous sytem lymphomas diagnosed and treated in our haematological department between 1998-2008.PATIENTS and *Methods.*During this period from 427 pts with newly diagnosed non-Hodgkin's lymphomas (nHl) in 19 pts PCNSL was diagnosed (4.44%, 14 cerebral and 5 spinal cord lymphoma cases).A significant male predominance (12:7) was registered with an age distribution of 34-77 (mean of 60.6,median of 64)years.No patients were immunocompromised. All CNS lymphoma specimens taken with neurosurgical resection or stereotaxic biopsies were confirmed histopathologically.All cerebral lymphoma cases proved to be diffuse large B-cell origin, whilst in spinal cord lymphomas low grade subtypes of nHl dominated.TREATMENT.Spinal cord lymphomas were treated mainly with local radiotherapy (30-40 Gy),except for patients with follicular lymphomas getting rituximab-containing polychemotherapy (R+CHOP-regimen) before irradiation.In cerebral lymphoma (every pts had supratentorial localization) the following combined therapy protocol was used:up to three courses of high dose metothrexat(HD MTX 3 g/m^2 in a single dose for 4 hours lasting drop-infusion) were given at a 4-week intervals.Leucovorin-rescue started after 24 h finished of MTX infusion.Intrathecal combination of metothrexat 15 mg,cytosin-arabinosid 35 mg and dexamethason 4 mg was given three times after HD MTX infusion.In complete response after chemotherapy (evaluated by cranial MRI or CT, recently PET/CT) whole-brain irradiation used in a total dose of 30 Gy.In partial response a boost irradiation for the tumour bed as rescue was also given.In relapse or resistant cases a salvage regimen was applied:after HD MTX course followed by high dose cytosin-arabinosid (HD Ara-C) 3 g/m^2/doe b.i.d.infused over 4 h i.v., repeated in three cycles every four weeks. *Results.* Complete remission had achieved in seven pts with cerebral and in four with spinal cord lymphoma (11/19, 58%),however one relapsed patient despite salvage therapy became resistant and later expired.Primarily nine pts were not valuable for response:five received only one or two HD MTX cycles and then died of causes related to therapy,four others died due to progression of disease.Mean of overall survival (OS) was 13.2 (2-66,median of 5) months,whilst mean time to progression (TTP) was 4.5 (2-6.5,median of 4) months.The 2-year survival for all pts was 31%. Acute toxicity of chemotherapy was usually haematological, moreover in 8 pts impaired renal function and sepsis developed.No severe adverse effect of radiotherapy could be observed.*Conclusions.*In PCNSL the basic treatment HD metothrexat together with intrathecal combination of cytosin-arabinosid, metothrexat and dexamethason followed by whole-brain irradiation of at least 3Gy could produce a medium response rate in our study.In relapse or progression other salvage regimens containing HD Ara-C alternating with HD MTX could reduce the treatment failure,as well.

1305

TREATMENT OF CUTANEOUS T-CELL LYMPHOMA WITH VORINOSTAT IN COMBINATION WITH OTHER AGENTS: A CASE SERIES

L.J. Geskin,[1] C. Sanz-Rodriguez[2]

[1]University of Pittsburgh School of Medicine, PITTSBURGH, USA; [2]Merck Research Laboratories, MADRID, Spain

Background. Vorinostat, a histone deacetylase inhibitor, was approved by the FDA in October 2006 for cutaneous manifestations of cutaneous T-cell lymphoma (CTCL) in patients with progressive, persistent, or recurrent disease on or following two systemic therapies based on the results of a Phase IIB trial which demonstrated an objective response in ~30% of patients with ≥Stage IIB disease. *Aims.* To present a case series of patients with advanced CTCL treated with vorinostat in combination with other therapies. *Methods.* Records from three patients were reviewed for the durable response of vorinostat in combination therapy. *Results.* Patient A, a 77-year-old female with Stage IVA Sézary Syndrome, intolerant of bexarotene, who failed extracorporeal photopheresis (ECP), began vorinostat monotherapy (400 mg/day). Clinical improvement of erythroderma and pruritus was noted within 5 weeks and clinical response was maintained for 26 months. Grade 3 thrombocytopenia led to dose reduction of vorinostat to 300 mg/day and subsequently the patient relapsed. IFN-γ was added and improvement in symptoms was observed within 4 weeks with ongoing response (>9 months). Patient B, a 64-year-old male with Stage III Sézary Syndrome, on bexarotene, ECP, topical steroids and narrow-band (NB) UVB, had failed three other systemic therapies including IFN-α. Vorinostat (200 mg/day) addition led to resolution of pruritus and improvement of erythroderma within 4 weeks, normalization of blood counts within 3 months and response is ongoing (>7 months). Patient C, a 47-year-old male with Stage IIA folliculotropic mycosis fungoides, had shown significant clinical response to combined NB-UVB therapy and bexarotene. Bexarotene was discontinued due to elevated creatinine phosphokinase, with disease relapse. Vorinostat (400 mg/day) was added to ongoing NB-UVB therapy and a clinical response (resolution of pruritus and improvement of all skin lesions) was achieved within 12 weeks. Patient C had complete clinical response within 5 months; NB-UVB was discontinued with ongoing (>8 months) complete clinical response. In these three patients, vorinostat was generally well tolerated; most adverse events were transient and Grade 1 or 2 in intensity. Consistent with previous reports, all three patients experienced mild-to-moderate anemia and thrombocytopenia. *Conclusions.* This case series documents the clinical application of vorinostat in combination with multiple other agents and supports the efficacy of vorinostat in inducing relatively quick, meaningful and prolonged responses. Clinical responses were noted in patients with progressive disease on their current therapies, which is suggestive of synergistic effects. These combinations should be further evaluated systemically in order to establish the place of vorinostat in treatment algorithms for CTCL.

1306

USE OF TEMOZOLOMIDE IN THE TREATMENT OF ADVANCED CENTRAL NERVOUS SYSTEM (CNS) NON HODGKIN LYMPHOMA

A. Ferrari,1 E. Conte,[2] V. De Sanctis,[3] M.C. Cox,[2] A. Moscetti,[2]
M. Pacilli,[4] F. Saltarelli,[4] G. La Verde,[4] M.A. Aloe Spiriti,[4]
R. Maurizi Enrici,[5] B. Monarca[4]

[1]Un. La Sapienza II facoltà Az.Osp. Sant'Andrea, ROMA; [2]University La Sapienza II facoltà Az. Ospedaliera Sant'Andrea Hematology, ROMA; [3]University La Sapienza II facoltà Az. Ospedaliera Sant'Andrea Radiotherapy, ROMA; [4]University La Sapienza II facoltà Az. Ospedaliera Sant'Andrea Hematology, ROMA; [5]University La Sapienza II facoltà Az. Ospedaliera Sant'Andrea Radiotherapy, ROMA, Italy

Background. The CNS localization of NHL actually represents a rare event associated with poor prognosis, both as systemic progression and primary localization in immunocompetent patients. Recent data define temozolomide as an active and effective drug in this patient group. *Aims and Methods.* From January 2004 to August 2007 we treated 4 patients, median age 57 years (range 34-72), 3M:1F. The histologic findings were: enteropatic T NHL, DLBCL with mediastinal sclerosis, Burkitt-like primary CNS NHL, primary immunoblastic B-cell NHL. Two patients had systemic NHL (stage IV) without CNS involvement at the time of diagnosis: both obtained a clinical response with first line therapy. One patient showed a systemic with CNS progression 11 month later and another patient showed CNS progression 8 months after autologous SCT performed as third line therapy; the latter had already been treated with whole brain radiotherapy (30 Gy) for previous isolated meningeal progression. Two patients presented a primary CNS NHL: both were treated with HDMTX±ARA-C schedule and then with whole brain radiotherapy (30 and 45 Gy) for no response to chemotherapy. All patients were treated with temozolomide 150 mg/m^2 five consecutive days every 21-28 days for a median number of 5 courses (range 2-10). Three out of four patiens started temozolomide for progression after radiotherapy and one patient as maintenance therapy after response obtained with radiotherapy. Anti-CD20 monoclonal antibody therapy (Rituximab) was administered during the whole treatment with temozolomide in three out of four patients; all the patients were supported with corticosteroids. *Results.* Three patients experienced a radiological response and clinical improvement with good control of neurological impairment. Among these three patients, one had a systemic progression of disease after 2 months of temozolomide, the other two presented a CNS progression after 2 and 6 months, respectively. The patient who started temozolomide in response after radiotherapy mantained clinical response for a time period of 12 months, when he showed a CNS pro-

gression. All the patients died in a median time of 11 months (range 4-19) from the last relapse/progression. No patient suspended treatment because of hematological or non-hematological toxicity. *Conclusions.* In this group of patients temozolomide resulted a safe treatment able to determine clinical improvement, even if of short duration.

1307

CLINICAL FEATURES AND TREATMENT OUTCOME OF PRIMARY GASTRIC MUCOSA ASSOCIATED LYMPHOID TISSUE LYMPHOMA

M. Todorovic,[1] N. Suvajdzic,[1] M. Perunicic,[1] B. Balint[2], M. Krstic,[1] D. Boskovic1

[1]Clinical Center of Serbia, BELGRADE; [2]Institute for Medical Research, University of Belgrade, BELGRADE, Serbia

Background. Gastric low grade B cell lymphomas arising from mucosa associated lymphoid tissue (MALT) are the most frequent lymphomas among those located in the primary digestive tract. *Aims.* To determine clinical characteristics and treatment outcome of gastric lymphoma after chemotherapy and immuno-chemotherapy. *Methods.* Thirty four patients with primary gastric mucosa associated lymphoid tissue (MALT) lymphoma (Ann Arbor stages I to IV) were enrolled. All had upper gastric endoscopy, abdominal ultrasonography, CT and Helicobacter pylori status assessment (histology and serology). All patients with gastric MALT lymphoma received poly- or monochemotherapy (Cyclophosphamide, Doxorubicine, Vincristine, Prednisolone - CHOP; Chlorambucile, Vincristine, Procarbasine, Prednisolone - LOPP; or Chlorambucile) or combined immuno-chemotherapy (Rituximab-CHOP) as the first line treatment. The reason for giving chemotherapy or immunochemotherapy to all patients as first line treatment was the BCL-10 postivity of all patients with clinical stage I + II. Four patients were operated (subtotal gastrectomy). Patients who were Helicobacter pylori positive, received triple anti-Helicobacter pylori treatment combined with chemotherapy or immuno-chemotherapy as first line management. After anti-Helicobacter pylori treatment and initial chemotherapy, patients were re-examined every four months. *Results.* Histological regression of the lymphoma was complete in 22/34 (64.7%) and partial in 9 (26.5%) patients. Median follow up time for these 31 responders was 60 months (range 48-120). No regression was noted in 3 patients. Among the 25 (73.5%) Helicobacter pylori positive patients, the eradication rate was 100%. Distribution of IPI value showed that the most number of patients had IPI score 3. The actuarial survival curve estimated high survival rate (83%) in the first 12 months of the following period. The use of the Cox proportional hazard model determinated negative independent prognostic predictive factors for overall survival (OS): high IPI score, elevated ESR (erythrocyte sedimentation rate) and low platelets (χ^2=13.397, df = 3, p=0.0039). Multivariate logistic regression showed the significance of elevated CS and low value of hemoglobin as independent prognostic factors that had negative influence for the outcome (dead or alive) of the disease (df=1, p=0.0049), whilst the elevated ESR had negative impact for achieving CR (df=1, p=0.0107). *Summary and Conclusions.* These data show that adequate patient selection for treatment option offer them good chance for long-term survival. Cox proportion hazard model differentiate IPI score, ESR, and platelets as predictors of survival.

1308

QUALITY CONTROL AND EXPERIMENTAL USE OF FIBRIN GLUE PREPARED BY RECYCLED CRYOPRECIPITATION IN LIVER SURGERY

M. Jevtic, B. Balint, G. Ostojic

Military Medical Academy, BELGRADE, Serbia

Background. Fibrin glue (FG) is a two component biologic system with adhesive, sealant and topical hemostatic properties, containing fibrinogen (Fg), factor XIII (FXIII), fibronectin (Fn), thrombin, some antifibrinolytic agent and ionized calcium. *Aims.* to investigate of quality and clinical efficacy of FG, in experimental setting prepared with our own method (by recicled cryoprecipitation). *Methods/Results.* In this study, FG component 1 was prepared by recycled cryoprecipitation from single-donor plasma. The mean concentrations of Fg, FXIII and Fn were: 54.2+19.9 g/L, 8.03+2.3 IU/mL and 3103.1+148.91 mg/L, respectively. Horizontal tensile strength of FG was 1.076+0.18 N/cmE2 in the average. Using a rat model, the efficacy of the FG-treatment in liver surgery was evaluated on the basis of the 24 hour survival ratio and hematological parameters of the experimental animals and control group. Survival of rats subjected to partial and total lobectomy and FG-treated was significantly higher than in FG-nontreated animals. Survival of animals subjected to liver incision was not significantly different, however the differences in hematological parameters were significant in favor FG-treated animals. *Summary and Conclusions.* The rerults obtained confirmed that high-quality FG can be prepared by recycled cryoprecipitation from single-donor plasma, with sufficient yield of fibrinogen, FXI-II and fibronectin which have efficient hemostatic therapeutic properties.

1309

A CYTOGENETIC STUDY OF VARIANT PHILADELPHIA REARRANGEMENTS IN CHRONIC MYELOID LEUKEMIA

A. Bennour,[1] H. Sennana,[1] M.A. Laatiri,[1] M. Elloumi[2], B. Meddeb,[3] A. Khelif,[1] A. Saad[1]

[1]Farhat Hached Hospital, SOUSSE; [2]Hedi Chaker Hospital, SFAX; [3]Aziza Othmana hospital, TUNIS, Tunisia

Background. Variant translocations involving 9q, 22q, and at least one additional genomic locus occur in 5-10% of patients with chronic myeloid leukemia (CML). The mechanisms for the formation of these variant translocations are not fully characterized. Studies on the prognosis of these variant translocations revealed conflicting results; in addition, deletions in the derivative chromosome 9 are reportedly more frequent among variant translocation cases. *Aims.* To help shed light on these controversial subjects, we sought to analyze all the variant translocation cases of CML identified by the cytogenetics laboratory in Farhat Hached hospital between the years of 2001 and 2007. *Methods.*We analyzed cytogenetic and fluorescence *in situ* hybridization (FISH) data from 23 CML patients with variant translocations tested at our laboratory, Cytogentic studies was performed with R-banding. For FISH studies, we used the BCR/ABL dual-color, single-fusion extra signal (ES) probe from Abbott,Vysis, furthermore a Spectrum Aqua probe (Aq) covering the argininosuccinate synthase gene (ASS) 5' to the ABL sequence on chromosome 9q34 was also included with the BCR/ABL ES probes to assist in the detection of 9q deletions and the identification of the Ph chromosome in the interphase nuclei. Whole chromosome paintings were also used to explain the genesis of these variant translocations. *Results.* Among 301 CML patients with Philadelphia chromosome (Phi), 23 patients had variant translocations (7,64%). Our results demonstrated that all chromosomes could be implicated in variant Phi rearrangements and we defined 33 breakpoint which distribution did exhibit a nonrandom pattern, and has been reported to locate preferentially in the CG-richest regions of the genome, typically represented by the light bands in R-banded chromosomes, some breaks have already been described. Deletions der (9) were observed in 10 cases with FISH data available (43,47%), consistent with the literature and higher than in typical translocation cases (12-15%). Sequential changes of 9q deletions are possible and could be acquired as the disease progresses in addition to simultaneous formation of the Philadelphia chromosome with the deletion. Variant translocations may be formed by either a one-step or a two-step mechanism. *Conclusions.* Proper assessment of the prognostic significance of variant translocations requires better categorization of these translocations based on their mechanisms of genesis and the deletion status.

1310

IMPACT OF S TRAIT IN THE DETERMINING OF ARTERIAL HYPERTENSION: RESULTS OF A PROSPECTIVE STUDY ABOUT 86 PATIENTS WITH HYPERTENTION

S. Mseddi, S. Mseddi, M. Frikha, J. Gargouri, Z. Labiadh, M. Elloumi, T. Souissi

Hedi Chaker Hospital, SFAX, Tunisia

Introduction. Many studies have shown that arterial hypertension (HTA) is more frequent and more serious in black population, even in Tunisia. Many factors were sought if responsible for this phenomenen, including the sickle cell trait. In this work, we intended to do a screening of S trait, and to study the characteristics of the hypertension in correlation with the ethnic group (white or black) and with the S trait, in all patients followed for HTA in Mansoura, a region of south Tunisia where there is a high frequency of S trait and of black individuals. *Patients and Methods.* For all patients followed for HTA in this region, we determined : the patient coulor, HTA characteristics (age of onset, antihypertensive drugs received, the quality of the equilibrium of arterial pressure) and we performed a falciformation test and a hemoglobin electrophoresis at alkaline pH, to determine the presence of S trait (A/S) or a normal profile (A/A). Results were analized by t, χ^2 and Fisher tests. *Results.* Eighty six individuals are included in the study. Fourty three are black

and 43 are white. Seven A/S patients are found (8,15%). S trait frequency in HTA patients is 11,62% in blacks and 4,65% in whites. This difference is not statistically significant (p=0,4). HTA characteristics in different groups separated according to the race and the presence or absence of S trait, shows that the mean age of HTA onset and at the moment of the study, is significantly younger in A/S than in A/A patients [42,9 *vs* 53,2 years (p=0,036) and 49,1 *vs* 61,4 years (p=0,011)]. A difference is also found when A/S black patients are compared to A/A white [40,6 *vs* 54,1 ans (p=0,02) and 46,6 *vs* 62,4 ans (p=0,003)] and to A/A black patients [40,6 *vs* 52,2 ans (p=0,08) and 46,6 *vs* 60,4 ans (p=0,033)]. However, this HTA needs less antihypertensive treatment and has a better blood pressure stability in A/S than in A/A patients. *Comments*. The frequency of S trait in HTA patients in Mansoura (8,15%) is not significantly different from that in the general population (10,83%) in a study performed in a representative sample (p=0,4). According to this study, the S trait is not more frequent in patients affected with hypertension, and therefore it does not predispose to HTA. However, this HTA breaks out at a significantly lower age in A/S patients especially the black ones, but it is not more serious according to the criteria used in this study, which are the needed treatment and the arterial blood pressure stability.

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Withdrawn by the authors

1312

CHRONIC LARGE GRANULAR LYMPHOCYTE LYMPHOPROLIFERATIVE DISORDERS (LGL-LD) - IMMUNOPHENOTYPIC CHARACTERISTICS

N. Kraguljac Kurtovic, V. Stanojevic, A. Bogdanovic, T. Dragovic-Ivancevic, B. Mihaljevic, M. Gotic

Clinical Center of Serbia, BELGRADE, Serbia

Background. LGL-LD which origin from morphologically recognized lymphocytes called LGL, represent rare and in biological and clinical sense, very heterogenous group (Jaffe *et al.,* 2001). According to lineage of pathologic lymphocytes, the group was divided on two basic classes - T-LGL-LD ($CD3^+$/T-Ag^+/$CD1a^-$/TdT^-) and NK-LGL-LD ($CD3^-$/$NKAg^+$). The main diagnostic procedure for detection and classification of LGL-LD is immunophenotyping, but final diagnosis (dg) of this disorders should be confirmed on cytomorphologic, clinical and molecular level. *Aims*. To study in detail immunophenotypic characteristics of pathologic lymphocyte populations gained from the group of patients finally documented as T-LGL-LD or NK-LGL-LD. During the study, the accent was on estimation of the specific patterns of aberrant Ag expression, as a characteristics of pathologic LGL. *Methods.*Immunophenotyping and multiparameter flow cytometry (IMFC) were performed by using standard two- and three-color monoclonal antibody panels, with specificity for T-, NK-, B-, and lineage non-specific antigens (Ag). Native peripheral blood or bone marrow specimens were labeled and analyzed by using the gating strategy which included lymphocyte gate (FSClow/SSClow), in combination with CD3 or NK-Ag gating, in some cases. Ag expression patterns were defined as aberrant according to normal Ag expression patterns detected on peripheral blood T- and NK-lymphocytes of healthy control group (n=10). *Results*. Study was conducted in time period 1997-2008 y., in a series of 720 untreated adult patients (pts) with suspected dg *de novo* Chronic LD. According to immunophenotypic characteristics, cytomorphologic and clinical criterions, final dg of LGL-LD fulfilled 21/720 (2.9%) pts, whereas 15/21 (71%) pts were with dg T-LGL-LD and 6/21 (29%) pts with dg NK-LGL-LD. T-LGL-LD were classified in three main classes according to immunophenotypic profile: $CD2^+CD3^+TCRaCD8^+CD4\text{-}CD25^-$ (10/15 pts) *vs* $CD2^+CD3^+TCRCD4^+CD8^-CD38^+CD25^-$(4/15 pts) *vs* $CD2^+CD3^+TCRa/b^+CD4^+CD8^+CD25^-$ (1/15 pts). High frequency of Ags expression on lymphocytes of T-LGL-LD, was disclosed for: CD5 (13/15 pts), CD7 (12/15 pts), CD57 (6/8 pts) and HLA-DR (6/8 pts). Abberant Ags expression pattern was revealed with high frequency for, CD7 (10/15 pts) and CD5 (9/15 pts), but with lower frequency for CD2 (3/15 pts). Patients with NK-LGL-LD were classified in one main class according to immunophenotypic profile - $CD2^+CD7^+/CD16^+56^{/+}CD3^-$ (6/6 pts). Expression of CD8 Ag was documented in a small subpopulation in 3/6 pts, whereas the aberrant Ag expression patterns were disclosed for: CD56 (2/3 pts), CD16 (3/5 pts), CD7 (3/6 pts), and CD2 (1/6 pts). *Conclusions*. IMPC is important diagnostic procedure which enables precise diagnosis of T- and NK-LGL-LD regarding differentiation from B-LD. Combination of aberrant expression patterns for CD7 and CD5 Ags in T-LGL-LD, and CD56, CD16 and CD7 Ags in NK-LGL-LD, represent marker characteristics of pathologic lymphocytes, and make possible reliable differentiation from reactive T i NK proliferations.

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PROGNOSTIC IMPACT OF ZAP-70 EXPRESSION AND OF OTHER BIOLOGICAL MARKERS IN CHRONIC LYMPHOCYTIC LEUKEMIA TREATED WITH FLUDARABINE CONTAINING REGIMENTS

I. Nichele, A. Ambrosetti, C. Sissa, M. Chilosi, R. Zanotti, O. Perbellini, F. Frattini, G. Pizzolo

Univeristy of Verona, VERONA, Italy

Background. In chronic lymphocytic leukemia (CLL) the prognostic significance of ZAP-70 and of other biological markers has been validated by large retrospective studies, mostly including patients conventionally treated. The impact of these prognostic factors, with the notable exception of cytogenetics, has been recently questioned in CLL patients more aggressively treated. *Aims.* we retrospectively investigated the impact of ZAP-70 expression and of other prognostic markers on predicting treatment outcome in CLL patients who received fludarabine containing regiments. *Methods.* we evaluated 76 patients with CLL, diagnosed between 1985 and 2005 at our Institution. Of them, 47 were males and 29 females (ratio 1,6) with a median age at diagnosis of 57 years (range 33-79). At the beginning of treatment 19 patients were at stage A according to Binet classification, 35 at stage B and 22 at stage C. ZAP-70 expression was detected in all 76 patients by immunohistochemistry on bone marrow biopsies taken at diagnosis. Fifty eight patients were ZAP-70 positive (76,5%) and 18 were ZAP-70 negative (23,5%). In 35 patients the mutational status of IgV(H) was also analysed: 27 were unmutated, 8 mutated. CD38 expression by flow cytometry and thymidine kinase (TK) levels were measured in 56 and 47 patients respectively: 34 were CD38 positive (>30%), 22 negative, in 22/47 TK levels were elevated (>9,1U/L). In 35/76 patients fludarabine containing regimens were given as first line therapy, in the remaining as second line treatment. Patients received either fludarabine alone (F=17), or fludarabine plus cyclophosphamide (FC=59); in 3/59 cases rituximab was associated to FC (FCR). The median number of chemotherapy cycles administered was 4 (range 3-6). All patients were treated at disease progression according to NCI-WG criteria. Response to treatment was evaluated after 1 to 2 months after the end of chemotherapy by physical exam, blood counts, abdominal ultrasound or CT scan and by bone marrow biopsy (30/76 patients). Clinical response was also defined according to NCI-WG criteria. *Results.* complete response (CR) plus unconfirmed complete response (uCR) rates for patients receiving fludarabine containing regimens were 15,5% in ZAP-70-positive patients *vs* 44,5% in ZAP-70-negatives (p=0.05). Median actuarial overall survival (OS) and progression free survival (PFS) of all the 76 patients were 95 and 15 months respectively. There was no statistically significant difference in OS and PFS according to ZAP-70 expression. OS, but not PFS, were statistically improved in IgV(H) mutated patients (p=0,015). Binet stage, CD38 expression and TK levels did not significantly influence response and disease outcome. *Conclusions.* Although based on a limited number of patients, our results suggest that in CLL the prognostic adverse impact of ZAP-70 expression and of other prognostic biological markers can be, at least in part, counterbalanced by the efficacy of fludarabine chemotherapy regiments.

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XENOGENIC CARTILAGE MATRIX EXTRACTS SPECIFICALLY INDUCE CHONDROCYTIC DIFFERENTIATION OF HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS

A. Bazarbachi,[1] H. El-Khoury[2], R. Hamdan-Khalil[2], S. Sindet-Pedersen,[3] M. El-Sabban[2]

[1]American University of Beirut Medical Center, BEIRUT, Lebanon; [2]American University of Beirut, BEIRUT, Lebanon; [3]The London Research Company, LONDON, UK

Background. Bone marrow-derived mesenchymal stem cells (BMMSC) represent a valuable source for regenerative medicine due to their multi-lineage differentiation potential. Cartilage has limited ability to self-repair. Recent approaches of tissue engineering are based on the use of biodegradable scaffolds in combination with chondrocytes. We have previously shown that xenogenic bone matrix extracts (BME) induce osteoblastic differentiation of human BMMSC. *Aims and Methods.* In this report, we investigate the effect of acellular cartilage matrix extracts (CME) on *in vitro* expanded human adult BMMSC. *Results.* We demonstrate that BMMSC treated with either bovine meniscus or joint CME exhibited characteristic chondrocytic morphological changes accompanied by the expression of chondocyte specific markers such as SOX9, increase levels of aggrecan protein, and deposition of glycosaminoglycans (GAGs), explicitly demonstrating that these CME induce chondro-

cytic differentiation of BMMSC *in vitro*. Interestingly, CME did not induce osteoblastic differentiation of BMMSC. Conversely, BME induced osteoblastic but not chondrocytic differentiation of BMMSC. *Conclusions.* L Hence, xenogenic organ derived extracts induce tissue-specific differentiation of BMMSC presumably through providing stem cells with structural and soluble mediators that mimic the *in vivo* microenvironment. These results provide a novel tissue engineering-based treatment of cartilage defect, using autologous BMMSC pretreated with CME.

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MESENCHYMAL PROGENITOR CELLS IN RED AND YELLOW BONE MARROW

A. Gurevitch,[1] Y. Slavin[2], B. Resnick,[1] G. Feldman[3]

[1]Hadassah University Hospital, JERUSALEM, Israel; [2]The International Center for Cell Therapy & Cancer at the Tel Aviv (Sourasky) Me, TEL AVIV, Israel; [3]University of Montreal, MONTREAL, Canada

Background. Marrow cavities in all bones of newborn mammals contain functionally active hematopoietic tissue supported by hematopoietic microenvironment - the composite referred to as red bone marrow (BM). From the early postnatal period onwards, the hematopoietic microenvironment mainly in tubular bones of the extremities is replaced by mesenchymal cells that accumulate lipid drops, referred to as yellow or fatty BM, whereas hematopoietic tissue - gradually disappears. It has been shown that an acute anemia leads to an increase in hematopoietic components in the areas of mixed red and yellow BM, whereas evacuating of yellow BM from long bones of anemic animals results in development of active hematopoiesis in vacant sites. Working hypotheses. We hypothesized that: 1) substitution of red with yellow BM in tubular bones results from a gradual loss of mesenchymal stem cells (MSCs) capable of developing bone and microenvironment supporting hematopoiesis; 2) mesenchymal cell population remaining in the areas with yellow BM contains mesenchymal progenitor cells that can develop functionally active hematopoietic microenvironment in conditions of hematopoietic insufficiency. *Methods.* To test these hypotheses, we used the experimental model of ectopic BM transplantation allowing evaluation of functional activity of mesenchymal progenitor cells *in vivo* according to their ability to produce bone and hematopoietic microenvironment (Figure1-A). BM from distal third of the tibia taken from rats of different ages was transplanted under the kidney capsule to normal (young untreated), total body irradiated, or old recipients. *Results.* Transplantation of red BM from young donors to normal recipients resulted in formation of osteo-hematopoietic foci (Figure 1-C).

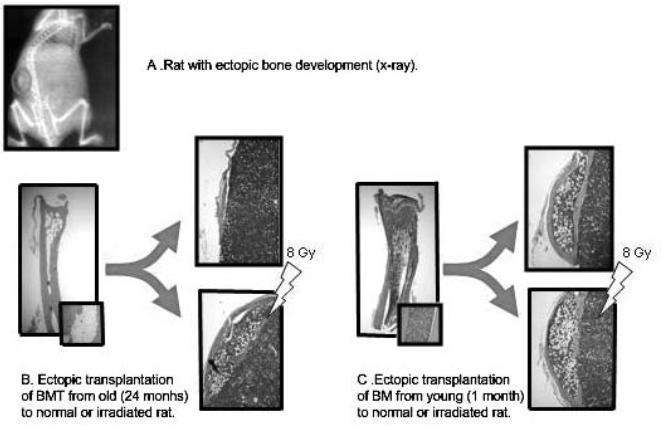

Figure 1. Ectopic bone marrow transplantation.

The older was the donor (i.e. the more fat contained the BM transplant) the less was the percentage of foci with hematopoietic microenvironment developed in normal recipients. Eventually, BM transplants from 2 years old donors formed small bones not supporting hematopoiesis in 100% of normal recipients (Figure 1-B), thus confirming hypothesis 1. However, in recipients with hematopoietic deficiency (irradiated or old), BM transplants of any age produced bone and hematopoietic microenvironment supporting hematopoiesis, verifying hypothesis 2. *Conclusions.* Results of this study not only provide evidences for both hypotheses but also imply that: 1) MSC population in tubular bones yet containing active hematopoietic tissue gradually becomes depleted of MSCs, starting from young age; 2) hematopoietic microenvironment is incapable of self-maintaining and its renewal depends on the presence of precursor cells; 3) fat in bone cavity, in contrast to subcutaneous fat, does not contain MSCs. Moreover, our data may indicate the existence of bi-potential stromal precursor cells producing either bone in normal or bone together with active hematopoietic microenvironment in irradiated or old recipients. Destroying of structural integrity of fatty tissue seems to be essential factor permitting or even stimulating mesenchymal progenitor cells in yellow BM to develop functionally active hematopoietic microenvironment. This study opens a spectrum of opportunities for an extension of active hematopoietic territories by substituting fat contents of BM cavities with hematopoietic tissue. In particular, this approach might be used to improve hematopoiesis compromised by cytotoxic treatments or irradiation.

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EXPRESSION OF GLUCOCORTICOID RECEPTOR IN NK LYMPHOPROLIFERATIVE DISEASES

A.F. Marinato, E.M. Rego, A.B. Garcia, R.P. Falcão

Medical School Ribeirão Preto, University of São Paulo, RIBEIRÃO PRETO, Brazil

Human glucocorticoid receptor (hGR) presents different isoforms, among then hGRalfa is responsible for the primary response to glucocorticoids whereas hGRbeta exerts a negative effect on hGRalfa function. Therefore, the hGRalfa/hGRbeta isoforms ratio is directly responsible for cellular sensibility to corticoids. The purpose of this study was to determine the hGR expression in NK and T/NK malignancies. Mononuclear cells were obtained from the peripheral blood of 16 healthy individuals and from the bone marrow or peripheral blood of 19 patients with NK lymphoproliferative neoplasms in leukemic presentation (three indolent NK-cell leukemia, two aggressive NK-cell leukemia, one blastic NK-cell lymphoma, nine T-cell large granular lymphocyte leukemia, two hepatosplenic T-cell lymphoma and two unspecified peripheral T-cell lymphoma). Using quantitative flow cytometry, we analyzed normal and leukemic NK ($CD3^-$, $CD56/16^+$) and T/NK ($CD3^+$ $CD56/16^+$) cells. The samples were labeled with anti-CD3, anti-CD56 and/or anti-CD16 antibodies, permeabilized and then incubated with saturating concentrations of anti-hGRalfa and anti-hGRbeta. A Facscalibur cytometer and CellQuest Pro software were used for acquisition and analysis. The expression intensity was measured by the Mean Fluorescence Intensity (MFI). Both, healthy and leukemic NK and T/NK cells expressed alfa and beta hGR isoforms. In normal individuals hGRalfa was detected in 94.2 to 99.9% of NK cells (median 99.3%) and in 95.2 to 99.8% of T/NK cells (median 98.8%). The hGRbeta was detected in 63.2 to 99.2% of NK cells (median 93.6%) and in 62.3 to 99.7% of T/NK cells (median 94.9%). Among leukemic cells, hGRalfa was expressed in 72.0 to 100% of NK cells (median 99.6%), in 64.4 to 99.3% of T/NK cells (median 98.0%), and hGRbeta was positive in 73.4 to 99.6% of NK cells (median 95.7%) and in 22.0 to 99.1% of T/NK cells (median 87.1%). The MFI of hGRalfa in normal lymphocytes ranged from 197.2 to 263.7 (median 232.3) and in leukemic cells from 136.3 to 291.9 (median 242.3) (p=0.27). The MFI of hGRbeta in normal cells was higher (144.5 to 226.0; median 198.3) as compared with leukemic cells (36.0 to 238.0; median 121.8) (p=0.0001). The alfa/beta ratio in normal NK lymphocytes ranged from 0.97 to 1.54 (median 1.22) and in neoplastic NK cells from 1.10 to 6.63 (median 1.95) (p=0.0001). The higher alfa/beta ratio in the NK and T/NK lymphoproliferative diseases suggested that neoplastic NK cells are more sensitive to glucocorticoid cytotoxicity than the normal NK lymphocytes. This finding must be correlated in future studies with *in vitro* and *in vivo* sensibility to corticoids.

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MEASURE BY FLOW CITOMETRY EFFECTS OF CONTINUOUS POSITIVE AIRWAY PRESSURE (CPAP) ON OXIDATIVE STRESS IN LEUKOCYTES (WBC) OF OBSTRUCTIVE APNEA PATIENTS (OSA)

R. Garcia-Delgado, S. Garcia-Segovia, A. Fernandez-Ramos

Hospital Virgen de la Victoria, MALAGA, Spain

Background. OSA is one of the most important risk factors of cardiovascular disorders due to a cell hypoxia/re oxygenation phenomenon. Under this chronic hypoxia conditions, oxidative stress is increased. with an increase in the production of ROS (reactive oxygen species), a decreased of reduced glutathione (GH), the major intracellular antioxi-

dant, and furthermore, it is demonstrated a production of plasma lipid peroxides that produces a decreased of membrane potential. *Aims.* We studied peripheral WBC derived from patients with OSA before and after a month long CPAP treatment. *Material and Methods.* Peripheral total blood from 21 obese patients with severe OSA were studied before and after de CPAP treatment. Flow citometry was used to measure ROS generation, GH levels and lipids peroxidation in neutrophiles, lymphocytes and monocites. *Results.* Our results are presented in the Table 1.

Table 1.

	IMF L		IMF M		IMF N		IMF T	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
GSH	81,62	166,15	222,80	479,67	203,14	457,79	153,81	321,45
ROS	90,55	48,41	188,87	102,09	122,16	68,61	116,24	64,61
H_2O_2	23,29	15,15	45,98	32,40	34,78	21,70	31,37	21,37
PMM	15,32	20,61	30,27	40,27	21,78	27,72	20,39	26,40

GSH, reduced glutation; ROS, reactive oxygen species; PMM, mitochondrial membrane potential; IMF, mean fluorescent intensity; L, lymphocytes; M, monocytes; N, neutrophiles.

ROS and lipid peroxidation were found to be lower, and GH higher in WBC of patients after CPAP treatment. Thus, oxidative status of cells was decreased. *Conclusions.* CPAP treatment seems to be a useful way to decrease oxidative stress and the vascular risk in patients with OSA. Flow citometry is a successful method of measuring oxidative status in leukocytes in OSA.

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IMPACT OF JAK2 V617F MUTATION ON CLINICAL OUTCOME OF PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA: A RETROSPECTIVE STUDY

M. Wojcik, M. Renc, A.B. Skotnicki

Hematology Department, KRAKOW, Poland

Background. Essential Thrombocythemia (ET) is a chronic myeloproliferative disorder (CMPD), present at approx. 30/100000 population. Although clinically it is often asymptomatic, it can manifestate itself as coagulation disorder events, either of haemorrhagic or thrombotic nature. Recently, a V617F mutation of Janus Kinase 2 (JAK 2) has been found in many cases of CMPDs, including the ET. As it is not present in all of the cases, its exact role in pathogenesis is not yet clear. However, efforts have been made to assess whether the presence of the V617F mutation has any impact on many clinical and laboratory features of the ET. *Aims.* The objective of the study was to assess if the presence of JAK2 V617F mutation had any impact on hematological parameters and/or clinical outcome of ET patients. *Methods.* A group of 40 patients (24 female and 16 male) that had been treated in our hospital between 2003 and 2007, has been studied retrospectively. These patients were treated with Anagrelide, administered continuously at dose of 0,5 to 3 mg/day, depending on platelet level. Analyzed features included leukocyte count, platelet count and frequency of vascular occlusive events. *Results.* The JAK2 V617F mutation was confirmed positive in 25 cases (63%), 15 were negative. After analyses, the presence of mutation was significantly correlated with both elevated leukocytosis and presence of vascular occlusive events. Nineteen (76%)of mutation-positive patients had their leukocyte count elevated above 10×10^9/l for at least 6 months (peak value 18×10^9/L), of which six developed vascular occlusive events - four cerebrovascular and two peripheral vein thromboses. Negative patients presented neither of these. The platelet level remained below 600×10^9/l upon treatment in both groups. *Conclusions*. Elevated leukocytosis is one of thrombosis risk factors in TE patients. Correlation of JAK2 mutation presence and elevated leukocytosis shows yet another aspect of worse outcomes of mutation-positive cases. As approx 2-4% of all JAK2 mutations are homozygous, further studies on larger groups are needed to assess is this might be more frequent in cases of thrombotic events.

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MANAGEMENT OF IMMUNE THROMBOCYTOPENIC PURPURA: A STUDY OF 170 CASES

M. Medhaffar,[1] A. Ben Hmida,[1] I. Frikha,[1] M. Ghorbel,[1] M. Abid[2], H. Bellaj,[1] N. Ajmi,[1] S. Hdiji,[1] M.I. Beyrouti[2], M. Elloumi[1]

[1]Hédi chaker hospital, SFAX; [2]Habib bourguiba Hospital, SFAX, Tunisia

Background. Immune thrombocytopenic purpura (ITP) is an acquired disease characterized by an immunological peripheral platelet destruction. ITP occur at any age and had a more chronic evolution in adult. We report the therapeutic results of 170 ITP cases. *Patients and Methods.* Between January 1996 and December 2006, we retrospectively analyzed the data of 170 patients (children and adults) with ITP diagnosis. The first treatment was Glucocorticoïds: Prednisone 1mg/kg/day during 4 weeks followed by a degression, dose and duration were adapted to the elderly and the diabetics. The evaluation at days 28: complete response (CR) if platelet count is higher than 100 G/L, partial response (PR) if platelet count is higher than 50 G/L and failure if platelet count is lower than 50 G/L. For patient with chronic evolution (up on six month) we evaluated various therapeutic lines such as high dose of glucocorticoïds (bolus of methylpredisolone), Vincristine, Danatrol and splenectomy. *Results.* 114 adults (79 women and 35 men) and 56 children (32 girls and 24 boys) were followed over eleven year period. Seven children had not recquired treatement with a good evolution in few days. 131 (80%) patients (39 children and 92 adults) achived good response (CR+PR) to prednisone 44 (33%) patients (32 adults and 12 children) relapsed in a median of 5 months of response. 12 patients received bolus of solumedrol in 2nd and 3rd line with only two PR. 5 patients received Vincristine with only one CR. 2 patients received Danatrol with two persistent CR. 27 patients (16 adults and 11 children) underwent splenectomy in 2nd, 3rd and 4th line, the response rate was 87% in adults and 63% in children. A chronic evolution was noted in 47% of adults and 26% of children. Finally, we have noted any death related to thrombopenia or treatement. *Conclusions.* Glucocorticoïds is the first-choice treatement for ITP resulting in a response in 70-80% of cases. However relapse occurs in the third of cases. Splenectomy remains the treatement of choice in 2nd line in our country comparing to the cost and results of mabthera?. We have noted more frequent chronic evolution and a better response to splenectomy in adults.

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PHENOTYPING AND AGGREGOMETRIC EVALUATION OF THE PLATELET FUNCTION IN CMPD AND AML

H. Bumbea,[1] A.M. Vladareanu,[1] S. Radesi,[1] V.M. Popov[2], M. Onisai,[1] M. Begu,[1] D. Casleanu,[1] I. Voican,[1] C. Ciufu,[1] A. Nicolescu,[1] C. Marinescu,[1] V. Vasilache,[1] M. Dervesteanu,[1] E Kovacs,[3] T Savopol[3]

[1]Emergency Universitary Hospital Bucharest, BUCHAREST; [2]County Hospital, PITESTI; [3]Carol Davila University of Medicine, Department of Biophysics, BUCHAREST, Romania

Background. Extensive bleeding or recurrent thrombosis are some of the most frequent causes of death in acute myeloid leukemia (AML) and myelodisplastic syndrome (MDS)respectively chronic myeloproliferative diseases (CMPD). Previous studies on platelet behavior associated with this disease identified impaired activation and aggregation processes, with quantitative and qualitative changes in chronic myeloproliferative disorders (CMPD) and myelodisplastic syndromes (MDS). The aim of this study was to examine platelet function in these diseases associated with functional impaired platelet. *Methods.* Whole blood flow cytometry analysis for platelet surface proteins (Glycoprotein Ib-IX [CD42b,CD42a], Glycoprotein IIb-IIIa [CD41,CD61], P-selectin [CD62P], granulophysin [CD63]) was fulfilled in 25 patients with acute myeloid leukemia (AML), 42 patients with chronic myeloproliferaive diseases (CMPD)], 13 cases of myelodisplastic syndrome and 16 healthy volunteers were included in the study. Also, aggregometry by chemoluminescence method was done. Between the CMPD cases 26.2% (11 cases) were diagnosed with polycythemia vera, 26.2% (11 cases) were diagnosed with myeloid metaplasia, 19% (8 cases) were diagnosed with chronic granulocytic leukaemia, 14.3% (6 cases) were diagnosed with unclassified myeloproliferative disease, 14.3% (6 cases) were diagnosed with essential thrombocythemia. The AML subtypes according to FAB classification were 33.3% AML4, 18.2% AML2, 15.2% AML1, 9.1% AML0. *Results.* The platelet phenotyping analysis reveled a statistical significant difference between the 4 analyzed groups for the adhesion marker CD42b marker ($p<0.05$) and lightly over the statistical significance for aggregation marker CD41 ($p= 0.74$) without any other signif-

icant difference i (p>0.05) in the expression of the activation markers (CD63, CD62p) or other adhesion marker (CD42a) and aggregation marker (CD61). The direct comparison of the acute myeloid and chronic myeloproliferative groups identified a strong significant difference (r=286, p=0.04) between the CD42b expression with a lower level in AML patients (median= 63.53) *vs* CMPD patients (median = 32.45). A similar result occurred for the CD41 antigen (r=312, p=0.06) which presented lower expression in CMPD group (median = 50.50) *vs* the AML group (median AML= 76.11) Figure 1.

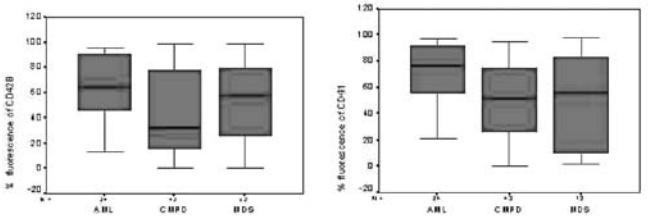

Figure 1. Expression of platelet activation markers in AML patients, versus CMPD, and MDS patients. Results are presented in box plots indicating the mediana as the horizontal line, 25 th-75th percentiles as the group distributions as boxes and 2.5-97.5% cumulative frequncies as whiskers. Outliners [identified by the 1.5 x inter-quartile range (IQR) criterion] are plotted as empety squares. N, number of case; AML, acute myeloid leukemia; CMPD, chronic myeloproliferative disease; MDS, myelodysplastic syndrome.

No significant difference was identified for the other platelet phenotyping markers. *Conclusions*. CD42b is one of the major adhesive receptors expressed on circulating platelets, interact with many ligands, the adhesive protein vWF, the coagulation factors, and the membrane glycoproteins (P-selectin and Mac-1). In our data appears a significant decrease in AML patients *vs* CMPD controls while anti CD42a antigen antibody which reacts with the whole CD42a/CD42b complex presents no particular difference in the above mentioned groups. This result might be interpreted as a particular reduction of α chain of GpIb due to down-regulation during platelet activation; possible due to a broken activation mechanism that could involve many other surface proteins. The reduction of the CD41 surface expression may be a CD42b decreasing result. Another hypothesis is that the CD41 antibody (used for the fluorescent probe) reacts with the GpIIIa unit only in the intact complex but not with the GpIIb or GpIIIa separately a process of splitting of the αIIb chain (CD41) and the β3 subunit (CD61) of the GpIIb-IIIa with disintegration or internalization of the GpIIIa may occur.

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MICROCHIMERISM - LOOK INTO THE INCIPIENT AML RELAPSE?

O. Horky, J. Mayer, L. Kablaskova, M. Borsky, D. Ohlidalova, M. Krejci, D. Dvorakova

University Hospital, BRNO, Czech Republic

Background. The reoccurrence or increase in autologous hematopoiesis after an allogeneic hematopoietic cell transplantation (allo-HCT) has been linked with incipient leukemia relapse, however, clear definition of such *emergency* in terms of microchimerism has not been defined yet. *Aims*. Since the usual minimal residual disease (MRD) analysis is more sensitive than conventional chimerism analysis and MRD-guided intervention is routinely used after transplantation, here we wanted to explore if more sensitive chimerism analysis (microchimerism) could be used for earlier detection of imminent relapse. *Methods.*We compared conventional polymerase chain reaction (PCR) of minisatellite, microsatellite, or sex specific regions with following fragment analysis (FA) (limit of quantification ~1%), with real-time quantitative PCR (RQ-PCR) of insertion/deletion and sex polymorphism (limit of quantification ~0.1%). The values of chimerism were correlated with the status of the disease (continuous complete remission; molecular relapse of the disease in the cases with the possibility of specific PCR MRD monitoring; and hematological relapse, HR). Only patients with follow-up of at least one year after allo-HCT, and with an informative polymorphism for RQ-PCR analysis, were included in this comparison (n=27, follow-up 1-7.9 years, median 3.0 years). *Results*. RQ-PCR analysis predicted full HR in 9/10 cases, with median of 60 days (0-322 days), whereas conventional FA only in 50% of cases. On the other hand, however, RQ-PCR analysis failed in 2/6 cases of early HR (borderline marrow blast count), and 3/6 cases of molecular relapses. Nevertheless, in these situations, relapses were judged on examination of bone marrow only and not peripheral blood, and retrospective analysis of bone marrow samples for microchimerism enabled detection of autologous cells as well. Rarely (n=2), stable mixed microchimerism without following relapse was seen, however, detailed analysis of sorted cell populations showed that it was restricted to the T-cells. *Conclusions*. Microchimerism detected by RQ-PCR turned out to be extremely sensitive and reliable method for early detection of incipient AML relapse. We recommend to test not only the peripheral blood, but also the bone marrow. Warning signs are reappearance of autologous cells or increase of so far stable microchimerism, confirmed in a subsequent sample. At our department, these experience now led to the introduction of early intervention routine policy (DLI with/without IL-2) based on an microchimerism examination.

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CENTRAL DIABETES INSIPIDUS REVEALING ACUTE MYELOGENUS LEUKAEMIA WITH MONOSOMY 7 AND ABNORMALITIES OF CHROMOSOME 3: A DISTINCT ENTITY

V. Peri, I. Nieves Pla, J.M. Bosch Benitez, L. Bartolome Hernandez, M. Caballero Gomez, R. Fernandez Martin, F. Fernandez Fuertes, M. Tapia Martin, J.D. Gonzalez San Miguel, M.C. Losada Castillo, J.A. Ruano Leon, S. Soler Martinez, J.M. Diaz Cremades

Hospital Insular de Gran Canaria, LAS PALMAS, Spain

Abnormalities of chromosome 3q21q26 are found in a variety of haematological diseases such as AML, myelodysplasic syndromes and chronic myeloid leukaemia, and are strongly associated with normal or high platelet count, morphological abnormalities of trombopoiesis, myelodysplasia and poor prognosis (The so-called 3q21q26 syndrome). Central diabetes insipidus (DI) in AML has been described in over 70 patients. To the best of our knowledge, at least 21 cases were evaluated cytogenetically. In 19/21 cases, abnormalities of chromosome 7 (mostly monosomy) were detected, and in 11/21 cases, abnormalities of chromosome 3 were identified. In 10 patients, both structural changes were observed. Case report: a 51-year-old man who presented with pallor, polyuria and polydipsia. His skin and oral mucosa were dried. The white blood cell count was 4.900/mm^3, haemoglobin 13.2gr/dl, and platelet count 324.000/mm^3. Differential count revealed 18% blasts. Blood chemistries revealed sodium of 149 meq/l, serum osmolality was 310 mOsm/kg and urine osmolaity was 142 mOsm/kg. Central DI has been diagnosed on the basis of the water deprivation test. NMR of the brain showed no evidence of leukaemia infiltration. A diagnostic lumbar puncture was negative. Treatment with DDAVP was started with good response. Bone marrow aspirate (BMA) revealed 79% blasts (CD34, CD13 and CD7: positive) a diagnosis of AML M0 with dysmegakaryopoiesis was formulated on the basis of WHO criteria. Cytogenetic analysis revealed inv (3)(q21;q26) and -7. EVI-1 gene over expression was detected at molecular investigation. He started induction chemotherapy (cytarabine+idarubicin) and the BMA showed resistance to therapy with 72% blasts. A second line of chemotherapy (fludarabine+cytarabine) was started without response, too. Now is under third line therapy. DI was persistet together with lack of response to chemotherapy.

Table 1.

AGE/SEX	FAB type	WBC (X10 9_)	PTL (X10 9L)	DYSPLASIA	NMR	CYTOGENETICS	CR/SURVIVAL (months)	REFERENCE
52/F	M4	12.2	104	NO	NORMAL	t(3;12)(q26;p12)	No/4	Nieboer et al, 2000
51/F	M1	255	302	YES (E,M)	ALT. PITUITARY STALK	-7, inv(3)(q21;q26)	No/1.5	Slater et al, 1992
48/M	M1	9.2	406	YES (M)	NORMAL	-7, inv(3)(q21;q26)	Yes/8	La Starza et al, 1994
39/F	M2	80.8	460	YES (M)	NORMAL	-7, inv(3)(q21;q26)	No/6	Lavabre-Bertrand et al, 2001
38/M	M2	78.4	286	YES (M)	NORMAL	-7, inv(3)(q21;q26)	No/8	Lavabre-Bertrand et al, 2001
42/F	M2	90.9	455	YES (M)	NORMAL	-7, t(3;3)(q21;q29)	No/14	Lavabre-Bertrand et al, 2001
43/M	M4	42.1	29	NO	NORMAL	-7, t(3;3)(q21;q26)	No/9	Castagnola et al, 1995
31/F	RAEB-t	2.3	65	YES (E,M,G)	NORMAL	-7, t(3;3)(q21;q26)	Yes/18	Muller et al, 2002
29/F	M5a	34	107	YES (M)	NORMAL	-7, t(3;3)(q21;q26)	No/1	Massimo et al, 2002
44/M	M1	4.2	395	YES (M)	NORMAL	-7, t(3;3)(q21;q26)	No/7	Massimo et al, 2002
51/M	M0	4.9	324	YES (M)	NORMAL	-7, t(3;3)(q21;q26)	No	This report

Conclusions. Recently, 11 cases of AML with DI and rearrangements of chromosome 3q were published; in 10 cases a -7 was also detected.

Five cases had inv(3)(q21;q26), five cases had t(3;3)(q21;q26) and one case had t(3;12)(q21;p12). No cerebral leukaemic localization was documented, except in one case, and everyone has a good response to DDAVP. The platelets counts were normal or high (median: 266.000/mm^3). No response to first-line therapy or early relapse were observed in all patients (median survival: 7,6 months) that confirming the suggestion of a very poor prognosis related to DI and/or chromosome 3 abnormalities and/or -7. In this context, it is, however difficult to ascertain the role(s) played by each of these factors, as all of them are per se related to an unfavourable outcome. EVI-1 over expression should be investigated always in the rare AML cases that present with DI also in the absence of cytogenetic evidence of 3q involvement. FISH analysis should also be performed, which might disclose masked 3q abnormalities. A better definition of the genes located at the 3q21 region might also elucidate the pathogenic mechanism leading to this peculiar clinical association. Our findings further support the existence of a distinct AML entity characterized by the presence of DI, abnormalities of chromosome 3q, monosomy 7, dysmegakaryopoiesis, poor outcome and EVI-1 involvement.

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ACUTE MYELOID LEUKAEMIA IN CHILDREN: RESULTS OF A TUNISIAN SERIES

H. Ben Neji, R. Ben Amor, R. Jeddi, L. Aissaoui, K. Kacem, R. Ben Lakhal, H. Ben Abid, Z. Belhaj Ali, B. Meddeb

Aziza Othmana university hospital, TUNIS, Tunisia

Introduction. The outcome of children with acute myeloid leukaemia (AML) has improved during the last years. Long term survival (> 5years) rate is approximately 50%. The reduction of relapse risk is due to efficacy and intensity of chemotherapy. The place of stem cell transplantation is now controversed in first complete remission. *Patients and Methods.* Between January 2003 and December 2006, 20 patients younger than 18 years, with *de novo* AML (Down's syndrome and FAB M3 excluded) were diagnosed in our institution. The median age is 11 years (2-17 yr); WBC was >×10^9/L in 9 children. Karyotype was informative in 17 patients. Among these 6 were normal, 4 were t(8;21) ,1 inv(16) and 1 t(9;11). All the children were treated with chemotherapy derived from LAME 02 protocol: one course of induction based on aracytine and mitoxantrone. 3 courses of consolidation therapy with high dose cytarabine in the second one for children in CR. Patients in CR with an HLA identical family donor have allogeneic stem cell transplantation. CNS prophylaxis is administered to patients with the M4 or M5 FAB subtypes and to patients with an initial WBC>50×10^9/L. There was no maitenance treatment with IL2. *Results.* 17 patients achieved complete remission after 1 course of induction (85%).Two patients had a second induction and a remission was obtained in one case. One patient died during induction therapy. 11 children had a matched sibling donor. Because of problems of feasibility, Only 6 had stem cell transplantation (all in CR1). The 3-years OS and the relapse free survival were respectively at 59% and 67%. Two of 6 allo-grafted, patients died by transplant toxicity (GVHD). In fact, the relapse rate is at 33.3% with a median of 43 months. No relapse was noted in children allografted, all relapses occurred in the group treated with chemotherapy only (p=0.06). The 2- years relapse free survival for children with an initial WBC <20×10^9/L is 90% and 38% for children with a WBC≥ 20×10^9/L (p=0.06). Five children who relapsed had a WBC >20×10^9/L. *Conclusions.* Despite of the low number of patients, our results are equivalent to those reported in large studies. The particularity of our population (North Africa) is the presence of numerous related donors (55% in our study). This suggest the extending of transplant (in CR1) to more patients, sibling those who have worse criteria at diagnosis (WBC>×10^9/L, high risk karyotype), with better management of graft complications.

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FLT-3 MUTATIONS IN CHILDHOOD ACUTE LYMPHOBLASTIC AND MYELOGENOUS LEUKEMIA

M. Braoudaki,[1] M. Karpusas[2], K. Katsibardi,[1] C. Papathanassiou,[1] G. Lambrou,[1] F. Tzortzatou-Stathopoulou[1]

[1]*University of Athens, ATHENS;* [2]*University Research Institution for the study of genetic & malignant diseases, ATHENS, Greece*

Background. Fms-like tyrosine kinase 3 (FLT3) is a commonly mutated gene in childhood acute leukemia. Known activating FLT3 mutations include 2 types: juxtamembrane domain (JM) mutations (manifested as internal tandem duplications, ITDs and deletion/insertion mutations) and activation loop (AL) mutations (most commonly single amino-acid substitutions of protein residues D835 or I836). FLT3 is consistently expressed in MLL rearranged acute lymphoblastic leukemias (MLL-ALL), in hyperdiploid ALL samples (containing >50 chromosomes) and in acute myelogenous leukemia (AML). *Aims.* The aim of this study was to investigate the incidence of both types of known FLT3 mutations in childhood ALL and AML. *Methods.* Bone marrow samples from 86 patients with pediatric ALL and 12 patients with pediatric AML at presentation and or relapse between 1999 and August 2006 were analyzed. Of the ALL patients, 2 had ALL with MLL rearrangements and 9 had ALL with high hyperdiploidy (DNA index >1.14). Genomic DNA was extracted according to the standard phenol-chloroform protocol. Gene segments in the vicinity of FLT3-JM (spanning region including exons 14 and 15) and FLT3-AL (exon 20) were PCR amplified using the previously published oligonucleotide primer combinations. For the preliminary detection of the FLT3-AL mutations at positions D835/I836, the amplified PCR product solutions were digested with EcoRV restriction enzyme. Following digestion, the uncut fragment from a 1.5% agarose gel was reamplified with the original primers prior to sequencing. Direct sequencing was performed to all PCR products. *Results.* In the current study, 2 cases (2.3%) harboured the FLT3-AL mutations, whereas no FLT3-ITD mutations were detected. Specifically, in this study group there is a 2.3% (2/86) mutation incidence in terms of total ALL cases and a 22.2% (2/9) incidence in the subgroup of hyperdiploid cases. In the MLL case subgroup no mutations were detected, which is not statistically significant. In addition, no mutations were detected among the 12 AML patients, which is not statistically significant either. Of the 2 patients with the mutation, one is at remission following 1 relapse, whereas the other died within 26 months following diagnosis from an infectious disease at the age of thirteen. *Summary and Conclusions.* The current study showed comparable incidences of FLT3 mutations to those reported in previous studies of childhood ALL. The high frequencies of FLT3 mutations in AML cases reported by others were not confirmed. Analyses of a larger number of patients are required to verify this. The prognostic value of the results cannot be evaluated since the clinical outcomes of the two patients are not compatible.

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IMMUNOPHENOTYPIC FEATURES OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN OF THE DONETSK REGION

V. Vilchevskaya, A.A. Ryabko, V.V. Tyutyunnik

V.K.Gusak Institute of Urgent and Recovery Surgery, Academy of Medical Science, DONETSK, Ukraine

The patients (n=74) of the Donetsk Region with the acute leukemia diagnosis treated at the Department of children oncohematology from January 2005 to December 2007 were studied. At the time of establishing the diagnosis, the investigation of the immunotype of blast cells membranes was carried out. The investigation was done by flow cytometry (FACSCALIBUR BD) using the broad panel of monoclonal antibodies: CD45, CD14, CD1a, CD2, CD3, CD4, CD8, CD5, CD7, CD19, CD20, CD22, CD10, CD34, CD79a, HLA-DR, CD13, CD33, CD117, CD13, CD15, CD11c, IgM, CD25, CD41a, TdT, CD71, CD38, CD24, MPO, GLyA, CD77, anti-Î, anti-Ï, CD64, CD23. It was established that the ratio of the lymphoid (n=68) and myeloid forms (n=6) as well as the ratio T - (n=14) and B-variants (n-54) with the Acute Lymphoblastic Leukemia (ALL) subgroup were in compliance with the world indices. When analyzing B-linear forms the distinctions were revealed: prevalence of relatively more matured forms of pre-B (35% *vs* 19-21%) and a decrease in the portion of common-B ALL (46% *vs* 56-61%). An increased rate of ALL with co-expression of myelomarkers (34% *vs* 15%) was established as well. The pro-B and common-B variants were distinguished for the predominance of CD33+ compressions and pre-B ALL that of MPO$^+$. As compared to the results of treating various B- ALL subtypes, the common-B ALL group had the greatest number of relapses (n=5) that does not correspond to the literature data according to which the common-B variant is the most prognostically favorable. Thereat 44% of children with common-B ALL referred initially to a low risk group had a disease relapse. The immunophenotypic characteristic of these patients was a more low density of CD10, CD19, CD34, HLA-DR markers expression in comparison with the patients who had no relapses. The degree of significance of the results obtained is low (p=0,456) which is due to a great sparseness of individual indices and too small selection. However, the obtained results suggest the necessity of consideration of an average fluorescent intensity of the expressed markers in stratification of the patients with the common-B sub variant of acute lymphoblast leukemia in the low risk group. Thus, the obtained results bear evidence

of the fact that children with leukemia in the Donetsk Region distinguish themselves for predominance of relatively more mature forms among B-Linear ALL (pre-B) and an increased frequency of myeloid co-expressions in lymphoblasts. Immunophenotyping is a necessary component of the primary examination of children. It allows doctors to study the nature of this disease more effectively and to select patients who need more intensive therapy.

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MTHFR POLYMORPHISM ALLELE FREQUENCIES IN GREEK CHILDREN WITH ALL. CASE-CONTROL STUDY

E. Stiakaki, N. Karathanasis, D. Choumerianou, M. Kalmanti

University Hospital of Heraklion, University of Crete, Medical School, HERAKLION, Greece

Background. Recent studies have reported the correlation between polymorphisms in genes encoding enzymes that control folate metabolism and ALL susceptibility in children. Methylenetetrahydrofolate redustase (MTHFR) plays an important role in folate metabolism and genetic changes that affect its catalytic activity may influence ALL. A single nucleotide polymorphism (SNP) at position 677 (C'T) has been described and has drawn great interest not only for its correlation with ALL but because of its involvement in the metabolism of chemotherapeutic agents, such as methotrexate, used for ALL treatment. *Aims.* To investigate the frequencies of MTHFR C677T alleles in a case-control study from the island of Crete. *Methods.* DNA was isolated from 36 children diagnosed with ALL and 36 healthy blood donors. The MTHFR C677T SNP was analysed with PCR followed by digestion with the restriction endonuclease Hinf I. PCR products were electrophoresed on a 3% agarose gel and genotypes were determined and confirmed by experience lab personnel blinded to all study data. The x2 criterion was used to compare the observed numbers of each genotype with those expected for a population in Hardy-Weinberg equilibrium. Statistical analysis was performed with the use of SPSS software. *Results.* In ALL subjects the frequency of the C allele was 0.68% and of the T allele 0.32%. In controls the frequency of the C allele was 0.65% and of the T allele 0.35%. Genotype distribution did not differ from that expected by the Hardy-Weinberg equation, neither in ALL subjects nor in controls. Crosstabs analysis showed no difference between ALL subjects and controls regarding the genotype frequencies (p=0.87). No difference between genotypes was also observed between high and low risk subgroups of ALL (p=0.55). *Summary and Conclusions.* The frequency of MTHFR C677T SNP does not differ between ALL patients and healthy controls. More polymorphisms on the same and other genes involved in folate metabolism need to be analysed, and creation of haplotypes may reveal a positive correlation among gene polymorphisms, susceptibility to ALL and the determination of following treatment in the Greek population.

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RELATION BETWEEN JAK2V617F MUTATION AND MYELOPROLIFERATIVE AND ANGIOGENIC INDEX IN ESSENTIAL THROMBOCYTHEMIA

E. Cacciola, E. Di Francesco, F. Pezzella, D. Tibullo, R.R. Cacciola

Hematology, CATANIA, Italy

An acquired somatic mutation, JAK2 V617F, has been reported in approximately half of patients with essential thrombocythemia (ET). To date, the relationship between JAK2 mutation and the outcome of disease is unknown. Therefore, we investigated the association of this mutation with myeloproliferative and angiogenic index such as platelets, white blood cell (WBC), haemoglobin (HB), hematocrit (HCT) and vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF), respectively, in fifty ET patients (24 males and 26 females, mean age 58 years) who fulfilled PVSG and WHO. Their mean duration of disease was 7 years. Of 50 patients, 18 were on hydroxyurea whereas 32 were not receiving any cytoreduction. All patients were on antiplatelets. None of the patients had splenomegaly. JAK2 mutation was analyzed by melt curve analysis. Platelets, WBC, HB and HCT were measured by automated analyser. VEGF and FGF were assayed by ELISA. Considering that VEGF and FGF may be produced by platelets, we adjusted VEGF and FGF per platelet (VEGFPLT/106 and FGFPLT/106).All patients had thrombocytosis (684±299×10^9/L), normal WBC (7.8±2.2×10^9/L), HB (13±1.8 g/dL) and HCT (39±5%) and elevated VEGFPLT (1.8±1.6 pg/106 *vs* 0.5±0.4 pg/106) ($p<0.0001$) and FGFPLT (0.10±0.09 pg/106 *vs* 0.034±0.002 pg/106) ($p<0.0001$). A positive correlation there was between JAK2 and HCT (p=0.012) whereas no correlation was found between the mutation and the other myeloproliferative index and the angiogenic factors. These data suggest that the acquired JAK2 mutation is likely involved in the hematopoietic expansion but not in angiogenesis. Further investigation needs to establish the exact prognostic role of this mutation in ET.

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JAK2V617F MUTATIONS: IMPLICATIONS IN THE DIAGNOSIS AND PROGNOSIS OF THE PATIENTS WITH CLASSIC BCR-ABL NEGATIVE MYELOPROLIFERATUIVE NEOPLASMS (MNS): SINGLE CENTER LONG TERM FOLLOW UP-STUDY

I. Panovska-Stavridis,[1] N. Matevska,[2] M. Ivanovski,[1] S. Trajkova,[1] M. Lozance,[1] A. Stojanovik,[1] A. Dimovski,[2] L. Cevreska[1]

[1]Medical Faculty-Skopje, SKOPJE; [2]Faculty of Pharmacy, SKOPJE, Macedonia

Identification of the activating V617F mutation in the JAK2 tyrosine kinaseas gene as a marker of myeloproliferation in patients with chronic myloproliferative disorders (CMPD) provided a major breakthrough in the understanding of the pathogenesis and proving the clonality of these disorders. It laid the foundation for new approaches to the diagnosis, classification and treatment of CMPD. In the upcoming WHO classifications, the term CMPD has been replaced by myeloproliferative neoplasms (MN) and the diagnostic criteria for classic BCR-ABL negative CMPD have been revised. The prognostic significance of the JAK2V617F mutation is still unknown with inconclusive clinical results. The purpose of our study was to estimate the prevalence of JAK2V617F mutation in the patients with classic MNs diagnosed and followed at our Institution prior to 2002, and to investigate whether MNs patients that carry JAK2V617F mutation differ in clinical course and outcome from JAK2V617F negative MN patients. The study group consisted of 64 living MNs patients diagnosed according to standard WHO criteria for diagnosis of MNs (26 patients were diagnosed as PRV, 34 as ET, 6 as MF and 8 were classified as atypical MPDs). The median follow-up of the patients was 7,8 years. DNA samples were obtained from unfractionated blood samples and the frequency of V617F JAK2 mutation was analyzed by allele-specific PCR assay. In order to determinate the mutant allele burden, mutation positive samples were analyzed by DNA sequencing. Our results showed that JAK2 V617F mutation was present in 79% of patients with PRV, 58% with ET, 69% with MF and in 33% with atypical MPDs. The mutant JAK2-V617F allele burden was greater than 50% in 36% of the PRV patients, 28% with MP and 11% with ET indicating the loss of heterozygosity for the JAK2 allele. The observed higher mutant JAK2V617F allele presence in our study group was associated with the long disease duration (median follow-up of mutation positive patient was 11,9 years). Correlations between the two JAK2-V617F different classic MNs groups were made using standard statistical tests. We compared the clinical and laboratory features at diagnosis and long-term prognosis, including the incidence of thrombo-hemorrhagic events, disease transformation and the survival of the patients. The two groups were comparable regarding all tested parameters except of the incidence of thrombotic history. The JAK2-V617F positive group has higher incidence of thrombotic complication 33,4%, compared with 14% in the other group ($p<0.05$). Although we observed a disparity in the incidence of the JAK2V617F mutation in different classic MNs entities in our population with respect to the expected frequency of JAK2V617F from the literature, our results confirm the diagnostic significance of the JAK2V617F mutation in MNs. The significantly higher incidence of trombotic complications in our patients with JAK2V617F mutation implies the possible role of the mutation in the pathophysiology of the thrombosis in patients with MNs and warrants further investigations. Our findings support the notations that MNs needs further molecular classification which will lead to improved risk stratification and facilitate the implementation of the molecular target therapy that is eagerly awaited.

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POLYCYTHEMIA VERA AND IMMUNE THROMBOCYTOPENIC PURPURA: A CASE REPORT SERIES

K. Tam, K.W. Tsang

University of British Columbia, VANCOUVER, BC, Canada

Background. Polycythemia vera (PV) is categorized as a myeloproliferative disorder that is characterized by increased red cell mass, elevated white blood cell count and thrombocytosis. Recently, PV has received renewed attention with identification of the JAK2 gene single point

mutation. PV is known to be associated with myeloid metaplasia, myelofibrosis, and acute leukemia, but not classically immune thrombocytopenic purpura (ITP). There is limited literature on the occurrence of PV with ITP. *Aims.* Here we report three patients diagnosed with PV and ITP with the intent of raising awareness that these two disorders can occur together. *Case Report.* In one case, the patient was initially diagnosed with JAK2 mutation positive PV who 6 months later developed symptomatic severe thrombocytopenia. This patient required simultaneous treatment for both PV and ITP. At this time his predominating hematologic disorder is refractory ITP which has posed a therapeutic dilemma given his concurrent diagnosis of PV. In the other two cases, both patients initially presented with ITP and then subsequently, were diagnosed with PV. One patient did not require treatment for his ITP and presented with JAK2 mutation positive PV 3.5 years later. His PV has been controlled with hydroxyurea and ASA while his platelet count has remained normal. In the other case, the patient had significant ITP requiring treatment and presented with JAK2 mutation positive PV 8 months after resolution of the ITP. Unfortunately, she was diagnosed with recurrent lung carcinoma and required radiation and chemotherapy, during which her blood counts were within normal. She continues on ASA for her PV while her platelet count has remained normal. *Summary and Conclusions.*We present three interesting cases of patients with the concurrent diagnosis of PV and ITP. None of the patients were found to have an underlying lymphoproliferative disorder which might explain the ITP. The occurrence of both PV and ITP is likely coincidental in these patients. We feel that it is important to recognize that these two unrelated hematologic disorders can occur both simultaneously and sequentially.

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CYTOGENETICS ABNORMALITIES AND JAK2-V617DF MUTATION IN PATIENTS WITH BCR-ABL NEGATIVE MYELOPROLIFERATIVE DISORDERS IN FIVE HOSPITALS IN JAKARTA

T.D. Atmakusuma, K.L. Tambuna, S.H. Effendy, B.K. Karsono, M.S. Sugyono, I.R. Rinaldy

University of Indonesia, JAKARTA TIMUR, Indonesia

Background. JAK2-V617F mutation, published in early 2005, has been included into a proposed revised WHO criteria for polycythemia vera (PV), essential thrombocythemia (ET) as well as a primary myelofibrosis (PMF). PV, ET, and PM belongs to a BCR-ABL- negative myeloproliferative disorders (MPD). *Aims.* Since no data of JAK2-V617F mutation as well as cytogenetis abnormalities of BCR-ABL-negative MPD have been reported in Indonesia, this review is to report data in some hospitals in Jakarta. *Methods.* A retrospective review on patients with PV, ET and MF was conducted in five hospitals in Jakarta, Indonesia. BCR-ABL fusion gene was performed with PCR-multiplex set primer of CA3-,C5e-,B2B-, and BCR-C. JAK2-V617F mutation (JAK2-1849 G>T mutation) was performed with amplification refractory mutation system (ARMS)-PCR method using 2 couples primer (Fo, Ro, Fwt, and Rmt). *Results.* 60 patients of PV and 23 patients of ET from 5 hospitals in Jakarta,Indonesia, were included into data analysis. In PV group, 81.70% were male and 18.30% were female, 23.30% of patients with liver and/or spleen enlargement, the maximum Hb concentration was 23.50 g%, maximum leukocyte count was 48,000/mm^3, maximum platelet count was 1,415,000/mm3, and maximum NAP score was 495. Of 12 patients of BCR-ABL-negative PV, abnormal karyotyping shown were 46,XY, del(4q),del (5q)/ 46,XY,del (2q),del (5q), del(6q)/46,XY,del (2q) and 46,XY,del(5q), the remainings were normal karyotyping. JAK2-V617F mutations were found in 4 of 12 BCR-ABL-negative PV patients (33,33%). All 60 patients were responded well to the treatment (phlebotomy, hydroxyurea, phlebotomy plus hydroxyurea, hydroxyurea plus anagrylide). Meanwhile, in ET group, 39,10% were female, 60,90% were male, 26,10% of patients with spleen or spleen and liver enlargement, and the maximum platelet count was 2,023,000/mm3 and maximum score of NAP was 424. Of 8 patients of BCR-ABL-negative ET, 7 patients revealed abnormal karyotyping, including 46,XX,del (2q),del(4q),del(5q),del(6q)/46,XX, del(2p),del(5q),del(6q)2 46XX,del(4q), 46,XX,del(2q),del(5q),del(6q)/46,XX,del(5q)/ 46, XX,del(6q)/46,XXdel (2q),46,XX, del (6q)/46,XX,-5,+6,del(6q), 46,XX, del(5q), del (6q), 46 XY,del(5q), and 46,XY,del(6q), and the remainings were normal karyotyping. JAK2-V617F mutations were found in 4 of 8 BCR-ABL-negative ET patients (50%). All patients responden well to the treatment (hydroxyurea, anagrelide, or both). *Conclusions.* 2 of 12 BCR-ABL-negative PV patients showed abnormal karyotyping and 4 of 12 BCR-ABL negative PV patients showed JAK2-V617F mutations (33,33%). Meanwhile 7 of 8 BCR-ABL-negative ET patients revealed abnormal karyotyping and 4 of 8 BCR-ABL negative ET patients revealed JAK2-V617F mutations (50%).

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CLINICAL ACTIVITY OF LUMILIXIMAB IN COMBINATION WITH FCR IS INDEPENDENT OF ZAP70 EXPRESSION ON CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

N. Pathan,[1] A. Estrellado,[1] J. Ranuio,[1] S. O'Brien[2], T. Kipps,[3] A. Cesano,[1] H. Mu,[1] S. Harris,[1] S. Tangri[1]

[1]Biogen Idec, SAN DIEGO; [2]The University of Texas/MD Anderson Cancer Center, HOUSTON; [3]Moores Cancer Center University of California San Diego, SAN DIEGO, USA

Background. Lumiliximab is a CD23 monoclonal antibody under investigation for the treatment of patients (pts) with relapsed chronic lymphocytic leukemia (CLL). Lumiliximab binds specifically to human CD23, a glycoprotein expressed on a majority of CLL cells. As ZAP70 is a poor prognostic factor of CLL, expression of the ZAP70 antigen by malignant B cells was analyzed in a subset of pts with relapsed CLL enrolled in a phase I lumiliximab monotherapy study. *Aims.* The ability of lumiliximab to induce apoptosis ex-vivo in patients expressing ZAP70 was evaluated in a subset of patients enrolled in the phase I monotherapy study. Additionally, pretreatment ZAP70 expression was analyzed in pts treated with a combination of lumiliximab, fludarabine, cyclophosphamide, and rituximab (L+FCR). *Methods.* In the lumiliximab monotherapy study, ZAP70 expression was quantified by Western blot assay using lysates from purified CLL cells and apoptosis was evaluated by flow cytometric assessment of caspase-3 activation on pretreatment and day 2 treated samples (*ex vivo*). In the L+FCR study, frequencies of CLL cells and ZAP70 positive cells were estimated by four color flow cytometry using standard commercially available antibodies. CLL cells were identified by first gating on viable CD45$^+$ cells and subselecting with antibodies to CD5 and CD19 receptors (CD5$^+$CD19$^+$ phenotype). ZAP70 expression was reported as% positive in CLL cells. *Results.* Analysis of data from the monotherapy study suggested induction of apoptosis by caspase-3 activation and decrease in CD5$^+$CD19$^+$ cells in pts expressing high levels of ZAP70. Moreover, apoptosis was also observed in a patient that expressed activated ZAP70. Results from the L+FCR study demonstrated clinical activity in pts having high or low levels of ZAP70 expression. *Conclusions.* Lumiliximab potentially provides clinical benefit in CLL pts with both low and high levels of ZAP70, a characteristic associated with aggressive disease and poor prognosis.

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SERUM FREE LIGHT CHAIN REFERENCE INTERVALS. A STUDY OF 133 BLOOD DONORS

M. Callis,[1] L. Garcia,[1] M. Gironella,[1] M.J. Rodrigo,[1] E. Garcia,[1] D. Castella[2]

[1]Hospital Universitari Vall d'Hebron, BARCELONA; [2]Banc de Sang i Teixits, BARCELONA, Spain

Introduction. Before the implementation of the free light chain (FLC) determination in our center, a control study with blood donors was undertaken. The serum references had been published by Katzmann and cols. who set 95% reference intervals and a diagnostic range for clonality. *Material and Methods.*From april to june 2007, 133 samples were collected in two lots and frozen, from anonymous blood donors, aged 18-65, who filled a health questionary. The second batch was screened with proteins studies, immunofixation and levels of immunoglobulins. Samples were defrosted and FLC quantified in a Dade-Bhering BNII nephelometer, using antibodies Freelite® from The Binding Site Ltd. *Results.* Results are given for the whole pool of blood donors, and for a subgroup of 50 controls with normal immune system, as assessed for the aforementioned studies (Table 1).

Table 1.

	Mean FLC	min	max	95% Interval	Reference Intervals
Whole pool blood donors					
κ chains	11.2 mg/l	1.56	21.7	**4.42 – 19.70**	**3.3 – 19.4 mg/L**
λ chains	12.5 mg/l	1.19	30.7	**3.52 – 19.80**	**5.7 – 26.3 mg/L**
κ/λ ratio	1.0	**0.19**	**6.18**	0.47 – 2.19	**0.26 –1.65**
Selected donors with normal immune system					
κ chains	13.2 mg/l	7.93	21.10	**8.78 – 19.70**	**3.3 – 19.4 mg/L**
λ chains	14.2 mg/l	8.85	30.70	**9.19 – 19.80**	**5.7 – 26.3 mg/L**
κ/λn ratio	0.96	**0.59**	**1.42**	0.64 – 1.41	**0.26 –1.65**

The 95% reference interval for the γ light chain was 4.42 - 19.70 mg/L and for the κ light chain was 3.52-19.80 mg/L, the latter down-displaced from the reference range. As shown in the histogram there is a cluster of low levels κ-FLC controls, who consequently disrupt the κ/γ ratio, giving rise to false positives for clonality. This contingency is solved applying the subgroup with a normal immune system in which the 95% reference interval are similar to the reported by Katzmann and the γ/κ ratio of 0.59-1.42 falls entirely within the diagnostic range. *Conclusions*. We validated the diagnostic range of the serum free light chain (FLC) in a population of blood donors, with normal immune system and age somewhat younger that the one utilize to set the references, which better emcompass the population with monoclonal gammopathies. Age, renal function and immune status seems to be the main features to be aware of, when interpreting FLC results. We alert upon the need to demonstrate an elevated FLC when diagnosing the corresponding clonality, particularly for the γ chain.

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FREQUENCY OF CYTOKINE PRODUCING MONOCYTES IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH THALIDOMIDE

S. Filipe,[1] A. Matos[2], J.M. Morgado[2], I. Silva[2], M.J. Inácio[2], C. Geraldes,[3] A. Paiva[2], M.L. Pais[2], A. Teixeira[1]

[1]Hematology Service of Coimbra University Hospital, COIMBRAM [2]Histocompatibility Center, COIMBRA; [3]Hematology Service of CHC, COIMBRA, Portugal

Background. Multiple myeloma (MM) is a currently incurable B-cell malignancy caused by the expansion of neoplasic plasma cells (PC), accumulated in the Bone Marrow (BM). Because immune alterations observed in MM patients seems to correlate with patients' survival, is important to understand how the cells of the immune system may be altered and how therapy could contribute for it. Thalidomide (Thal) was shown to be a potent immunomodulating agent with a broad spectrum of immunologic effects. Several hypotheses have been proposed to explain the clinical effectiveness of Thal in MM, including inhibition of IL-1β, IL-6 and IL-12 production, inhibition of TNF-α signalling, down-regulation of selected cell surface adhesion molecules, antiangiogenic activity and direct induction of apoptosis or growth arrest in MM cells. *Aims*.With the purpose to contribute to the understanding of thalidomide's effects over the cells of the immune system from MM patients, we evaluated the capability of monocytes from BM and Peripheral Blood (PB) to produce pró-inflammatory cytokines. *Methods.* We studied 6 MM patients treated with Thal (Thal was the first line treatment in 1 case and the second line treatment in the other 5 cases). These patients were compared with 6 MM patients receiving other therapy, with 6 patients not receiving MM therapy and with 4 individuals without haematological malignancies. The BM and PB samples were incubated during 6h in the presence or absence of IFN-γ and LPS.The frequency of monocytes (identified by their reactivity with anti-CD33 mAb) producing IL-1β, IL-6, IL-12 and TNF-α, and the amount of refered cytokines per cell, were quantified by flow cytometry. *Results*. We observed, in BM samples and after stimulation with LPS plus IFN-γ, that the group of patients receiving treatment with Thal presented lower frequencies of cytokine producing monocytes for all studied cytokines, when compared with MM patients with other therapy, with patients without therapy and with individuals without haematological malignancies. Additionally, Thal treated patients also presented lower amounts of cytokine per cell when compared with the other studied groups. Opposing to the observed in BM samples, in PB samples no significant differences were observed between Thal treated patients and the other studied groups. *Summary and Conclusions.*These results suggest that Thal may has an inhibitory action over the cells involved in innate immunity, like monocytes, by inhibiting the production of acute phase inflammatory proteins (IL-1β, IL-6 and TNF-α). *This work is supported by Pharmion.*

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EFFECT OF THALIDOMIDE-DEXAMETHASONE THERAPY ON BONE MARROW ANGIOGENESIS IN NEWLY DIAGNOSED MULTIPLE MYELOMA

S. Ladeb,[1] K. Mrad[2], A. Abdelkefi,[1] L. Torjemane,[1] I. Safra,[3] S. Hdiji,[4] N. Ben Romdhane,[5] M. Mdhaffer,[4] H. El Omri6, T. Ben Othman,[1] K. Ben Romdhane,[7] A. Ben Abdeladhim[1]

[1]Centre National de Greffe de Moelle Osseuse, SIDI REZIG MEGRINE; [2]Laboratoire d'anatomopathologie,Institut Salah Azaiez, TUNIS; [3]Laboratoire d'Hematologie.Institut Pasteur, TUNIS; [4]Service d'HematologieHopital Hedi Chaker, SFAX; [5]Laboratoire d'Hematologie, Hopital la Rabta, TUNIS; [6]Service d'Hematologie.Hopital Farhat Hached, SOUSSE; [7]Laboratoire d'Anatomopathologie,Institut Salah Azaiez, TUNIS, Tunisia

Background. Bone marrow angiogenesis is increased in multiple myeloma. It has been reported to decrease after thalidomide therapy in studies including patients with smoldering myeloma, newly diagnosed and relapsed myeloma. The degree of decrease was significantly more important in responders. The purpose of this study is to evaluate bone marrow microvessel density (MVD) before and after Thalidomide-dexamethasone therapy in patients with newly diagnosed multiple myeloma , to test its predictive value on response and to establish relation between variation of MVD after therapy and response. *Methods.* thirty patients with newly diagnosed multiple myeloma were evaluated for bone marrow angiogenesis before and after Thalidomide-dexamethasone therapy. The post-therapy marrows were performed 20-35 days from the end of therapy. Prognostic factors, including the international staging system and plasma cell percentage were studied in all patients and correlated with the degree of bone marrow MVD. Paraffin-embedded bone biopsy blocks were used for MVD determination, microvessels were identified by immuno-histochemical staining for CD34. Paraffin sections of well-vascularized tonsil served as positive controls, sections stained with non-immune rabbit immunoglobulin as negative controls for all samples tested. Bone marrow angiogenesis was estimated by microscopic evaluation in terms of MVD as previously described. MVD of more than 20 was classified as high-grade and the rest as low grade. *Results.* the median age of the group was 51.5 years (range 38-60 years) and 63% were male. All patients presented with III Durie-Salmon stage. The median bone marrow MVD at the start of therapy was 20.5 (range 3-85).The median post therapy MVD was 12.5 (range 3-91), reflecting a median decrease of 13 (range1-79). MVD decrease was observed in 17 patients. Thalidomide therapy resulted in 7 complete responses, 14 partial responses, 6 stable diseases and 3 progressions. MVD decrease was observed in 16 patients among the 21 responders and in only 1 patient among the 10 non responders (p=0.0016).The mean MVD decrease in responders was 14 (range 5-79). The mean MVD decrease in no responders was 8. No association was found between pre-therapy MVD and the ISS. A moderate correlation was found between pre-therapy MVD and degree of bone marrow plasma cell involvement (r=0, 46; p=0,01). No association was found between pre-therapy MVD and response. *Conclusions.* Thalidomide-dexamethasone therapy in newly diagnosed multiple myeloma resulted in a decrease of bone marrow MVD. The decrease of MVD was significantly more frequent in responders (p=0.0016). No association was found between pre-therapy MVD and response.

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FAILURE OF AN *IN VITRO* MODEL OF OSTEOCLASTOGENESIS IN HUMAN PLASMA CELL LEUKEMIA (PCL)

R. Rizzi,[1] S. Colucci[2], G. Brunetti[2], A. Oranger[2], G. Mori,[3] P. Curci,[1] G. Specchia,[1] M. Grano[2], V. Liso[1]

[1]Hematology, BARI; [2]Human Anatomy and Histology, BARI; [3]Biomedical Science, FOGGIA, Italy

Background. In multiple myeloma (MM)-lytic bone disease, we previously showed that T cells support the formation of osteoclasts (OCs) with longer survival by means of an *in vitro* osteoclastogenesis model consisting of human unstimulated and unfractionated peripheral blood mononuclear cells (PBMCs). By contrast, in T-cell-depleted MM PBMC cultures, exogeneous macrophage-colony stimulating factor (M-CSF) and receptor activator of nuclear factor-kB ligand (RANKL) were necessary for the formation of OCs, which did not exhibit however a longer survival. On the other hand, in the same culture systems derived from patients with MM without lytic bone disease, OCs developed only after the addition of the exogeneous cytokines. *Aims.* In the present study, our purpose was to use this *in vitro* osteoclastogenesis model for develop-

ment of OCs in cultures derived from human primary plasma cell leukemia (pPCL). *Methods.*Peripheral blood (PB) was obtained from 2 patients with pPCL. The controls were 32 patients with MM with or without lytic bone disease, and 32 subjects with non-neoplastic disease without any skeletal involvement, as included in our previous study (BLOOD 2004;104:3722). The diagnosis of pPCL was established according the IMWG criteria.The samples were collected after informed consent provided according to the Declaration of Helsinki. Both the patients with pPCL were women; one of them was 57- and the other 58-year-old. Skeleton standard radiography demonstrated two small lytic lesions of the skull and one of a rib in a patient, whereas it was negative in the other. Unfractionated as well as T-cell-depleted PBMCs obtained from our patients were isolated by centrifugation over Histopaque 1077 density gradient and cultured for about 30 days in the appropriate conditions, and in the presence and in the absence of M-CSF and RANKL (according to the *Methods.*we described in BLOOD 2004;104 3722). *Results*. At the end of the culture period, in the unfractionated PBMC cultures from both the patients with pPCL no spontaneous osteoclastogenesis was observed, whereas OCs developed following the addition of M-CSF and RANKL. In T-cell depleted PBMC cultures from the same patients, the formation of OCs occurred only in the presence of the exogeneous cytokines. Thus, the osteoclastogenesis findings demonstrated in patients with pPCL overlapped the results obtained in the culture systems derived from MM patients without lytic bone disease, and from the subjects with non-neoplastic disease without any skeletal involvement. *Summary and Discussion*. In line with the aim of our study, we used a culture system derived from human pPCL for *in vitro* development of OCs as paradigm of *in vivo* resorption. We did not observe spontaneous osteoclastogenesis in the cultures derived from PBMCs of the patients with pPCL, whereas spontaneous formation of OCs with longer survival occurred in the same culture system derived from PBMCs of the patients with MM-lytic bone disease. Since pPCL is rare, information about its intrinsec biology is still scanty; our findings can contribute to the study of the biological features of this disease.

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FREQUENCY OF CD4+CD25HIGH REGULATORY CELLS (T-REG CELLS) IN PATIENTS WITH MULTIPLE MYELOMA

D. Bourantas,[1] E. Xatzimichael,[1] G. Fili,[1] G. Baxevanos,[1] N. Kolaitis,[1] G. Vartholomatos[2], K. Bourantas[1]

[1]University Hospital of Ioannina, IOANNINA; [2]University Hospital of Ioanina, IOANNINA, Greece

Background. Regulatory T cells (T-reg cells) represent a population of $CD4^+$ T cells highly expressing CD25 which has a key role in immune homeostasis as it is involved in many aspects of immunoregulation. Previous studies report that several autoimmune diseases have low T-reg cell numbers while increased numbers of T-reg cells have been reported in solid tumors as well as in hematologic malignancies. *Aims.* Since MM is a disease associated with immune dysfunctions the purpose of our study was to evalute $CD4^+CD25^{high}$ T cells proportions in patients with multiple myeloma (MM) compared to healthy donors. *Materials and Methods.* Peripheral blood was taken from 15 healthy donors and 13 patients with MM (2 newly diagnosed-11 patients during the course of the disease). Both in controls and in MM patients T-reg cells numbers were analysed by flow cytometric analysis by evaluating the proportions of $CD4^+$ T cells expressing high levels of CD25. *Results.* The proportion of $CD4^+CD25^{high}$ regulatory T cells was increased in patients with MM (mean 8.07±3.3%) compared with controls (mean 4.4±3.04%), (p=0.008). *Summary.* Our findings showing increased $CD4^+CD25^{high}$ T cells in patients with MM compared to controls suggest that elevated T-reg cells numbers may contribute in suppressing antitumor immune responses and favor development of the disease.

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THE ROLE OF HEPCIDIN IN THE PATHOGENESIS OF MULTIPLE MYELOMA RELATED ANEMIA AND THE VALUE OF HEPCIDIN IN CLINICAL FOLLOW-UP

R. Geyik,[1] M. Yilmaz[2], M. Pehlivan[2], V. Okan[2], H. BuyukHatipoglu,[3] A. Celik[4]

[1]Gaziantep University School Of Medicine, Department of Internal Medicine, GAZIANTEP; [2]Department of Hematology, GAZIANTEP; [3]Department of Internal Medicine, GAZIANTEP; [4]Department of Biochemistry, GAZIANTEP, Turkey

Background. Anemia is an important cause of morbidity and mortality in multiple myeloma (MM). Hepcidin, produced in the liver where its production is induced by IL-6, is an acute phase reactant which has a central role in the chronic disease anemia. Hepcidin causes anemia by means of preventing iron from the intestines and secretion from the macrophages. IL-6 level increases in MM. Increased cytokines and acute phase reactants have important roles in the anemia seen in MM. *Aims.* The etiopathogenesis of the anemia in MM is still unclear. Thus, we researched the role of the hepcidin in the anemia seen in MM, its relation with the cytokines and its role in disease activity. *Methods.* 29 recently diagnosed MM patients were included in the study. Serum levels of hepcidin, TNF-α, IL-6 and the hematologic parameters having prognostic value (β2 microglobulin, CRP, LDH ve ESR) were measured. 6 patients died during the therapy, so the same parameters were measured for the remaining 23 patients after 3-4 cyles of chemotherapy. *Results.* The results were compared with the results of 25 healthy controls. The post-treatment serum levels of hepcidin and IL-6 were significiantly decreased (respectively, p=0.008 and p=0.021). However, the post-treatment levels were significantly high as compared to the control group. Significant increase was found in post-treatment hemoglobin (Hb) (p= 0.043). Pre and post- treatment levels of the hepcidin and IL-6 were significantly and positively correlated. Pre- treatment levels of the Hb and the hepcidin were significantly and negatively correlated; however, after the treatment this correlation disappeared. There were no correlations between the serum levels of hepcidin and the known prognostic parameters for MM including LDH, β2 microglobulin and CRP. There is a significant correlation between the increased serum IL-6 and the hepcidin levels. IL-6 and hepcidin levels decrease as inflamation diminishes. *Conclusions*. Hepcidin seems to have a direct role or act a mediator in the pathogenesis of anemia seen in MM. However, because of the close relation of hepcidin with the other prognostic factors, both Hb and IL-6, it may be considered to be used in patient follow-up in MM patients. Besides, hepcidin might be speculated to be used as an acute phase reactant.

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PLATINUM-BASED PROTOCOLS IN THE TREATMENT OF RELAPSED OR REFRACTORY HODGKIN´S DISEASE

J.N. Rodríguez, G. Rodríguez, E. Martin, D. Vicente, A. Rodríguez, M.V. Moreno, J.A. Quesada, A. Fernández-Freire, J.C. Diéguez, A. Fernández-Jurado

Hospital Juan Ramon Jimenez, HUELVA, Spain

Background. The platinum-based protocols (ESHAP or EDHAP) have been widely used in relapsed or refractory cases of non-Hodgkin lymphoma (NHL) and even as a conditioning regimen prior to transplantation as well. In contrast to these data, their use in Hodgkin´s disease is scanty. *Aims.* We present our experience in 11 patients with relapsed or refractory Hodgkin's disease treated with these protocols, nine of them prior to transplantation. *Methods.* Eleven patients (5 males, 6 female), mean age 34 years (17-58) are presented. Histologic subtypes included 3 lymphocyte predominance, 7 nodular sclerosis and 1 mixed cellularity. Six cases were relapsed and 5 resistant to previous therapies. Eight patients were treated with ESHAP cycles and only one with EDHAP. No modifications on the conventional doses or schedule of these protocols were made. In another two cases with lymphocyte predominance histology Rituximab (375 mg/m^2, day 1) was added to the conventional protocol (ESHAP and DHAP, one each). *Results.* A total of 36 cycles were administered, mean number of cycles was 3,3 (2-5). Ten patients received complete doses and in one case corticosteroids were reduced 50% due to previous hypertension. No delayed administration of cycles was observed. Toxicity included: Anemia (n=10, one had previously anemia) in 6 patients (60%), mean cycle to observe 2,17; neutropenia (n=6, excluding those in which cycles incorporated G-CSF as mobilization) in 3 (50%), mean cycle 1, only 2 of them required G-CSF; thrombocytope-

nia in 6 (54,5%), mean cycle 1,33; mild increase of creatinine levels in 3 (27,3%), mean cycle 2,67; hypomagnesemia in 3 (27,3%), mean cycle 2,67; febrile syndrome in 1 (9,1%); emetic syndrome in 2 (18,21%). Response was observed in 8 cases (72%) (4 CR and 4 PR). Nine patients received an hematopoietic progenitors transplantation post-platinum treatment: Eight autologous transplantation, and the other a non-myeloablative allogenic one from his sister. In those cases of autologous procedures enough number of $CD34^+$ cells (data from 7 patients: mean $8,94 \times 10^6$/Kg; extremes: 3,18-18) could be obtained from peripheral blood (7 cases) or bone marrow (1 case) without significative problems. Three patients (autograft) have relapsed after transplantation (one patient is now being transplanted). *Conclusions.* In our experience, platinum-based protocols could be a safe, generally well tolerated and worthwhile option for the treatment of patients with refractory or relapsed Hodgkin´s disease. Toxicity is mainly related to cytopenias and mild renal failure. These protocols do not seem to affect the number of $CD34^+$ cells collected in cases of autologous transplantation.

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5 YEARS TREATMENT RESULTS OF A TUNISIAN PROSPECTIVE NON RANDOMIZED ADULT HODGKIN LYMPHOMA STUDY: SINGLE INSTITUTION EXPERIENCE ABOUT 114 PATIENTS

R. Ben Lakhal, R. Mansouri, H. Ben Neji, R. Jeddi, L. Aissaoui, R. Ben Amor, K. Kacem, N.H. Kraiem, H. Ben Abid, Z. Belhadjali, B. Meddeb

Aziza Othmana Hospital, TUNIS, Tunisia

Aims. To evaluate the clinical characteristics and the treatment results of Hodgkin lymphoma (HL) patients treated by a Tunisian prospective adult HL study. *Patients and Methods.* Between 2002 and 2006 114 newly diagnosed HL patients were enrolled in the Tunisian prospective study (MDH 2002) at the haematology department of Aziza Othmana hospital (Tunis). The MDH 2002 is based on: 1) The use of the EORTC prognostic factors in early stages and the international prognostic scoring (IPS) in advanced stages. 2) The use of ABVD regimen : 3 cycles for favourable early stages (G1), 6 cycles for unfavourable early stages (G2) and stage IIIA(G3) and 8 cycles for favourable advanced stages (IPS<3) (G5) involved fields radiotherapy is combined to chemotherapy for early stages and stage IIIA. 3) The use of intensive chemotherapy (escalated BEACOPP regimen level 4) for unfavourable advanced stages (IPS ≥ 3): G4. 4) Refractory and relapsed HL was proposed for intensive therapy and stem cell autotransplantation The following prognostic factors were analyzed with regard to their impact on overall survival (OS), event free survival (EFS) and relapse free survival (RFS) at 5 years: age, sex , histology, stage, B symptoms erythrocyte .sedimentation rate, bulky mediatinal disease, peripheral bulky disease, number of involved lymph node regions, IPS, Treatment group. *Results.* Median age at presentation was 33 years (16-68 years). There were 45 females and 69 males (M/F : 1,5) 52% of patients presented with advanced disease (33% with stage IV and 46% with IPS ≥ 3) . B symptoms were present in 72% of patients. At 5 years OS , EFS and RFS were respectively 87% , 75% and 90%. 4 toxic deaths with escalated BEACOPP regimen were observed. In univariante study age <25 years and advanced stage were 2 predictive adverse prognostic factors for OS (p=0.02, p=0.05) , EFS (p=0.0006, p=0.02) and for RFS (p=0.009 , p=0.01). IPS ≥ 3 was an adverse prognostic factor only for the OS (p=0.05). Treatment groups and bulky mediastinal disease influenced respectively RFS (p=0.01) and EFS (p=0.05). Induction failure therapy was a significant adverse prognostic factor for the OS and the EFS (p=0.0001). *Conclusions.* Our results are similar to these observed in other developing countries. Intensified induction chemotherapy for all advanced HL and beter management of acute toxicity can improve our results. Treatment of refractory HL has to be conclusively defined

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LATE EFFECTS OF TREATMENT FOR HODGKIN LYMPHOMA: A SINGLE CENTER EXPERIENCE

A. Sau, G. La Barba, S. Pulini, A. Patriarca, D. Carlino, G. Melatti, A. Spadano, G. Fioritoni

Spirito Santo Hospital, PESCARA, Italy

Background. Hodgkin lymphoma (HL) is today a potentially curable malignancy. In the last two decades the survival rates in pediatric patients reached values up to 90% in early stages of disease. Nevertheless, survival is well related to the occurrence of late treatment side effects. *Aims.* Our aim was to evaluate the therapy related to long term complications in a cohort of 29 young patients affected by HL, treated at our Center. *Methods.* From 1983 to 2004 a total of 29 children were diagnosed and treated consecutively for HL at our Center. Sixteen out of 29 were male and 13/29 were female. The median age at diagnosis was 15 years (range 6-18 years). Twelve/29 were in early stage at referral (stage I-IIA) and 17/29 were in advanced stage. Looking to treatment, 3 received only chemotherapy and 26 received both radio- and chemotherapy. The treatment was designed according to different protocols. Briefly 9 patients were enrolled into AIEOP LH 04 protocol, 9 into AIEOP LH 89 protocol, 1 in AIEOP LH 83 protocol and, at least, 1 was treated with 6 ADVB cycles. *Results.* After treatment 27/29 were on complete remission (CR). Only 2 cases were resistant to therapy and both died for disease progression. Among the CR patients, 2 experienced disease relapse, respectively at 6 and 12 months at the end of treatment. One of them died due to disease progression. To date, 26 patients are still alive and on CR. 5/26 (19%) showed laboratory features of hypothyroidism. Only one patient showed clinical and laboratory features suggesting thyroiditis, and only one patient need hormone replacement therapy. Moreover we found 2 cases of Chronic Restrictive Pulmonary Disease (CRPD), one followed by symptomatic tuberculosis 10 years after HL diagnosis and 2 cases of oligo-azoospermia. No secondary neoplasm or cardio-myopaty were recorded. The overall survival at 10 years, in our cohort, was 87.9% with a 30 months median follow-up (range 10-300). *Summary and Conclusions.* Our study confirms previously published data on the incidence of thyroid dysfunction and its correlation with a previous thorax and neck radiotherapy. The lack of secondary neoplasm is, perhaps, due to the brief median follow-up and the reduced number of patients undergone to high dose extend field radiotherapy. Patients with HL require special forms of extended follow-up and should be specifically counseled about minimizing risk factors for cardiovascular disease, smoking cessation, avoidance of sun exposure and for women who have received thoracic radiation, breast screening. Given the difficultly in conducting randomized trials, clinicians may benefit from regularly referring to well-conducted consensus studies that include updates and referencing websites.

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SALVAGE THERAPY WITH MINI-BEAM IN REFRACTORY AND RELAPSE HODGKIN'S DISEASE: A LONG-TERM FOLLOW-UP OF A SINGLE INSTITUTION

E.R. Rodrigo Alvarez, I. Rivas, G. Salvatierra, A. Lopez de la Guia, D. Hernandez, M.J. Sanjurjo, F. Hernandez Navarro, M.A. Canales

Hospital La Paz, MADRID, Spain

Background. Although Hodgkin's disease usually presents a good response to initial treatment, a non-depreciable proportion of patients does not response (15%) or relapse (20%) after achieving complete response. *Aims.* The purpose of our study has been to evaluate the long-term efficacy of mini-BEAM treatment as salvage therapy before autologous hematopoietic stem cell transplantation (HSCT) in case of refractory or relapse Hodgkin's disease. *Patients and Methods.* We have analyzed 44 patients with refractory or relapse Hodgkin's disease, treated in our institution with mini-BEAM (BCNU, etoposide, cytarabine and melphalan) regimen before autologous HSCT between 1978-2008. Twenty-eight were male (64%) with a median age of 34 (18-62) at time of treatment. Eight (18%) were refractory to front-line therapy, sixteen were partial responders, and 20 had relapsed. The median number of mini-BEAM courses administered was 3. *Results.* The overall response rate is 84% (54% complete response). Thirty nine out of 44 patients followed transplant after mini-BEAM, and four died becuase of disease progression. The most frequent complication has been febrile neutropenia in 15 patients (34%), bacteremia in 8 (18%) and mucositis in 3 of them (7%). With a maximum follow-up to 172 months since beginning of treatment with mini-BEAM, 28 patients (63%) are still alive, 19 of them in complete response. *Conclusions.* Mini-BEAM regimen followed by autologous HSCT is an effective regimen for salvage in case of refractory or relapsed Hodgkin's disease with an acceptable toxicity.

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THE VALUE OF TUMOUR BURDEN ESTIMATION THROUGH MORPHOLOGICAL AND CLINICAL CHARACTERISTICS IN THE PROGNOSIS OF CHILDREN AND ADOLESCENTS WITH HODGKIN LYMPHOMA

M.B. Barros,[1] E.M. De Matteo[2], C.M. Minicelli,[1] F.S. Soares,[3] I.Z. Zalcberg,[1] R.H. Hassan[1]

[1]Instituto Nacional do Câncer, RIO DE JANEIRO, Brazil; [2]Ricardo Gutierrez Children´s Hospital, BUENOS AIRES, Argentina; [3]Hospital A.C. Camargo, SÃO PAULO, Brazil

Background. Hodgkin lymphoma (HL) exhibits a high rate of therapy response, although 20% to 30% of patients ultimately relapse. Current clinical and radiological characteristics used for patient stratification in most treatment centres, lead to a wrong stratification in almost one third of patients. Prognostic factors have been identified in adult patients with HL, and several prognostic indices have been developed to guide therapy decisions. However, these factors may not be relevant for children. *Aims.* To determine if clinical and morphological characteristics, as well as oncoprotein immunoexpression could predict treatment response in paediatric HL, their prognostic impact was investigated in a series of 65 Brazilian patients. *Methods.* CD15, CD20, p53, p21 and Ki67 expressions were determined by immunohistochemistry. Epstein-Barr virus (EBV) was detected by RNA *in situ* hybridization. *Results.* Patients distributed equally between low/high stages. Nodular sclerosis (NS) (82.8% of the cases) grading revealed 49% grade I and 51% grade II patients. Morphologic analyses showed median values of 40 H-RS cells, 4 mitosis and 36 eosinophils (in 10 high-power fields). 48% of the cases were EBV^+. An expression level of 50% (cut-off) was employed to consider p53 overexpression, identified in 35/56 analyzed cases (62.5%), while p21 expression was observed in 36/52 analysed cases (69.2%). Analysis of the p53/p21 balance showed 10 (19.2%) $p53^-/p21^-$, 29 (55.7%) $p53^+/p21^+$, 7 (13.4%) $p53^-/p21^+$ and 6 (11.7%) $p53^+/p21^-$ cases. Thus, p53 was considered non-functional in 16/52 cases (30.8%). A high PI defined by Ki67 expression in >50% of H-RS cells was observed in 50% of the cases. In univariate analyses, the number of involved anatomic sites (>5), NS grade, number of H-RS cells and a high proliferative index (>50% H-RS Ki67+) showed significant impact on disease free survival (DFS). Immunophenotype, eosinophils count and p53/p21 expression did not impact survival. In the Cox analysis, only the number of involved anatomic sites showed independent prognostic impact on DFS (p=0.03); the number of neoplastic cells showing a borderline significance (p=0.08). *Conclusions.* Our results suggest that development of biological markers allowing tumour burden estimation to be included in prognostic systems could improve the efficacy of risk-tailored therapy in HL. Thus, the number of involved anatomic sites (nodal and extranodal) could be a promissory prognostic factor in paediatric HL and prospective studies need to include this variable for confirmation. In addition, the number of H-RS cells alone or in combination with PI deserves further evaluation in a larger series to clarify its clinical value.

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PREVALENCE OF HEPATITIS B OR C VIRUS INFECTIONS WITH LYMPHOID TUMOURS IN GAZIANTEP TURKEY

V. Okan,[1] M. Yilmaz[2], A. Bayram,[3] C. Kis,[4] S. Cifci,[4] H. BuyukHatipoglu,[4] M. Pehlivan[2]

[1]Gaziantep University Medical School, GAZIANTEP; [2]Department of Hematology, GAZIANTEP; [3]Department of Microbiology and Clinical Microbiology, GAZIANTEP; [4]Department of Internal Medicine, GAZIANTEP, Turkey

Introduction. The etiology of most lymphoproliferative disorders is unclear. Several hypotheses have been proposed for the pathogenesis of these disorders. One of these mechanisms is the infection of the tumor clone by an oncologic virus. To date several viruses have been shown to be associated with lymphoproliferative disorders including, EBV, HHV-8 and HTLV-1. Recently some evidences have arisen about the roles of Hepatitis B and particularly hepatitis C viruses in the pathogenesis of lymphoproliferative disorders. Based on this information we researched the prevalence of Hepatitis B and C virus in lymphoproliferative disorders. *Material and Methods.* 334 newly diagnosed lymphoproliferative patients (200 male 134 female) and 802 (133 female, 881 male) healthy controls who were randomly recruited from the blood bank of the university were included in the study. All patients and control group had no history of drug abuse, no history of blood transfusions, and none of them were homosexual. Diagnosis of lymphoproliferative disorder was made through a morphologic evaluation of lymph node and/or bone marrow biopsies on which immunophenotypic analysis for surface B and T lymphocytic markers was also performed in all cases. Comparisons were made according to the WHO classification of lymphoid tumors. HBsAg, anti-HBs, HBeAg, anti-HBe, AntiHBc IgG ve anti-HBc IgM levels evaluated with commercial enzyme linked immunosorbent test (ELISA). Serological testing for anti-HCV was determined by a third generation ELISA assay: HbsAg and anti-HCV seropositivity for HBV DNA and HCV RNA was confirmed by a PCR assay. *Results.* In patients with lymphoid tumors the seropositivity of HbsAg and/or anti-HCV was 8.7% (829/334). In control group this was 49/802; however, it did not reached statistically significance (p=0.232, OR: 1.361, 95%CI: 0.821-2.255). Seropositive patients were confirmed by PCR analysis. We found no significant sex-related or age-related differences both for Hepatitis B and C seropositivity. There was no significant difference between the seropositivity of Hepatitis B and C and both in NHL and Hodgkin lymphoma. However, in diffuse large cell lymphoma and follicular lymphoma subgroups HbsAg seropositivity was significantly higher as compared to control group (p=0.017×2, p=0.048×2). Seropositivity of Hepatitis C only showed a significant difference in follicular lymphoma subgroup as compared to controls (p=0.008×2). *Conclusions.* We did not found a significant difference between the patients with lymphoproliferative disorders and the controls for the prevalence of Hepatitis B and C viruses. However, significant difference was found in subgroups related to controls. Combining together these two viruses might have a role in development of subgroups of lymphoproliferative disorders. Nevertheless, larger epidemiological studies particularly focused on the subgroups are necessary to draw clear *Conclusions.*

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THROMBOPHILIA AND CRYPTOGENETIC FOCAL EPILEPSY IN CHILDREN AND YOUNG ADULTS

V. Pansini,[1] M. Luciani,[1] F. Prischich[2], M. Soldati,[1] G. Derossi[1]

[1]Children Hospital Bambino Gesù, ROME, VATICAN CITY; [2]Hospital S. Andrea, ROME, Italy

Background. The pathogenesis of epilepsy is frequently due to several factors and its cause is often impossible to find. Diagnosis by imaging, first of all Angio-MRI, could clarify some of the cases previously classified as idiopathic. Nevertheless, between idiopathic cases (29,3%, Jallon P. *et al.,* 1997), a thrombotic pathogenesis not possible to diagnose by neuroimaging could be assumed. Just few studies have been performed to investigate whether there is a role of hemosthatic mechanisms in inducing epilepsy in childhood and youth. Thrombophilia indicates a predisposition to develop thrombosis based on inherited or acquired disorders of hemostasis. Hence, not necessarily a mutation of a gene associated with thrombophilia is related to a clinical event. *Aims.* With the intention to contribute to the definition of pathogenetical mechanisms of idiopathic epilepsy (Angio-MRI negative) in children and young adults, the aim of this study is to demonstrate if there is a correlation between specific prothrombotic risk factors and cases of focal epilepsy. *Methods.* We studied 48 patients (32 males, 16 females), aged 1-32 years, average 11,5 years, affected by focal epilepsy. In all the patients any kind of focal idiopathic epilepsy was excluded. The diagnosis was made as follows: First phase: clinical history, neurological examination, EEG and neurological imaging. Second phase: in children with diagnosis of cryptogenetic or symptomatic partial epilepsy, the following tests have been performed: PB cells count, coagulation tests and thrombophilic screening including Factors VII, VIII, Proteins C and S anticoagulant, APC resistance, Factor V Leiden, Factor II Prothrombinic, total Homocysteine, Fibrinogen Dimers, Fibrinogen levels, PTT-s, PTT-r, prothrombin time, PT ratio and Antithrombin III. *Results.* 15 patients (12 male, 3 female) showed increased levels of Factor VIII (>120%) (range 123-226; madian 159); 10 patients (8 male, 2 female) showed Hyperhomocysteinemia (>15 umol/L) (range 15,9-47,3; median 24,5); 1 patient, female, had protein S levels < 50% (37%). Remarkably, just one patient had a heterozygous mutation of Factor V Leiden and another one a heterozygous mutation of Factor II prothrombinic. No significant correlations were found with other evaluated parameters. *Summary and Conclusions.* Our data do not seem to confirm the possible correlation between Factor V Leiden mutation and focal epilepsy as previously reported (Scantlebury MH *et al.,* 2002). The incidence of this mutation, as well as other mutations commonly evaluated (Factor II prothrombinic), seems to be the same, even lower, as that found in the general population (2-5%). However, in patients affected by focal epilepsy, this study shows a higher incidence of increased levels of Factor VIII and Homocysteinemia compared to the general population (11% and 5% respectively). Furthermore, the overall studied population shows an increased frequency of thrombophilic alterations compared to the gener-

al population requiring further investigations. The authors discuss the results in order to explain possible correlations between thrombophilic disorders and idiopathic epilepsy.

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PLASMA TOTAL HOMOCYSTEINE (THCY) LEVELS AND METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISM IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

B.A. Lwaleed,[1] A.L. Soares[2], A.P. Fernandes[2], J.E. Cardoso[2], M.O. Sousa[2], M.C. Lasmar[2], B.A. Novelli,[3] G.F. Lages[2], L.M. Dusse[2], L.M. Vieira[2], B. Lwaleed,[1] M.G. Carvalho[2]

[1]Southampton University Hospitals NHS Trust, SOUTHAMPTON, UK; [2]Faculty of Pharmacy, Federal University of Minas Gerais, BELO HORIZONTE, MINAS GERAIS, Brazil; [3]Santa Casa de Misericódia de Belo Horizonte Hospital, BELO HORIZONTE, MINAS GERAIS, Brazil

Background. Thrombotic episodes are accountable for approximately 80% of deaths in type 2 diabetic patients. Hyperhomocysteinaemia is a well recognized independent risk factor for atherosclerosis and thromboembolism. Increased homocysteine levels may occur due to a number of factors including inherited gene polymorphism of methylenetetrahydrofolate reductase (MTHFR) C677T. *Aims.* We evaluated plasma total homocysteine (tHcy) levels and frequency of the MTHFR C677T gene polymorphism in asymptomatic healthy volunteers and type 2 diabetic patients with hypertension but without nephropathy. *Methods.* A total of 53 subjects were investigated. These included asymptomatic healthy volunteers (n=16), patients with type 2 diabetes (n=7), subjects with hypertension (n=12) and patients with both type 2 diabetes and hypertension (n=18). Plasma total homocysteine levels were measured using the AxSYM® Homocysteine assay. The MTHFR C677T genotype was analysed by polymerase chain reaction. Renal function, serum lipids and other metabolites were assessed using standard methods. *Results.* There was no significant difference in tHcy levels between the groups studied. The frequency of MTHFR C677T gene polymorphism observed was similar to those obtained for the general Brazilian population. In patients with type 2 diabetes and hypertension but without impaired renal function we observed no meaningful correlation between increased tHcy levels and the presence of MTHFR C677T gene polymorphism. In conclusions, type 2 diabetics who are homozygous or heterozygous for the MTHFR C677T gene polymorphism showed normal tHcy levels. *Summary.* Our results suggest that diabetes without associated adverse risk profile is not an independent correlate of increased tHcy levels.

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HEREDITARY FUNCTIONAL PROTEIN C AND PROTEIN S DEFICIENCY IN A COHORT OF THROMBOPHILIC PATIENTS

M. Ionita,[1] R. Pacurar[2], H. Ionita,[1] D. Nicola,[1] L. Cheveresan,[1] I. Ionita,[1] M. Cheveresan,[1] D. Tanasie,[1] I. Suceava,[1] D. Calamar,[1] S. Balgradean[2]

[1]University of Medicine and Pharmacy Victor Babes, TIMISOARA; [2]County Hospital, Hematology Department, TIMISOARA, Romania

Background. A hereditary thrombophilic state must be differentiated from an acquired one and it is suspected in a patient with characteristic anamnestic data: thromboembolic episodes at an early age (15-45 years) with familial distribution, occurrence in unusual sites such as mesenteric, cerebral and axillary veins, reccurence in the same site or, characteristically, in separate sites, with or without triggers, despite an adequate therapy, warfarin-induced skin necrosis.Because of the discusions about the necesity of screening tests in hereditary thrombophilia, about the management of the patients and their family, it is necesary to enlarge the database with new studied cases. *Aims.* In this study we report the results of the measurement of functional activity of activated protein C and protein S in a strictly selected cohort of patients suspected of hereditary thrombophilia. *Methods.* Were studied a group consisted of 38 patients examined in the Hematology Department of the City Hospital, Timisoara within four years. The selection criteria were: age under 50, recurrent thrombosis, sometimes in different sites from one episode to another, without an apparent cause, all of them having no clinical, imagistic or laboratory signs of paraneoplastic, inflammatory or infectious activity. Global plasma activity of protein C and S were determined using an ACL 2000 nephelometric centrifugal analyzer and IL Test Kitts provided by Instrumentation Laboratory (IL SpA, Viale Monza 338-20128 Milan, Italy). *Results.* The studied cohort (38 pts) included 28 males (74%) and 10 females (26%) with recurrent thrombotic events in personal and family history. The affected sites are: 5 pts presented clinical signs of pulmonary embolism (PE), 25 patients thrombosis of legs veins (TLV) from which 20 superficial thrombophlebitis and 5 deep vein thrombosis, 5 patients were diagnosed with axillary thrombosis (TAV), 3 patients thrombosis of the mesenteric veins (TMV), 1 patient - inferior cava vein thrombosis (TCV) and one with renal arteria thrombosis (TRA). Note that 2 of the TLV were complicated with PE. Five patients (13%) presented functional protein C deficiency, other 6 patients (16%) presented functional protein S deficiency and one patient (3%) was diagnosed with associated deficiency of protein C and protein S.No deficiency was seen in 68% of the patients. None of the patients with functional protein C or S deficiency had associated functional deficiency of antithrombin III or resistance to activated protein C. All the positive results were confirmed after six month by repeating all tests, in the same conditions. *Conclusions.* This study demonstrates that the incidence of functional protein C and S deficiency in patients with recurrent thromboembolic disease increases when the selection is carefully made, making the test useful and cost-efficient. Finding a hereditary protein C and/or protein S deficiency has to be followed by searching it in blood-related individuals in order to take an adequate prophylactic and therapeutic attitude.

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ALTERATION IN COAGULATION IN ACUTE PANCREATITIS

P. Paraskevopoulou

General Hospital, ATHENS, Greece

Background. Pancreatitis is a severe disease with high mortality, morbidity and with the obvious risks of bleeding or thrombosis. Some alterations in the coagulation cascade could determine the severity of the disease. *Aims.* With this study we try to investigate the alteration of which factors of coagulation increase the possibility of a negative development of pancreatitis. *Methods.* 20 patients with acute pancreatitis were studied. They were subdivided in two groups; Group A (n=15) with mild pancreatitis and group B (n=5) with severe pancreatitis. We consider as mild the patients with CR p<150 mg/L and APACHE score <8 and as severe all the patients with CRP >150 mg/L and APACHE score > 8. In all patients TAT ,Antithrombine (AT) and Protein C were measured. In group A we measured these parameters during the 1st, 2nd and 3rd days of treatment while in group B during the 1st, 2nd, 4th, 6th and 7th days of treatment. Antithrombine, Protein C were measured with reagents of Dade Behring in an automated analyzer BCS. TAT was measured with Elisa of Dade Behring. Normal ranges for Antithrombine: 75-125%, Protein C:70-140%, TAT: 0,5-4,1 μg/L. *Results.*

Table 1.

	Group A (n=15·3)		Group B (n=5·5)	
	n	%	n	%
TAT	10.7±9.3	↑ 62.2	12.58±8.3	↑92
AT III	87.3±14.7	↓ 15.5	63.05±13.1	↓80
Protein C	88.3±25.2	↓ 20	64.5±17.5	↓76

Conclusions. TAT was found statistically increased by 62% in group A and by 92% in group B. Antithrombine and Protein C were decreased by 80% in group A and by 76% in group B from the first day of care and remained at these levels until the 7th day. All of these constitute indications of a high risk of thrombosis, a severe complication for these patients. High levels of TAT are an indication of increased coagulation activity but this does not correlate directly with thrombophilic tendencies. Nonetheless these three factors could be used as indicators of severe development of pancreatitis.

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ANALYSIS OF THROMBOPHILIC PARAMETERS IN WOMEN WITH ADVERSE PREGNANCY OUTCOME IN COMPARISON WITH HEALTHY WOMEN,REVEALED DIFFERENCES IN FVIII:C AND D-DIMERS LEVELS BETWEEN THESE POPULATIONS

P.A. Kotsi, A. Sarantopoulos, I. Anastasopoulou, C. Tsoukala, A. Karafoulidou

Laiko General Hospital, ATHENS Greece

Background. Adverse pregnancy outcome is a common problem in

everyday practice. 20% of women will experience at least one automatic abortion in their life.5% of women will experience at least two automatic abortions. In 40-55% of cases, the cause of this complication remains obscure, after the obstetrical (10%), chromosomal (4%) and hormonal screening (28%). Hereditary thrombophilia it has been correlated with adverse pregnancy outcome. *Aims*. Analysis of thrombophilic parameters in women with history of adverse pregnancy outcome in comparison with age matched healthy women as controls. *Methods.* 175 women were studied- mean age 32 years (18- 48 y), 75 healthy and 100 cases with history of adverse pregnancy outcome (1-2 abortions n=52, 3 or more abortions n=18, 2nd trimester abortion n= 8, still birth n=6, small for dates n=7, vascular complications n=9). All the women were examined with family history and thrombophilia testing and were free of other pathologic conditions. Laboratory testing included screening tests PT, APTT, FIB (stago), d-dimers (vidas), ATIII:C,PC:C, PS:C,PS:F, PS:T, normalized APC-R, FVIII:C, FIX:C (stago), LA testing and determination of FVL, G20210A, and MTHFR (C677T) (vienna lab, elucigene) mutanions status. One way anova was used for statistical analysis of results. *Summary and Conclusions.* Higher mean levels of FVIII:C (p=0,03) and d-dimers (p=0,01) were found in the group of women with pregnancy complications history compared with the control group. The incidence of FVL mutation may contribute to that difference but the difference of the presence of the mutation between groups was not significant p=0.14. Higher levels of FVIII:C and d-dimers may play role in the evaluation of a woman with history of pregnancy complications as additional risk factors. Our data need evaluation in larger and prospective studies.

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EVALUATION OF LEUKOREDUCTION IN PACKED CELL UNITS FILTERED BY HOME-MADE BEDSIDE FILTERS, PRE AND POST REVISION OF PRODUCT TECHNOLOGY AND MATERIALS.

M. Nikogoftar,[1] F. Atashrazm,[1] B. Sadeghi[2], H. Abolghasemi,[1] S. Abroun[3]

[1]Iran Blood Transfusion Organization, TEHRAN; [2]Dept.of Hematology, School of Medical Sciences, Tarbiat Modares University, TEHRAN; [3]Dept.of Hematology, School of Medical Science, THEHRAN, Iran

Leukocytes are present in all blood products which prepared by standard *Methods.*and cause a wide variety of side effects after transfusion. The use of filter technology for leukoreduction has been widely practiced and according to AABB accreditation, leukoreduced blood components must contain less than 5×10^6 leukocyte per unit, but sometimes there are more than 5×10^6 by using leukoreduction filters. In this study we did absolute leukocyte count in filtered (home made bedside filter) packed cell units by True count method as standard method and CD45 MoAb method. 93 packed cell units from recruitment donors, stored at 4 c and filtered by two type homemade filters, as manufacturer instruction. The pre revised filters were made before technical revision and the post revised were made after that. Furthermore eight packed cell units were filtered by European Certificated control group filters. Sample preparation was done according to True count kit and CD45 MoAb procedures and analyzed by Flowcytometry and results were analyzed in SPSS by χ^2 test. The mean of leukocyte count/unit by anti CD45 and True count method were 9×10^6 and 10×10^6 respectively in 55 filtered bags by pre revised filters, and 4.2×10^6 and 4.8×10^6 in 30 filtered bags by post revised filters, whereas the mean of leukocyte count/bag in eight filtered bags by control filters was 2.3×10^6.For comparison of test groups and control, we select 8/55 and 8/30 test units randomly (the equal number with control group).The mean of leukocyte count/bag in pre revised group of test was $7.9\pm5.4\times10^6$ and in post revised group was 4.2×10^6 but in control was 2.3×10^6 (p<0.05). According to the results the mean of leukocyte count/bag in pre revised group was more than AABB standard. 38.2% of bags had less, and 61.8% had more leukocyte count than standard value (48.9 to 74.6% with CI=95%), which confirmed the necessity of revision in product technology of home made filters. The manufacturer revised the product and material technology and new filters (post revised group) reduced leukocytes within standard limits.(leukocyte count in 6 bags were out of standard value).In post revise filter group 20% had more than 5×10^6 Leukocytes and 80% had less than this value. (5.7-34.3% with CI=95%).There is meaningful difference between control and pre revised test group (p=0.03).In post revised test group, despite meaningful difference, mean of leukocyte count/bag were within normal standard. The results of this research caused home made filter production with higher quality.

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MOLECULAR CYTOGENETIC ANALYSES OF 12P13.3 JARID1A GENE IN A CASE OF MYELODYSPALSTIC SYNDROME WITH A COMPLEX T(12;21)(P13;Q22)

A. Buijs, A.W. Dekker

University Medical Center Utrecht, UTRECHT, Netherlands

We report on a case of myelodysplastic syndrome (MDS) showing a rare translocation t(12;21)(p13;q22). 12p13 ETV6 (TEL) and 21q22 RUNX1 (AML1) are frequently rearranged in both myeloid and lymphoid malignancies. Deletions of 12p13 have been identified in ~10% of AML. Other genes in the 12p13 and 21q22 regions identified to be rearranged in myeloid leukemia or solid tumors are JARID1A and ERC1, and ERG, respectively. To molecularly characterize genes involved in the t(12;21) we used a FISH approach. The ETV6-RUNX1 gene rearrangement, specific for t(12;21) in pre-B ALL, could be excluded. By using BAC probes from 12p13.3 region FISH analyses demonstrated that probe RP11-283I3, spanning exons 11-31 of JARID1A, was translocated to derivate chromosome 21. Furthermore, on 12p13.3 an interstitial deletion of 1.0 to 4.8 Mb was identified distal to ETV6 containing ERC1, indicating loss of heterozygosity (LOH). On chromosome 21 the breakpoint could be narrowed to a 1.4 Mb region distal to the RUNX1 gene. Further molecular studies may reveal involvement of 12p13.3 JARID1A as a result of this complex t(12;21)(p13.3;q22.12) or may identify LOH of 12p13.3 as a marker for AML.

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DISCORDANCE BETWEEN MORPHOLOGICAL AND CYTOGENETICAL REMISSION IN A PATIENT WITH AML AND T(2;11)(P21;Q23)

W. Kroes,[1] E. den Ouden,[1] M. Baasten,[1] J. Kerkhoffs,[2] R. Willemze[1]

[1]LDGA / Leiden University Medical Center, LEIDEN; [2]Haga Hospital, THE HAGUE, Netherlands

We report here on a 58 year old male presenting with biphenotypical acute myeloblastic leukemia .Conventional cytogenetic techniques on bone marrow at initial diagnosis, before treatment, showed the following karyotype: 46,XY,t(2;11)(p21;q23),del(5)(q21),add(12) (p12)[9]/46, XY,t(2;11)(p21;q23)[5]/46,XY[1].FISH analysis with a DNA probe for the MLL-gene demonstrated that the breakpoint at 11q23 was telomeric to the MLL gene. T(2;11) (p21;q23) is a rare but recurrent translocation observed in MDS and AML. This translocation is specifically associated with a deletion of the long arm of chromosome 5. After induction treatment the patient was in complete morphological remission. However, cytogenetic studies revealed the t(2;11) in all metaphases, without the del(5q) and the add(12p). Evaluation after the second therapy cycle showed again a complete morphological remission but persistence of t(2;11) in all analyzed metaphases. To investigate the possibility of a constitutional chromosome aberration chromosomal analysis was performed on skin-fibroblasts and peripheral blood. The skin-fibroblasts revealed a normal karyotype in all 100 analyzed metaphases. PHA stimulated blood showed a mosaic karyotype: 46,XY,t(2;11)(p21;q23) [11]/46,XY[21]. This suggests that the t(2;11) is an acquired aberration that persists in spite of morphological remission. The patient underwent a bone marrow transplantation and is still in complete morphological remission after 10 months. To our knowledge this is the first description of an AML case with a t(2;11) showing discordant morphological and cytogenetical remission.

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ACUTE MYELOID LEUKEMIA IN PATIENTS AGED ≥70. EXPERIENCE AT A SINGLE CENTRE

J.N. Rodríguez, E. Martin, G. Rodríguez, M.V. Moreno, J.A. Quesada, A. Chacón, M.J. Romero, J.C. Diéguez, A. Amian, A. Fernández-Jurado

Hospital Juan Ramon Jimenez, HUELVA, Spain

Background. The management of old patients with acute myeloid leukemia remains controversial, specially in those cases that can be considered very old patients (aged 70 or older) in which the dilemma therapeutic abstention vs treatment (with low or high intensity) can be considered. *Aims.* We present our experience with this group of patients in the period 1990-2007. *Methods.* During the period of study 74 cases were diagnosed (FAB M3 cases were excluded). Patients were divided into 3 groups according to the treatment: supportive treatment, low intensity treatment (low doses Ara-C: 10 mg/m²/12h s.c. days 1-21) and high

intensity treatment (adapted ICE: Idarubicin 10 mg/m^2 days 1 and 3; Ara-C 100 mg/m^2/12h days 1-3; Etoposide 100 mg/m^2 days 1-3). *Results.* The mean age of patients was 76,32 years (70-89); sex distribution was 37 males and 37 females; mean Karnofsky index was 72,5 (30-100); 47 patients received treatment and 27 did not; overall survival was 6,3 months (median 2; 0,06-90+), significant differences were observed in the mean overall survival between the treated and no-treated groups (8,7 *vs* 2,3 months respectively; p=0,03). In the low intensity group (30 patients) an overall response of 33,3% (6 CR, 4 PR, 13 NR and 7 not evaluable) was observed while in the high intensity one (17 patients) this overall response was 53% (7 CR, 2 PR, 3 NR and 5 not evaluable); no statistical differences were observed between both groups (p=0,25). Considering overall survival in these same groups, no statistical differences were observed between them 7,2 (0,25-90+) *vs* 11,3 (0,25-52+) months (p=0,39) respectively between the low and high intensity groups. *Conclusions.* 1) Overall survival in the treated group is higher than in the non-treated one, differences reached statistical significance (p=0,03). 2) Though no statistical differences have been observed in the overall survival between both groups of treatment, this event could be explained by two reasons: the very long survival in one patient in the low intensity group and the still short follow-up of some patients in the high intensity one. 3) Comparing both arms of treatment, a higher proportion of CR can be observed in the high intensity group (41,2% *vs* 20%, respectively), however, if this circumstance will contribute to a longer survival is still unknown.

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CAN VERAPAMIL INFLUENCE THE EVOLUTION OF THE MALIGNANT HAEMOPATHIES?

R. Mihaila,[1] E.C. Rezi[2], A. Catana[2], O. Flucus[2], M. Deac,[1] R. Mihaila[3]

[1]*Lucian Blaga University, SIBIU;* [2]*Departmental Clinical Hospital, SIBIU;* [3]*Public Health Authority, SIBIU, Romania*

Background. The apparition of the refractory disease associates often with the supra-expression of the proteins involved in the multidrug resistance in many malignant haemopathies. Verapamil is an inhibitor of the P-glycoprotein - an efflux drug pomp and, in some studies made *in vitro*, it contributed to the reversing of the multidrug resistance. *Aims.* We proposed ourselves to study the effects of verapamil on the evolution of the patients with malignant haemopathies who are hypertensives, comparing with those normotensives, treated only with chemotherapy. *Methods.* We studied a group formed by 45 patients with malignant haemopathies which were divided into 2 groups: group A - with hypertension, who were treated with verapamil along with the chemotherapy, and group B - without hypertension, treated only with chemotherapy. At each group, we have analyzed the next parameters: age, gender, the diagnosis, the survival period until the moment of this study, the eventual adverse events, the medium values of cholesterol level, triglycerides, glycaemia, transaminases, the presence and the grade of arterial hypertension, the presence of the diabetes mellitus, of the obesity and atherosclerosis and the patient's evolution. The patients were included in the study only after they gave their agreement. The results were statistically analyzed using the t Student test and the chi test. *Results.* Among the 9 patients from group A (7 with chronic lymphoproliferative syndromes and 2 with chronic myeloproliferative syndromes) the medium age was 57±11 years; the gender repartition: 77,78% women and 22,22% men. The medium administrated dose of verapamil was 151±69 mg/dl. Under this treatment, the systolic arterial pressure decreased with 20,13% (p<0.05), and the diastolic with 13.56% (p>0.05). The 36 patients from group B had a medium age of 63±18 years; the gender repartition: 50% women and 50% men. The cholesterol level was significantly higher in group A, comparing with group B (p=0.004). At the rest of the biological testes there were no significant differences between the 2 groups. There were no adverse events. The medium survival period did not varied significantly between the 2 groups, but, even though the cholesterol level was higher at the initial moment at the patients from group A, the number of patients with a stable disease was significantly higher in group A comparing with group B - 77.78% *vs* 44.44% (p<0.0001), and the number of patients with progressive disease was significantly higher in group B comparing with group A - 30.56% *vs* 11.11% (p<0.0001). In group A there was a single death caused by the evolution of the haemopathy (after a record survival of 32 mouths with chronic myeloid leukemia discovered in blastyc phase), but in group B - 5 (p<0.0001). *Summary.* Verapamil could have a benefit not only by its anti-hypertensive effect, but also by its possible positive influence on the evolution of the malignant haemopathies - a pleiotropic effect based on the inhibition of the P-glycoprotein, by blocking the efflux of cytostatic drugs from the malignant cells.

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COMPARATIVE STUDY ON HEMATOPOIETIC TOXICITY OF CISPLATIN AND NON-TRADITIONAL ANTITUMOR AGENTS IN EHRLICH ASCITES CARCINOMA

M.F. El-Azab

Suez Canal University, ISMAILIA, Egypt

Background. The use of chemotherapeutic agents in the treatment of cancer is complicated by the serious hematotoxicity. Hence, there is ongoing global research into the development of new agents which will have effective antitumor activity with minimum toxicity. *Aims.* This study was conducted to evaluate the hematotoxicity profile of thalidomide, rofecoxib, and captopril in comparison with that of cisplatin in EAC-bearing female Swiss albino mice. The modulatory effects of these drugs on cisplatin-induced hematopoietic toxicity were also assessed. *Methods.* The EAC cells were implanted subcutaneously at 2 sites bilaterally to produce solid tumors on the lower ventral side of Swiss mice. These mice were used to evaluate the effects of thalidomide (100 mg/kg, i.p.), rofecoxib (20 mg/kg, p.o.) or captopril (50 mg/kg, p.o.) as individual treatments or in combination with cisplatin (2 mg/kg, i.p.) on peripheral blood components and bone marrow cellularity. All treatments were started 24 hours after tumor cells inoculation. Complete blood count and bone marrow cellularity were measured as a time course on days 7, 14 and 21 post EAC-cells inoculation.

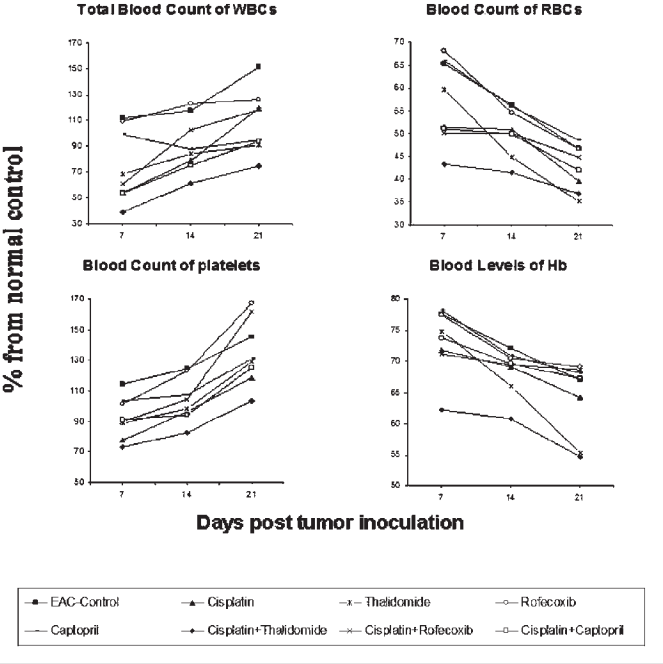

Figure 1. Sequential changes of hematological parameters in peripheral blood (Total WBCs, RBCs, hemoglobin, platelets) after treatment with cisplatin (2 mg/kg, i.p.) alone and in combination with thalidomife (100 mg/kg, i.p.), rofecoxib (20 mg/Kg, p.o.) or captopril (50 mg/Kg, p.o.) measured on days 7, 14 and 21 in EAC-bearing female Swiss albino mice.

Results. The progression of EAC-tumor was accompanied by a gradual decrease in hemoglobin content, erythrocytic count and bone marrow cellularity. In addition, a gradual increase in leukocytes and thrombocytes and reversal of the lymphoid-myeloid ratio in the differential leukocytic count were observed. Cisplatin treatment caused an inhibitory effect on peripheral blood components and bone marrow cellularity. Daily treatment with either thalidomide or captopril exhibited a depressive effect on leukocytes with reduction in granulocytes percentage on 3rd week with subsequent increase in lymphocytes percentage. Although a reduction in RBCs count and Hb level was observed in all treatment groups, cisplatin had the most depressive effect on RBCs count and Hb level on day 7. A significant reduction in platelets count after combined treatment of cisplatin with thalidomide was detected on days 7 and 14. The combination of cisplatin with either rofecoxib or cap-

topril produced a significant reduction in the percentage of erythroid progenitors and bone marrow cellularity on day 7. Treatment with cisplatin produced the most depressive effect on the percentage of lymphoid progenitors as well as bone marrow cellularity on day 7. A significant reduction in bone marrow cellularity in cisplatin/thalidomide-treated group was detected on day 21 as compared to the EAC-control. *Conclusions.* It is concluded that thalidomide, rofecoxib, and captopril had lower hematopoietic toxicities in comparison with cisplatin in the current EAC-model. The use of antiangiogenic agents may represent a promising strategy for cancer management.

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PREVALENCE OF BONE DISEASE IN CHILDREN AND ADOLESCENTS WITH SICKLE CELL ANEMIA: SINGLE INSTITUTE EXPERIENCE IN KINGDOM OF SAUDI ARABIA

S. Al Jaouni, N. Fida

King Abdulaziz University Hospital, JEDDAH 21589, Saudi Arabia

Background. Bone disease is an increasingly recognized serious cause of morbidity on young adults with hemoglonopathies disorders. Sickle cell anemia (SCA) is a prevalent genetic disorder in Saudi Arabia. Sickle hemoglobin leads to tissue hypoxia and adverse effect on bone, sickler has multiple bone problems include, bone pain participate vaso-occlusive crises, osteomalacia, osteopenia, spinal deformations, fractures, severe osteoporosis and a vascular necrosis. *Aims*. To assess the prevalence of bone disease among children with sickle cell anemia at our institute. *Methods.* Two hundred three (203) SCA patients were enrolled in the study, age ranges from 1-18 years old. (98 females & 105 males). These patient were treated and followed at King Abdulaziz University Hospital (KAUH), Jeddah Kingdom of Saudi Arabia (KSA). All patients were assessed clinically. Blood and urine samples were obtained for the determination of biochemical and hormonal profiles, included , PTH, 25 OH vitamin D3. Bone maturation was assessed by radiological bone age. Bone mineral density (BMD) by DEXA was determined on half of the adolescent patients. Bone formation markers (bone-specific alkaline phosphatase and osteocalcin) and bone resorption markers (Pyridinoline and deoxy pyridinoline) were analysed for patients whom had BMD and referred for treatment. *Results.* High prevalence of hypovitamnosis D, 25% in sickle cell children patients, 55% among adolescents. High prevalence of reduced low bone mass (LBM) among adolescents whom screened. *Summary*. Bone assessment was found to be suboptimal in children and adolescents in our institute. All sickles should be screened annually for bone disease. Calcium and vitamin D deficiencies may further compound the patients risk for bone disease. Early diagnosis and treatment can prevent a bone complications.

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COLD AGGLUTININ DISEASE - A CASE SERIES OF 5 ADULT CHINESE PATIENTS TREATED AT A REGIONAL HOSPITAL IN HONG KONG

T.K.H. Lau, Y.S. Liang, V. Li, C.S. Kho, S.Y. Liu, R.W. Chu, C.W. Chan

Pamela Youde Nethersole Eastern Hospital, HONG KONG, Hong Kong

Background. Cold agglutinin disease can be defined as an autoimmune haemolytic anaemia due to cold agglutinins, which are IgM antibodies that react with polysaccharide antigens on the red blood cell surface at temperatures below the core temperature of the body. It can arise secondary to infection or can be related to underlying malignancy, most often lymphoma. It is classified as idiopathic when it is not associated with lymphoma or other diseases. *Aims.* Little is known about the epidemiology of cold agglutinin disease in Chinese adults. We report five cases in Chinese. *Methods.* The clinical and laboratory features of five Chinese patients with chronic cold agglutinin disease diagnosed between December 2004 and October 2007 at a regional hospital in Hong Kong were analysed. *Results.* Five patients (M:F = 3:2, median age 72, range 50-87) were analysed. All patients except one presented with anaemic symptoms. Acrocyanosis was present in four patients. Presenting haemoglobin ranged from 4.7-7.8 g/dL.

Table 1.

Sex /age	presenting features	Hb (g/dL)	Anti-IgG	Anti-C3d	cold agglutinin titre	Underlying condition	Treatment	FU month
M87	Anemia	7.6	-ve	++	320	DLBCL	R-COOP	6, died
F72	Anemia, Acrocyanosis	6.8	-ve	+++	>2560	IgM MGUS	Clorambucil, R	37, alive
F50	Acrocyanosis	7.6	-ve	+++	>2560	Idiopathic	Chlorambucil	17, alive
M69	Anemia	7.8	+++	++++	>2560	Idiopathic	Chlorambucil, R	24, alive
M74	Anemia	4.7	-ve	++	320	AdenoCa of colon Bence-Jones proteinuria	Chlorambucil, surgery	3, alive

Diagnosis of cold agglutinin disease was established in each case by the demonstration of positive direct antiglobin test (DAT) for the presence of bound complement and an elevated cold agglutinin titre, which ranged from 320 to >2560 dilutions. Workup of the underlying condition revealed malignancy in three patients - lymphoma in one patient, adenocarcinoma of colon in one, and IgM MGUS in another. In two patients, no associated condition was detected. Bone marrow biopsy was performed in all patients, which showed erythroid hyperplasia without abnormal infiltration. All except one patient received chlorambucil resulting in decrease in transfusion requirement. Rituximab was given in two patients with good response. *Conclusions.* Cold agglutinin disease is an uncommon cause of haemolytic anaemia among Chinese. For patients without evidence of mycoplasma infection or infectious mononucleosis, and for patients whose cold agglutinin is monoclonal, workup for underlying malignancy with such investigations as urine and serum protein electrophoresis/immunofixation, bone marrow biopsy, and computed tomography should be considered. In addition to avoidance of cold exposure, the use of alkylating agent chlorambucil and immunotherapy with anti-CD20 are effective treatment options. Larger studies with longer follow up are required to study the epidemiology, clinical features and therapy outcome in Chinese.

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COMPLETE RESPONSE TO RITUXIMAB OF AN ACQUIRED PURE RED CELL APLASIA ASSOCIATED WITH HAEMOLYTIC ANAEMIA REFRACTORY TO IMMUNOSUPPRESSIVE TREATMENTS

P. Niscola,[1] L. Scaramucci,[1] D. Piccioni,[1] M. Giovannini[2], A. Tendas[2], L. Cupelli[2], T. Dentamaro,[1] G. Natale[2], A. Perrotti,[1] P. de Fabritiis[2]

[1]Sant'Eugenio Hospital, ASL Roma C, ROMA; [2]Hematology, Sant'Eugenio Hospital, Tor Vergata University, ROME, Italy

Aims. Acquired pure red cell aplasia (PRCA) simultaneously associated with autoimmune haemolytic anaemia (AIHA) has been very rarely reported so far. We describe the case of an elderly woman affected by idiopathic PRCA associated with AIHA which was unresponsive to two lines of immunosuppressive treatment and then received rituximab given as salvage therapy. *Case Report.* A 68-years-old woman was admitted because of severe malaise, pallor and fatigue. Her past medical history was unremarkable. Physical examination was unremarkable, with the exception of pallor and mild jaundice. She exhibited severe normochromic and normocytic anaemia (haemoglobin = 2.7 gr/dL). Reticulocytes were not detectable; white blood cell and platelet counts were normal. On the blood film, spherocytes and polychromasia were found. Haemolytic parameters were increased; serum haptoglobin was undetectable. Direct and indirect Coombs tests were positive. A warm-reactive antibody of the IgG isotype was eluted from the red blood cells (RBC). Serologic tests for parvovirus B19, HIV, Hepatitis virus, CMV and EBV were negative. Chest x-ray and total body CT scan were normal. A bone marrow (BM) aspirate and a trephine biopsy demonstrated normal representation of myeloid and megakaryocytic precursors, but nearly absent erythroid precursors. Basing on these findings, a diagnosis of AIHA with PRCA was made. Given the symptomatic anaemia and the very low haemoglobin levels, the patient received repeated administra-

tion of RBC concentrates without any reactions. Methylprednisolone, which was given at dose of 2 mg/kg for 4 weeks was ineffective, so that treatment with cyclosporine-A (CSA) was added during the following g 4 weeks but, unfortunately, without any benefit. Therefore, the patient was offered rituximab as salvage therapy for which she gave her informed consent. Rituximab was administered intravenously at the dose of 375 mg/m^2 as a 4-hour infusion, once weekly for a total of 4 doses, without any adverse reaction or side effects (July 2007). Progressive increase of haemoglobin levels and the achievement of transfusion independence followed the rise in reticulocytes after the fourth dose of rituximab. Serum haemolytic parameters normalized, as well. A BM aspirate demonstrated the full recovery of the erythroid matrix. The patient, 8 months after rituximab therapy, shows normal haemoglobin and reticulocyte levels, and she is no longer received immunosuppressive therapy. *Summary.* Our report concerns the case of a PRCA associated with an AIHA, very exceptionally observed as primary and idiopathic condition in the elderly. Treatment of these conditions usually employs immunosuppressive drugs, mainly steroids and CSA, or immunomodulating agents. However, when conventional immunosuppressive treatments failed, salvage treatments are needed. In our experience, the patient achieved a late haematological response to this agent, outlining this finding no early effects by rituximab, for which it should be attributable to the B-cells ablation induced by the anti-CD20 therapy. The course of response gave indirect demonstration of the immunomediated origin of the two associated disorders. Therefore, our case, confirming few similar observations so far reported, suggests a role of rituximab for treatment of patients with antibody-mediated haematological disorders refractory to conventional immunosuppressive treatments.

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SAFETY AND EARLY EFFICACY OF THE TERMINAL COMPLEMENT INHIBITOR ECULIZUMAB (EC) IN A CHILDHOOD PATIENT WITH PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA (PNH)

G. La Barba,[1] A. Sau,[1] S. Pulini,[1] P. Salutari,[1] A. Spadano,[1] F. Fioritoni,[2] A.M. Risitano,[3] G. Fioritoni[2]

[1]Department of Hematology Spirito SantoHospital, PESCARA; [2]Department of Hematology/Spirito Santo Hospital, PESCARA; [3]Federico II University, NAPLES, Italy

Background. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hemolytic anemia, caused by a deficiency of glycosylphosphatidylinositol (GPI) anchored proteins on the haematopoietic cells, characterized by intravascular hemolysis, fatigue, thrombosis, poor quality of life, transfusion dependency and bone marrow failure. Onset usually occurs in adulthood. Few children and adolescents with PNH have been described and laboratory data on diagnosis, clinical course, survival and therapy in young patients are unavailable. Eculizumab (EC) is a novel humanized monoclonal antibody targeting the complement fraction 5. Overall results from two Phase III clinical trials (TRIUMPH and SHEPHERD) show that the terminal complement inhibitor EC significantly reduces hemolysis resulting improvement of anemia, fatigue and quality of life. *Aims.* We tested safety and clinical efficacy of EC in a childhood patient with PNH. *Methods.* A 18-year-old girl referred astenia, tachycardia and emission of dark urine. Laboratory data showed severe anemia (Hb 8 g/dL) with reticolocytosis (200.000/mL), lactate dehydrogenase about 2200 U/L, reduced seric haptoglobin (<0.07 g/L); urine analysis resulted highly positive for blood dipstick test while showing intense hemoglobinuria. The young girl presented periodic hemolytic crisis with worsering quality of life while needing blood transfusions. Flow cytometry analysis with anti CD59/CD55/CD66b antibodies demonstred a large cellular population of granulocytes (98%) and erythrocytes (87%) with no detectable expression of GPI-anchored proteins. So a PNH diagnosed has been made; given the transfusion dependence, a treatment by eculizumab was considered. The patient was vaccinated against Neisseria meningitidis. Then, she received infusion of EC as follows: 600 mg intra venous every 7±1 days for four weeks, 900 mg one week later and then 900 mg every 14±1 days for a total of 11 weeks of therapy. Clinical and biochemical indicators of hemolysis were measured weekly. *Results.* No infusion-related effect or adverse event have been observed to date; clinical benefit from EC was sustained throughout the treatment period. After starting eculizumab therapy, hemolysis rapidly improved as shown by a 78% decrease of lactate dehydrogenase (LDH) levels (from a median of 2130 U/L before treatment to 460 U/L at 11 weeks). The patient became transfusion-independent, and Hb levels increased from baseline stabilized at 11 g/dL in the absence of transfusions. Eculizumab was well tolerated. Throughout therapy no episodes of hemoglobinuria, dysphagia, headache or abdominal pain were recorded and the quality of life improved significantly. *Summary and Conclusions.* Beneficial effects of EC in PNH are observed in adult patients but few data on therapy in young patients have been described. In this report eculizumab appears to be well tolerated and early effective in a young patient improving her hemolysis, hemoglobinuria, the need of blood transfusions and the quality of life. This report suggests that EC treatment may be extended to younger patients; however, further larger studies are needed to test the safety and efficacy of EC in children.

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COMAPRISON OF CARDIAC FUNCTIONAL PARAMETERS IN SICKLE THALASSEMIA AND THALASSEMIA INTERMEDIA

A. Taher,[1] A.H. El Chafic,[1] H. Ismaee'l,[1] A. Inati[2], S. Alam,[1] R. Daher,[1] P. Jallad[1]

[1]AUB-MC, BEIRUT; [2]Rafik Hariri Hospital, BEIRUT, Lebanon

Introduction. Mild forms of hereditary hemoglobinopathies such as sickle-beta thalassemia (ST) and thalassemia inremedia (TI) might show acceptable hemoglobin levels and in many cases are transfusion independent. Although these mild variants are relatively well tolerable, the mild anemic state can result, over many years, in multiple system damage one of which is the cardiovascular system. *Aims*. In this study, the cardiac function of patients with ST was compared to that of TI using conventional pulse wave Doppler (PWD) echocardiography as well as tissue Doppler imaging (TDI). Amino terminal pro-brain natriuretic peptide (NT-proBNP) was also compared among the ST, TI, and healthy individuals. The relationship between cardiac function indices and hematologic variables (NT-proBNP, ferritin, and hemoglobin) was evaluated. *Methods.*Patients were referred to an outpatient clinic for transthoracic resting PWD echocardiography and TDI. Blood samples were collected from all patients to measure NT-proBNP, creatinine, hemoglobin, and ferritin levels. In addition, 33 healthy individuals were evaluated for comparison of NT-proBNP. Results were considered statistically significant when $p<0.05$. *Results*. Twenty seven patients with ST and nineteen patients with TI were studied. Thirty three healthy individuals serving as controls for standardization of creatinine and NT-proBNP were also included. Those with pulmonary hypertension (PHT) in the ST group were significantly of an older age; while those in the TI group showed a significant linear correlation between pulmonary artery systolic pressure (PASP) and ferritin level. TI patients were found to have a significantly higher E/Ea ratio and deceleration time. Left ventricular end diastolic diameter index (LVEDD/BSA) was elevated mildly and similarly in both groups, however, it is significantly and strongly correlated with hemoglobin (Hb) in ST while significantly correlated with E/Ea in TI. NT-proBNP in TI patients was significantly higher than controls, in contrast to ProBNP in ST patients which couldn't reach statistical significance in comparison with that of controls. ProBNP levels in TI patients were even much higher than that of ST patients with borderline significance ($p=0.078$) knowing that both groups having similar age, gender, and creatinine levels. NT-proBNP was significantly correlated with Hb in both patient groups. It was also significantly correlated with age in ST group but to E/Ea independent from age in TI group (r=0.502, $p=0.040$). Serum ferritin of TI patients (but not ST patients) correlated significantly with PASP (r=0.649, $p=0.012$) independent from age and Hb and was significantly higher than that of ST patients. *Conclusions.* We have found that TI and ST patients of relatively similar age, gender distribution and Hb levels have similar prevalence of PHT and no significant difference in mean PASP. In TI, we showed that left ventricular filling pressure (E/Ea) and iron load (ferritin) are correlated with ventricular geometry (LVEDDi) and PASP respectively; while in ST, anemia (Hb) and an age-related effect are the respective dominant factors. However why a similarly aged and anemic group (ST) does not have a similar set of correlations is a question with no known answer to us.

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ERYTHROCYTE MEMBRANE CHOLESTEROL CONCENTRATION IN PATIENTS WITH MALIGNANT NEOPLASMS RECEIVING OR NOT CHEMOTHERAPY

K. Lempesopoulos,[1] X. Tsarouhas[2], S. Pagonis,[1] S. Savvanis[2], L. Kavallierou,[1] A. Yalouris[2]

[1]A. Fleming, MELISSIA; [2]Elpis, General Hospital, ATHENS, Greece

Background. Cholesterol is a major component of the cell membrane. It plays an important role in its physiology affecting vital properties, such as membrane fluidity, cation transport, cell receptors, osmotic resistance e.t.c. Abnormal conditions that change serum cholesterol concentration can also alter erythrocyte membrane cholesterol concentration (EMCC) possibly resulting in differentiation of several membrane functions. *Aims.* To investigate whether changes in serum cholesterol concentration, usually observed in patients with malignancy, affect EMCC. Patients and *Methods.* Thirty five subjects (24 men and 11 women, age: 39.45±10.85) and 38 patients (25 men and 13 women, age: 68.76±12.59) with solid malignant tumor were studied. Their primary malignancy was located in breast in 9 patients, prostate in 8, colon in 7, lung in 3, liver in 3, bladder in 3, stomach in 2, pancreas in 2, and skin (melanoma) in 1. Fifteen of them had received no chemotherapy, while 23 were during the course or had recently finished chemotherapy. Serum total cholesterol concentration and EMCC were measured in all studied subjects. *Results.* Serum cholesterol was significantly lower in patients during/after chemotherapy (145.69±24.23) as compared to both controls (216.48±21.18, p=0.01), and patients without chemotherapy (211.33±33.06, p=0.01). EMCC was significantly higher (p=0.01) in patients without chemotherapy (174.01±31.34), and significantly lower (p=0.01) in patients during/after chemotherapy (73.23±14.28) as compared to controls (145.36±17.05). In six patients studied before and during chemotherapy the mean decrease of EMCC was 52.35%. *Conclusions.* Patients with malignancy have significantly higher EMCC than the controls although their serum cholesterol values do not differ. Chemotherapy seems to decrease both concentrations but mainly EMCC, resulting in a considerable depletion of erythrocyte membrane from its structural component. The possible consequences of this depletion to cell physiology have to be investigated.

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SUBOPTIMAL DOSES OF DEFERIPRONE IN THE TREATMENT OF REGULARLY TRANSFUSED THALASSEMIA MAJOR PATIENTS

M. Hadjigavriel

Thalassemia Center, Limassol Hospital Cyprus, LIMASSOL, Cyprus

Background. A small number of regularly transfused beta thalassemia patients are treated with suboptimal doses of the oral chelator Deferiprone, although there are no clinical trials that have established the efficacy of these doses. *Aims.* The objective of this study was to evaluate the efficacy of chelating treatment in a group of regularly transfused beta thalassemia patients treated with less than the standard (75mg/kg/day) dose of Deferiprone. *Methods.* 8 regularly transfused beta thalassemia major patients treated with suboptimal doses of deferiprone (less than 75 mg/kg/day, divided into three doses), were assessed for their chelation treatment and outcome after a mean follow up period of 4 years. *Results.* A group of 8 transfusion dependent thalassemia patients (5 male, 3 female), mean age 40 years (range 36-47) were treated with deferiprone monotherapy for a mean period of 4 years (range 2-5). Mean annual blood consumption of the patients during that period was147 ml/kg/body weight (range 123-166). All but one patient were chelated with combination of deferiprone and desferrioxamine, and 1 patient with s.c desferrioxamine prior to start deferiprone monotherapy. All patients had low levels of serum ferritin when they started deferiprone. Mean baseline serum ferritin was 259 ng/mL (range 85-687). Mean dose of deferiprone during observation period was 63 mg/kg/day (range 52-72). Mean serum ferritin at the end of the follow up was 214 ng/mL (range 123-356). Body iron stores during the observation period were assessed also by cardiac and liver MRI (T2 and T2*). One patient presented with abnormal baseline cardiac MRI (T2: 23.6msec - moderate iron deposition) and one patient with abnormal liver MRI (T2<18msec - severe iron deposition). Cardiac T2* at the end of the follow up was normal in all patients, meanT2* 36,4 msec, (range 25-44). Liver T2* was normal in all but 2 patients (T2* 4.3 and 3.7 msec respectively - mild iron deposition). All patients were free of haemosiderosis related events. Urinary iron excretion in this group of patients was difficult to be interpreted. *Conclusions.* Suboptimal doses of Deferiprone were given to some regularly transfused beta thalassemia major patients with very low levels of serum ferritin. These doses were not associated with an increased accumulation of body iron, as assessed by serum ferritin and MRI.

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ABSENCE OF MUTATIONS AT HAMP AND HEMOJULELIN GENES IN HFE-RELATED HEREDITARY HEMOCHROMATOSIS

F.A. Gonzalez,[1] C. Bieza[2], E. Arroyo[2], P. Ropero,[3] P. Baquero,[4] M.S. Mesa,5 A. Villegas[1]

[1]Hospital Clinico San Carlos, MADRID; [2]Facultad de Medicina, MADRID; [3]Hospital Clinico San carlos, MADRID; [4]Centro Superior de Investigaciones Cientificas, MADRID; [5]Facultad de Biología, MADRID, Spain

Background. Heredity Hemochromatosis is one of the more frequent recessive traits in Europe. Among its symptoms we find iron overload wich causes several liver, heart, and endocrine complications. Over 80% of the cases are produced by the C282Y mutation in homocygosis at HFE gen, and to a lesser extent, by this mutation in association with other mutations, like H63D, of the same gene. However a very little proportion of subjects carrying these mutations eventually suffer of significant iron overload so other genetic and environmental factors may be playing an important a role in this pathology. Some studies recently show that most of the patients present low levels of HAMP protein, being described some cases of juvenile HH caused by mutations at HAMP and Hemojuvelin gens. *Aims.* The aim of this study was to asses the existence of mutations at HAMP and Hemojuvelin gens in patients which were sent to our laboratory suffering from iron overload, as well as to find if these mutations could have an influence on the overload level. *Materials.* 101 patients presenting iron metabolism overload pattern (ferritin> 350 ug/dL or IST>45%) or relatives of them, with the following features: 25 were C282Y homocygotes, 31 double heterocygotes C282Y/H63D, 31 H63D homocygotes and 35 with no C282Y or H63D mutations. *Methods.* Mutations were searched in HAMP gene by SSCP (Single Stranded Conformation Polymorphism) in an ALF Sequencer. The four exons of Hemojuvelin gene were sequenced in a 310 ABI PRISM Genetic Analyzer. *Results and Conclusions.* There has not been found any alteration at HAMP and Hemojuvelin genes. Even though it is believed HAMP plays a significant role in iron homeostasis, finding its levels reduced in most cases of HH, it does not seem this reduction may be caused by mutations at HAMP and Hemojulelin genes.

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THALASSEMIA AND OTHER HEMOGLOBINOPATHIES IN MACEDONIAN CHILDREN

S. Glamocanin,[1] O. Muratovska,[1] K. Martinova,[1] Z. Trajkova-Antevska,[1] B. Conevska,[1] S. Koceva,[1] G. Efremov[2]

[1]University Children's Hospital, SKOPJE; [2]Research Center for Genetic Engineering and Biotechnology, SKOPJE, Macedonia

Background. β-thalassemia is a heterogeneous, inherited disease resulting from reduced or absent synthesis of the β-globin chain of hemoglobin. The results of five population surveys made in Macedonia in the period between 1966 and 1994 with total number of 22.136 screened subjects showed the incidence of β-thalassemia and other hemoglobinopathies in Macedonia is 3,8%, (0.7-12.3%), with the highest incidence (5-20%, mean 9,6%) in the southeastern part of country. The aims of this study are: 1.Detection of thalassemia and other hemoglobinopathies in the selected group of children examined at the University Children's Hospital in Skopje with diagnosis anemia and genetic characterization of the identified variants. Evaluation of treatment and effects of treatment. *Methods.* From January 2000 until December 2007, total number of 125 new diagnosed children with β-thalassemia and other hemoglobinopathies were detected. 115 of them were β-thalassemia heterozygotes and ten patients from different unrelated families were new diagnosed homozygotes or compound heterozygotes. The diagnosis was based on accepted clinical, hematological and biochemical criteria for β-thalassaemia. The new non radioactive-based polymerase chain reaction (PCR) method was utilized for identification of mutations. Evaluation of patients was made accordingly to the Recommended Annual Comprehensive Care Checklist for thalassemia patients. *Results.* 27patients (male/female=14/13), aged between 1 to 30 years with thalassemia were treated at the University Children Hospital in Skopje. 21 patients were β-thalassemia homozygotes and six were Compound Heterozygotes with different mutations. Five patients were double heterozygotes β-thal/ Hb Lepore (IVS-I-1/Lepore) and one patient was double heterozygote β-thal/ Hb Knossos (IVS-I-6/Hb Knos-

sos) (G.D. Efremov, RCGEB-MASA). The most common alleles were IVS-I-110, IVS-I-1, and IVS-I-6. A total of 26 patients were Macedonian, only one was Albanian. All patients were on a regular blood transfusion regimen combined with chelation therapy. Standard transfusional therapy was aimed at maintaining the hemoglobin blood concentration ≥9.5 g/dL. Chelation therapy was given according to following schedule: Desferrioxamine (DFO) 40 mg/Kg/day intravenously (i.v) two days after transfusion received 18 patients, (Group A); DFO subcutaneously by pump 40 mg/Kg/24hr, 5 days/week received 3 patients, (Group B). A combined chelation therapy protocol was introduced using Desferrioxamine 40 mg/Kg/day i.v for 2days/week and Deferiprone 75 mg/kg/day orally 5days /week (Group C). All patients were seronegative for hepatitis C. Eight patients in group A, three in group B and three in group C had been splenectomised. The results show that two chelation treatments, Group B and C, cause a similar reduction in serum ferritin. The decline in growth velocity and delay in bone age were more significant in patients older than 9-10 years. Low peak GH response was found in all three patients that underwent stimulation test. Eleven of sixteen patients older than 15 years had pubertal failure. Cardiopathy was registered in 15/27 patients. *Conclusions.* Population surveys for detection of β-thalassemia heterozygotes may establish preventive programs and reduce the incidence of β-thalassemia. Good managing of blood transfusion regimen combined with chelation therapy may reduce late complications.

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PURE RED CELL APLASIA (PRCA) IN A PATIENT AFFECTED BY REFRACTORY ANAEMIA DURING ALFA-EPO TREATMENT

A. Luraschi,[1] S. Montanara,[1] P. Fedeli,[1] P. Buscaglia,[1] S. Cozzi,[1] A. Castello[2]

[1]Verbania Hospital, VERBANIA; [2]PAVIA, Italy

Pure red cell aplasia(PRCA) during R-EPO treatment is a rare condition generated by erythropoietin induced antibodies and described in patients with chronic kidney disease during treatment with alfa-EPO subcoutaneous administered. Diagnosis of PRCA induced by EPO-antibodies requires two confirmatory investigations:bone marrow examination and demonstration of anti-EPO antibodies in patient's serum. Recently two cases of PRCA have been described in two patients with normal renal function affected by myelodisplastic syndrome treated with alfa-EPO and beta-EPO. In both cases bone marrow aspiration revealed the absence of erythroid precursors and in serum samples the presence of anti-EPO antibodies was demonstrated by immunoprecipitation assay. We describe one more case of PRCA in a patient with refractory anaemia treated with alfa-EPO. On november 2005 the patient,a 76 years old man affected by psoriasis,with normal renal function,presented normocitic anaemia and low erythropoietin level . Examination of bone marrow revealed a norma cellularity with erythroid hyperplasia and displasia;no ringed sideroblasts. On january 2006,due to a reduction in the haemoglobin level,he started alfa-EPO 150U/kg administered subcutaneously three times a week; Hb level improved to 11 gr/dL. Ten months later,the patient's haemoglobin suddendly decreased (4.2 gr/dL)with low reticulocite count.Bone marrow biopsy showed histology consistent with pure red cell aplasia. Viral infections(in particular parvovirus B19 and hepatitis B virus) were excluded and a chest TC scan excluded thymoma. In patient's serum samples the presence of anti-EPO antibodies was demostrated (Lab. Pasteur-Paris and CLIA immunochemistry laboratory-Richmond USA). Treatment with alfa-EPO was immediately stopped and patient received transfusion and steroid therapy. PRCA generated by erythropoietin-induced antibodies is not restricted to renal insufficiency patients. In patients with low risk myelodisplasia a suddenly decreased in haemoglobin level during EPO-treatment ,requires careful invastigation because it is not always connected with disease progression but may due to this rare and severe complication.

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DATA FROM THE REGISTRY OF THE PATIENTS WITH MYELODYSPLASTIC SYNDROME OF A ROMANIAN SINGLE CENTER. III ANALYSIS OF THE GROUP UNDER 50 YEARS OLD

R. Gologan, D. Vasilache, D. Georgescu

Fundeni Clinical Institute, BUCHAREST, Romania

Introduction. The myelodysplastic syndrome (MDS) is mostly observed in people older than 65 years and therefore there are few reports refering to patients less than 50 years old. However, the interest for this group of MDS patients is increasing because: they are leading an active life and therefore their disease have higher socio-economic consequences, their comorbidities are much more rare, there are important ethnic differences in frequency between western and extreme eastern countries, the age-related *genetic instability* could not be incriminated in the pathogenesis of their MDS, they are the main candidates for aggressive treatments (high doses chemotherapy, bone marrow transplantation). *Patients and Methods.* The cases with age under 50 years were extracted from the data-base of the MDS Registry of the Clinic of Hematology, Fundeni Clinical Institute, Bucharest, Romania comprising 404 primary cases, collected between 1982 and 2004. The registration form, using the FAB classification, was kindly provided by MDS Foundation (USA)(Chairman Prof. J.M. Bennett). The parameters included in the analysis were: age at presentation, sex, place of residence, values of hemoglobin, neutrophils, platelet count, neutrophil count, percentage of bone marrow blasts, prognostic scores, acute leukemia (AL) transformation. The frequency of different subtypes of MDS and the dynamics of the new cases during the analysed period were also determined. A comparison with the group of age above 60 years and with other similar reference studies was performed. *Results.* There were 66 (16,7%) cases from which 22 (62,6%) were under 40 and 19 (28.3%) under 30 years old, with a mean age of 34,7 years. A global predominance of the feminine gender and of the urban location with no geographic aggregation could be noticed. The mean values of hemoglobin, neutrophils and platelets were 7.6 g/dL, 2,500/μL and 142.000/μL, respectively. Patients with refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) accounted for 46.7% of all cases (RA 34,8%, RARS 11,9%), refractory anemia with excess of blasts (RAEB) 20,8%, RAEB in transformation (RAEB-T) 13,4%, chronic myelomonocytic leukemia 4,4% and unclassified 13,4%. The annual number of new cases increased three times during the analysed period, the increase being not constant, with a peak in 2000, and not uniform. The subtypes with the most important increase in time were RA and RAEB-T. The AL transformation could be registered in 25.7%, after a mean time of 5 months. *Conclusions.* This study indicates a higher proportion (16.7%) and a lower age (34,7 years) of young patients with MDS in Romania comparatively with western reports, considered almost in the middle among those reported for extreme western and eastern countries The tendancy of the annual frequency of the new cases indicated a three times increase. An urban predominant location, in contrast with that of the group above 60 years old, has been noticed. The female predominance appears as a characteristic feature of the patients with MDS from this group of age. The degree of anemia was obviously more severe than that reported in other studies.

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EXPRESSION ANGIOTENSIN-CONVERTING ENZYME (ACE, CD143) AND SHAPERONS BIP, CALNEXIN, CALRETICULIN BY LEUKEMIC DENRITIC CELLS IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS

V. Galtseva,[1] E. Pashin,[1] N. Parovichnikova,[1] A. Vorobiev,[1] M. Danilov[2], B. Sudaricov,[1] B. Sudaricov,[1] G. Savchenko[1]

[1]National Research Center for Hematology, MOSCOW, Russian Federation; [2]University of Illinois at Chicago, CHICAGO, USA

Background It has been shown that dendritic cells (DC) play a key role in the induction of adoptive immune response. AML blast cells can be induced to differentiate into leukemic dendritic cells (LDC), which are quite similar to monocytes derived DC by the expression profile of integrins and co-stimulatory molecules. LDC differ from DC by the absence of surface ACE expression on LDC in contrast to high level of ACE on DC. *Aims.* We propose that the absence of surface ACE expression in LDC is due to the block of normal ACE transport to the cell surface, normally controlled with shaperons. To confirm this hypothesis we quantified the level of intracellular and surface ACE in LDC and DC as well as mRNA expression levels of shaperons Bip, Calnexin, Calreticulin in DC and LDC. *Methods.* Blood samples were collected from 12 AML patients at diagnosis before induction chemotherapy and 9 healthy donors. Mononuclear cells were isolated using gradient centrifugation with Ficoll-Paque and were differentiated into dendritic cells by culturing with 180 ng/mL calcium ionophore for 4 days at 37 and 33°C (supposed to be activation factor for shaperons' induction). DC were stained for surface and intracellular ACE using two mAbs - 1D8 and 9B9 to be analyzed by flow cytometry, simultaneously the mRNA expression levels of ACE and shaperons Bip, Calreticulin, Calnexin were evaluated with RT PCR. *Results*. The surface ACE expression of LDC cultured at 37°C was 2.89±2.47% for 9B9 and 2.06±1.79%for 1D8, and was increased up to 49.8±15.4% for 9B9 and 38.2±18.4% for 1D8 with low-

ering culture temperature to 33C. The surface ACE expression of DC cultured at 37C was 48±9.85% for 9B9 and 4.8±2.5% for 1D8 , at 33C-34.9±4.7% for 9ɜ9 and 5.5±3.6% for 1D8. The intracellular ACE expression of LDC cultured at 37C was 64.2±12.7% for 9'9 and 56.15±15.8% for 1D8, and was lowered to 13.7±9.7% for 9B9 and 5.42±5.58% for 1D8 with lowering culture temperature to 33C. The intracellular ACE expression of DC cultured at 37C was 35±25% for 9B9 and 19.06±14.59% for 1D8, and was increased up to 62.2±27.3% for 9B9 and 37.04±11.58% for 1D8 with lowering culture temperature to 33C. The ACE mRNA expression levels measured with RT PCR in LDC and DC cultured at 37°C and 33°C haven't been changed and remained nearly the same 0.2% according to TB mRNA expression normalization. Bip, Calreticulin, Calnexin mRNA expression levels measured in relative units in LDC cultured at 37°C were lower than that of 33°C: 2.6, 2.3, 1.9 and 5.1, 2.4, 2,4 correspondingly. Bip, Calreticulin, Calnexin mRNA expression levels measured in relative units in DC cultured at 37°C also were lower than that of 33°C: 4.5, 1.8, 1.9 and 6.3, 2.1, 2.3 correspondingly. *Conclusions.* The data demonstrate the block of ACE transport at normal human temperature 37°C to the cell surface of LDC and thus provide the evidence of the altered function of LDC. It was determined that LDC cultured at 33°C had the increased ACE expression on the cell surface compared with 37°C temperature schedule, which may be the result of its augmented transmembrane transport by means of shaperons Bip, Calreticulin and Calnexin, which mRNA expression levels were also increased under 33°C cell shock culture conditions.

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NETRIN-1 DEPENDENCE RECEPTOR PATHWAY IN ACUTE MYELOID LEUKAEMIA

M. Dumontet,[1] M. Delloye[2], S. Brunet Manquat,[1] P. Mehlen[2], A. Bernet[2], C.M. Dumontet[1]

[1]*INSERM, LYON;* [2]*CNRS 523,8 LYON, France*

Background. Netrin-1 mediated pathways have been shown to be involved in cell survival. Alteration of these pathways has been shown to result in the occurrence of neoplasia in animal models. However the expression and function of netrin-1 and netrin-1 dependence receptors remain unexplored in haematological malignancies. *Aims.* to determine the level of expression and function of netrin- receptor components in AML *Methods.* We have analysed the level of expression of netrin-1 and netrin-1 receptors DCC, UNC5A and UNC5B in human acute myeloid leukaemia lines and fresh acute leukaemia cells. Q-PCR analysis of netrin-1 and netrin-1 receptors was performed on 11 acute leukemia lines (K562, HEL, Kasumi, KG1a, SC1, MOML13, MB4, JEKO1, U937, SUPB15, BV173) and 19 AML samples. The effect of a binding peptide (DCC-5Fbn) on fresh human AML cell survival was evaluated by annexin V staining. *Results.* Netrin-1 was found to be weakly expressed in AML lines comparison to solid tumors, the strongest expression being found in Kosumi and HEL lines. DCC expression was greatest in NB4 and HEL, UNC5a expression in HEL and NB4 and UNC5b expression in K562 and NB4. Expression of netrin-1 and netrin-1 receptors was heterogeneous amongst clinical samples, including some samples with levels significantly greater to those observed in cell lines. Alteration of binding of netrin-1 to its receptors by a titrating polypeptide (DCC-5Fbn) can be expected to induce cell death in cells with functional receptors as shown in the metastatic breast malignancy (Fitamant *et al.*, PNAS, 2008, in press). To test this hypothesis and to analyse the functionality of netrin-1 pathways in AML cells, fresh human AML cells were exposed for 24 hours to recombinant DCC-5Fbn, *in vitro* and analyzed for induction of apoptosis by annexin V staining. DCC-5Fbn was found to induce significant apoptosis in a fraction of human AML samples. *Conclusions.* Overall these results suggest that the netrin-1 dependence receptor pathway is present and functional in a subset of patients with acute myeloid leukaemia and might be used as a therapeutic target in this context.

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TRANSIENT LEUKEMIA IN DOWN SYNDROME NEWBORNS

S. Pulini, D. Carlino, G. La Barba, A. Sau, D. Onofrillo, C. Passeri, A. Natale, R. Di Lorenzo, G. Fioritoni

Department of Hematology, PESCARA, Italy

Background. Transient leukemia (TL), also known as transient abnormal myelopoiesis (TAM), occurs in approximately 10% of Down syndrome (DS) newborns. At presentation many infants could have only an incidental finding of blast cells in the peripheral blood, without clinical problems. In approximately 20% of cases the disease may be very severe, with hydrops foetalis, effusions or multiple organ failure and death. Despite its typical transient nature, 20-30% of TL-DS patients develop a non transient acute myeloid leukemia (AML-DS), usually within the first 4 years of age, and typically of megakaryoblastic or erythroblastic FAB phenotype. *Aims.* We report our recent cases of TL-DS of newborns, looking at their clinical presentation and the subsequent follow up. An important question is what are the determinants of its evolution in AML. *Methods.* Two 2-day newborns presented leukocytosis (90000/microl and 70000/microl respectively), in the second with anemia and thrombocytosis. In the peripheral blood films (40% and 70% respectively) and then in bone marrow, large cells with prominent nucleoli, basophilic cytoplasma with few granules were present. The immunophenotype of these blasts cells from the first neonate was characterized by the expression of CD33, 34, 117, 71, 7 while myeloperoxidase (MPO) was negative; in the second infant CD13, 33, 34, 117, 56, 7 and MPO were positive, HLA-DR negative. We have looked for molecular abnormalities: BCR/ABL, AML1/ETO, CBFβ/MYH11 and DEK/CAN were negative. Chromosome analysis showed a constitutional trisomy 21 without any other clonal abnormality. The little patients presented both congenital heart malformations. *Results.* The two newborn didn't receive any chemotherapy. Leukocytosis and percentage of blast cells disappeared during the first two months.At 10 and 33 months respectively they are in complete remission, without any signs of TAM. *Summary and Conclusions.* Blast cells in DS patients often show megakaryocytic antigens (CD41, CD42b, 61), but this expression is variable; the electron microscopy with immunogold labeling of CD61 would be very important, since always positive. Moreover they usually expresses C-Kit, like our cases did. TL-DS resolves spontaneously in the 60% of cases within 3 months; the most frequent causes of early death (20% of cases) are multiorgan failure associated with high leucocyte count, preterm delivery, liver fibrosis, effusions, coagulopathy, bleeding diatheses, cardiac and other congenital defects. The TL-DS newborns presenting with thrombocytopenia and pleural effusions seem to have a higher risk of subsequent AML-DS. The latter is probably related to somatic mutations of the gene encoding the hematopoietic transcription factor GATA1. According to recent pathogenic models, the GATA1 mutations constitute an early event and AML-DS could arise from latent TL clones surviving some months after an apparent spontaneous complete remission. Recent studies propose a screening for the presence of GATA1 mutation in DS neonates to identify children at more risk of severe complications. Someone suggests chemotherapy with low dose cytarabine, to improve prognosis, in infants with high leukocytosis, severe thrombocytopenia or liver dysfunction. Our two cases have clinical, biological and cytogenetic aspects of a good prognosis.

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EXTRAMEDULLARY MYELOID CELL TUMORS IN ACUTE MYELOID LEUKEMIAS - COULD IMMUNOPHENOTYPIC MARKERS PREDICT THEIR OCCURRENCE AND OUTCOME? ANALYSIS OF A SMALL GROUP OF PATIENTS

I. Voican, A.M. Vladareanu, H. Bumbea, S. Radesi, D. Cisleanu, M. Begu, C. Ciufu, V. Vasilache, C. Marinescu, A. Nicolescu, M. Dervesteanu, M. Onisai, R. Bucovanu, A.M. Vintilescu

Emergency Universitary Hospital Bucharest, BUCHAREST, Romania

Background. Extramedullary myeloid cell tumors (EMT), also known as granulocytic sarcoma, chloroma or myeloblastoma, are malignant tumors of myeloblasts and granulocytes in different stages of maturation. Their development represents a poor prognostic factor and correlates with a short survival. *Aims.* To identify particular characteristics for EMT occurrence in *de novo* and secondary AML and the correlation between immunophenotypical markers and different sites of leukemic involvement using a large panel of immunophenotypical markers: CD14, CD33, CD34, CD15, HLADR, CD65, CD117, CD38, CD7, NG2, MDR, CD9, CD13, CD45RO, CD4, CD56. *Methods.* We present a retrospective study on 16 consecutive cases with *de novo* (7cases) secondary (9cases) AML, admitted to our department between 01.03.2005 and 31.12.2007 and diagnosed according to WHO criteria. Immunophenotypical markers used were: CD14, CD33, CD34, CD15, HLADR, CD65, CD117, CD38, CD7, NG2, MDR, CD9, CD13, CD45RO, CD4, and CD56. *Results.* We analyzed 16 patients (6 females and 10 males) with a median age of 56 years, range [18-78]. The following morphologic types were identified: AML0 - 1case, AML1 - 2cases, AML2 - 7cases, AML4 - 2cases, AML5 - 2cases and acute leukemia with eosinophils - 2 cases. The EMT locations were: skin (1), lymph nodes (6), gastrointestinal tract (4), central nervous system (2), bone (1), synovia (1), muscle (2) and thyroid gland (1). All the affected sites had histopathological and immunochemistry confirma-

tion. Six patients presented unique determination of EMT; eight had multiple sites involving the same histological structure and in two cases EMT developed in organs with different histological structure. In four cases, the EMT preceded medullary involvement. No statistical correlation could be found between patients' age and the number of EMT sites (ro=0.10, p=0.71) as well as the particular EMT location (p>0.05). The fluorescence intensity of CD34 was correlated with age (ro=0.695, p=0.038). A weak correlation (due to the small number of cases) can be made between the muscular and the thyroid location (ro=0.681, p=0.05). We found no correlation between: age/sex and survival until EMT occurrence or AML type (*de novo* or secondary). No significant correlation was found between patients' survival reported to the moment of EMT occurrence and number or type of location or morphologic AML type. We found no significant correlation between immunophenotypical markers or the morphologic type and certain location of EMT, except for the two cases with neurological involvement with the expression of neural receptor CD56, probably due to the small number of analyzed cases. In our group, the overall survival reported to the EMT diagnosis was shorter in *de novo* AML compared to secondary AML. *Conclusions.* EMT can develop in patients with *de novo* or secondary AML, as first manifestation of the disease, preceding the onset in marrow and peripheral blood, or as late events in the evolution of the illness. In most of our cases, the EMT appeared after medullar involvement. The most common involved sites were: lymph nodes and gastrointestinal tract and rare and unusual location were thyroid gland and synovia. The neurological involvement could be associated with expression of neural receptor CD56.

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INCIDENCE OF JAK2 V617F TYROSINE KINASE MUTATION AND ITS CORRELATION WITH THROMBOSIS AND BLEEDING COMPLICATIONS IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISORDERS

Marton,[1] L. Iványi,[1] L. Kereskai[2], L. Pajor[2]

[1]Univ Teach Hosp Markusovszky, SZOMBATHELY; [2]Univ of Pécs, Fac Med, Inst Pathology,Lab Mol.Biol, PÉCS, Hungary

The JAK2 V617F tyrosine kinase mutation is present in various frequency in patients of Philadelphia-chromosome negative chronic myeloproliferative disorders (CMPD, i.e.polycythemia vera- PV, essential thrombocythemia- ET and primary myelofibrosis- PMF). The impact of this mutation on clinical phenotype is still debated. In myeloproliferative disorders a major cause of morbidity are thrombosis and bleeding. Interest is focusing on association between JAK2 V617F mutation status as a risk factor for thrombosis in the main CMPD subgroups. *Aims.* Of the study. In a retrospective survey was to evaluate possible relevance between JAK2 V617F mutational status and hematological abnormalities, particularly thromboembolism in patients with PV, ET and PMF. *Patients and Methods.* From DNA samples getting from peripheral blood or bone marrow the V617F mutation was detected by allele-specific.polymerase chain reaction and patients were genotyped by a DNA tetra-primer amplification refractory mutation assay. All CMPD patients were diagnosed by routine hematological methods and proved to be Philadelphia-chromosome negative by conventional cytogenetic analysis and molecular biological techniques. At time of diagnosis initial hemostatus (leukocyte-, platelet-counts, hgb-htc levels)LDH, age,sex, spleen size and thrombotic events as well as bleeding episodes during course of illness as variables were compared with JAK2 V617F mutation status, respectively. Statistical analysis was made by statistical software program for Windows. RESULTS.From June 2007 in 105 patients (pts) with CMPD diagnosed, treated and cared in our hematological centre (39 pts with PV, 52 with ET and 14 with PMF) JAK2 V617F allele burden screening was performed. Positive mutational status was detected in 31 PV pts (74.3%)associated with thrombosis/bleeding in 11 ones (11/31, 35.4%),whilst in 52 pts with ET among 35 JAK2 positive cases (67.3%) in 10 thrombotic/bleeding episodes could be registered (28.5%). In 14 pts with PMF JAK2 positivity existed in 6 with no thrombosis/bleeding events (42.7%). Arterial thrombosis, namely cardiovascular (myocardiac infarction), cerebral (stroke, ischemia) in ET, and venous (pulmonary or portal vein) in PV occured in rather equal frequency. However, life-threatening bleeding has not been appeared. JAK2 mutation and occurence of thrombotic events in ET and PV did not show any significant difference (28.5 *vs* 35.4%). Moreover, hematological and other variables mentioned above compared with JAK2 positivity and thrombotic episodes also did not show any significant connection. Despite a medium number of JAK2 positivity in PM, thrombosis was not observed at all. *Conclusions.* The incidence of JAK2 mutation our PV cases is slightly lower, in ET higher and corresponding in PMF compared with other literary data. In our series of CMPD cases the relatively low-intermediate incidence of JAK2 positivity in thrombotic patients suggests that there is no direct role of JAK2 mutation in development of thrombosis in PV and ET.

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ABDOMINAL VEIN THROMBOSIS ASSOCIATED WITH LUPUS ANTICOAGULANT AS THE FIRST CLINICAL FEATURE OF MYELOPROLIFERATIVE DISEASE

M. Jamrozek-Jedlinska,[1] A. Grzywacz,[1] K. Zawilska[2]

[1]J.Strus Hospital, POZNAN; [2]University of Medical Sciences, POZNAN, Poland

Background. Thrombosis occurring at young age and located at unusual sites is generally caused by inherited thrombophilia but other reasons should also be taken into consideration. *Case reports.* We present 3 cases (1 male, 2 female, aged 24-48) admitted to our department for thrombophilia testing because of idiopatic portal or splenic vein thrombosis. Inherited thrombophilia including: antithrombin-, protein C-, protein S deficiency, activated protein C resistance, factor V Leiden or protrombin gene G20210A mutation were not found. All cases presented with lupus anticoagulant and primary diagnosis of antiphospholipid syndrome has been established. Furthermore we extended laboratory procedures due to marginal erythrocytis (RBC 5.4-5.7) with microcitosis (MCV 76-80). Splenomegaly was of no value in differential diagnosis, as it could result from splenic or portal vein thrombosis. Platelet and leucocyte count were normal but this findings could be false because of hypersplenism. Histopatology of bone marrow revealed megakariocyte hyperplasia and fibrosis (+1). Molecular testing were positive for Janus kinase 2 (JAK 2) V617 F mutation (heterozygotic form) in all cases, suggesting myeloproliferative disease as the primary cause of thrombosis. Additionally paroxysmal nocturnal haemoglobinuria has been diagnosed in one case. *Conclusions.* Thrombosis requires detailed casuistic diagnostic procedures, as one risk factor detected could not exlude other ones, coexisting in examined case.

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COMPARATIVE ANALYSIS OF MESENCHYMAL STEM CELL CONTENT DETECTED BY FLOW CYTOMETRY IN UMBILICAL CORD BLOOD, G-CSF MOBILIZED PERIPHERAL BLOOD, ADULT AND PEDIATRIC BONE MARROW OF HEALTHY DONORS

M. Beksac,[1] H.E. Ersoy,[1] K.D. Dalva,[1] D.U. Uckan[2], S.M. Meriç,[1] M.S.B. Beksaç,[3] M.E. Ertem,[1] O.I. Ilhan,[1] M.B. Beksaç[1]

[1]Ankara University, ANKARA; [2]Hacettepe University department of Pediatrics, ANKARA; [3]Hacettepe University, Department of Obtetrics and Gynecology, ANKARA, Turkey

Background. Hematopoietic stem cells (HSCs) are present in human umbilical cord blood (UCB), bone marrow and G-CSF mobilized peripheral blood,all of which are regarded as valuable sources for cell transplantation and cell therapy. The content of mesenchymal stem cells (MSCs) in these sources have been documented by *in vitro* culture and *in vivo* transplantation models. Recently with the application of monoclonal antibodies against surface markers on MSCs-related antigens such as Stro-1, CD73, CD 90, CD 105 and CD29 have made quantification of MSCs possible. Aim of this prospective study was to standardize the flow cytometric immunostaining steps and antibodies by using the *in vitro* cultured MSCs as positive control and to compare the MSC percent in various sources of stem cell. *Methods.* These antibodies were used in immunophenotyping of MSC: GAM-PE (Serotec, UK), Stro-1 (R-D Systems, USA), CD44-PE (BD Biosciences, USA) ,CD45-FITC (Beckman Coulter, France),CD45-PC5 (BC), CD73-PE (BD Biosciences, USA), CD90-PC5 (BC), HLA-DR (mmunotech), CD34-PC5 (mmunotech), CD105-FITC (Serotec, UK), CD29-FITC (BC). Data obtained from 200.000 cells were acquired using the FC 500 flowcytometer system running CXP software (Beckman Coulter; Miami, CA USA). Human MSC cells were obtained following *in vitro* culture in DMEM-LG and 10% FCS. G-CSF primed blood (n:9) and marrow samples were obtained from healthy HLA identical sibling donors following informed consent. Cord blood was collected solely for the study (n:15). Bone marrow were taken from either adult (n:8) or pediatric (n:3) donors. *Results.* Among all the antibodies tested, CD90,CD105,CD73,CD29 were the antibodies reactive against *in vitro* cultured MSCs. The results of multicolour immunophenotyping (% of cells expressing these markers but are CD45-) are listed in Table 1. *Conclusions.* Each antibody detects a differ-

ent epitope of MSCs and the MSC content detected by each antibody is not constant. However Pearson correlation analysis showed a significant correlation among the expression of most of the antibodies (Stro-1, HLA-DR, CD 44, CD 90, CD 105, CD 29) but not with CD 34. As a result of our findings, we can conclude that cord blood and G-CSF primed peripheral blood contain similar amount of MSCs; BM , regardless of donor age, is at least 1 log richer (p<0.05) in MSC content compared to adult or cord blood. Inclusion of additional markers against progenitor cells ie the above mentioned and/or Aldehyde dehydrogenase, will improve the calculation of the repopulation capacity in the sources of stem cells.

Table 1.

	cord blood n:15	primed PB n:9	adult BM n:8	pediatric BM n:3	in vitro MSC n:6
CD73+	0,025	0,02	0,15	0,09	84,95
CD90+	0,01	0,04	0,205	0,21	55
Stro-1+	0,075	0,085	0,32	0,12	2,7
HLA-DR+	0,01	0,13	0,69	0,36	3,225
CD105+	0,02	0,05	1,17	2,66	98,8
CD29+	0,152	0,4	1,23	17,2	98,8
CD44+	1,45	0,55	12,39	31,19	77
CD34+	0,055	0,12	0,94	0,72	0,08

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MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FROM HLA IDENTICAL SIBLING DONORS IN 177 PATIENTS WITH ACUTE MYELOID LEUKEMIA

R. Ahmed Nacer, M. Benakli, R. Belhadj, F. Mehdid, M. Baazizi, N. Rahmoune, S. Madene, D. Ait Ouali, F.Z. Maifi, H. Belaidi, R.M. Hamladji

Pierre Marie Curie Center, ALGIERS, Algeria

Introduction. chemotherapy for acute myeloid leukaemia (AML) results in 65-80% complete remission, but only 20-30% of patients (pts) maintain a durable remission with standard consolidation therapies. The use of allogeneic hematopoietic stem cell transplantation (HSCT) increases the rate of long term survival although a substantial number of patients still relapse. We report the results of allogeneic HSCT from HLA identical sibling donors underwent in 177 pts with AML. *Patients and Methods.*: during an 98 months period (september 1998 to november 2006) 177 pts with AML (M1 30; M2:71; M3:14; M4: 30; M5: 12; M6:2; M7: 1; M0:7; NP:10) received 178 allogeneic HSCT and one boost from HLA identical sibling donors; median age 25 years (6 to 47); sex ratio 1,08; status disease at transplant, first complete remission (CR1): 155 pts, CR2: 15 pts, in relapse: 8 pts; median interval from remission to allograft 5,6 months (1 to 24). All patients received conditioning regimen with chemotherapy alone: Tutshka with additional VP16 20 to 30 mg/Kg body: 134 pts, Tutshka: 8 pts, Santos: 35 pts and Fludarabine based conditioning: 1 pt. The GVHD prophylaxis consisted of ciclosporine and (Seattle). Ten pts received bone marrow transplantation of median mononuclear cells 4,28×10^8/Kg body (range 2,3-8,1) and 169 pts peripheral blood stem cell (within one additional HSCT and one boost) of median CD34$^+$ cells 6,8×10^6/Kg body (range 8,03-26,5). At september 2007 maximal follow-up is 111 months and minimal 10,8 months. *Results.* The median time to engrafment was 15 days (9 to 24). One hundred and eleven pts (62,7%) are alive in remission after median follow-up 46,8 months (9,8 to 110). Acute GVHD occurred in 45 pts (27,6%) with 29 grade II-IV and chronique GVHD in 54 pts (37%), extensive : 33. Sixty six pts died (37,3%): 43 pts (24,3%) by transplant related mortality (TRM; infectious: 18, AGVHD: 11, CGVHD : 2, VOD : 8, cerebral hemorrhage: 2, metabolic disorder: 2). Twenty three pts (12,9%) relapsed and died except one pt is alive after successfully second transplant. Actuarial overall survival (OS) and event free survival (EFS) at 6 years are respectively 62% and 61%. When we compare the results between two groups of age: below 35 years (139 pts) and more than 35 years (38 pts), TRM is higher in second group (35,3% *vs* 44,7%; p=0,02). No difference between the two groups for OS (61% *vs* 53%; p=0,3) and EFS (62% *vs* 53%; p=0,4). *Conclusions.* Our results are similar to literature but with lowest relapse rate. Because of higher TRM in group more than of age 35 years we should purpose for this pts Tutshka conditioning regimen without VP16 or reduced intensity conditioning regimen.

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PICA: A FREQUENT SYMPTOM IN IRON DEFICIENCY ANEMIA

C. Beyan,[1] K. Kaptan,[1] A. Ifran,[1] E. Beyan[2]

[1]Gulhane Military Medical Academy, ANKARA; [2]Numune Education and Research Hospital, ANKARA, Turkey

Background. Pica is the habitual ingestion of non-nutritive substances the most common of which are earth or clay and ice. Pica is a peculiar manifestation of iron deficiency anemia (IDA) and generally disappears after correction of IDA. *Aims.* The aim of the study is to investigate the frequency and types of pica and the relationship of pica with patient characteristics. *Methods.* In this study, a total of 119 IDA patients (114 women and 5 men) whose mean age was 37,0±11,5 (mean±SD) (range 15-72 years) were investigated. Ninety four of the cases have pure IDA, 19 of them have additional vitamin B12 deficiency and six of them have pregnancy. *Results.* The frequency of pica was 34,4% (41/119) in whole patient population. Pica types and frequencies are in Table 1. The frequency was 38,3% in pure IDA patients, 15,79% in iron and vitamin B12 deficiency patients and 33,3% in pregnants. Pica was more prevalent in pure IDA patients when compared with patients having additional vitamin B12 deficiency (p=0,048). Pica frequency did not differ according to age groups. There was not a relationship between the severity of anemia and the frequency of pica. There was no difference between complete blood count parameters of IDA patients with and without pica except a borderline difference in mean platelet volume (p=0,057).

Table 1. Pica types and frequencies.

Pica Type	Frequency*
Geophagia	43,9% (18/41)
Pagophagia	21,9% (9/41)
Citrus shell eating	14,6% (6/41)
Raw rice eating	9,7% (4/41)
Coffee grain eating	7,3% (3/41)
Salt (sodium chloride) eating	4,9% (2/41)
Chewing gum eating	4,9% (2/41)
Paper eating	2,4% (1/41)
Ash eating	2,4% (1/41)
Cheese yeast eating	2,4% (1/41)
Gasoline drinking	2,4% (1/41)
Pencil tip eating	2,4% (1/41)
Toothpaste eating	2,4% (1/41)
Sunflower seed eating	2,4% (1/41)

Summary and Conclusions. As a conclusion, pica is a frequent finding in IDA, and patients with and without pica have similar characteristics.

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HB STANLEYVILLE II [ALPHA78(EF7)ASN?LYS]. FIRST CASE DESCRIBED IN SPAIN

P. Paloma,[1] F.A. González,[1] S. De la Iglesia[2], O. Briceño,[1] M. Polo,[1] M. Mateo,[1] C. Benavente,[1] A. Mora,[1] A. Peña,[1] C. Pérez,[1] A. Villegas[1]

[1]Hospital Clínico San Carlos, MADRID; [2]Hospital Dr. Negrín, LAS PALMAS DE GRAN CANARIAS, Spain

Background. Structural hemoglobinopathies are the result of mutations in the genes of globin, which determine a qualitative alteration in the expression of these genes. Most alterations does not originate any significant change, therefore they deal like silent or asymptomatic form. *Aims.* This work shows a new case of Hb Stanleyville II. *Methods.*and *Results.* The propositus is a woman 72 years old, Caucasian and from Canary Islands. Her haematological data are Hb 14.3 gr/dL; Hto 44.4%; MCV 85.8 fL; MCH 27.7 pg; RDW 15.1%; reticulocytes 1.2%; Hb A2 3.1% and Hb F 1.6%. Electrophoretic studies in cellulose acetate electrophoresis at alkaline pH=8.6 and isoelectrofocusing (IEF) shown an anomalous Hb which run like Hb S. In agar citrate electrophoresis (pH 6.0) the anomalous Hb does not appear. The analysis by reverse phase HPLC for globin chains showed an alpha X anomalous after alpha A. Molecular analysis by sequentiation of the PCR products genes alpha1 and alpha2 showed the mutation AAC'AAA at CD78 of 2nd gene alpha2 in heterozygotic state, which lead the change of Asparagine to Lysine. *Summary and Conclusions.* The substitution of an amino acid with neu-

tral charge like the Asparagine for other one with positive charge like the Lysine in the segment EF, which corresponds to the external surface of the tertiary structure of the chain of globin, determines the change of charge in the chain. This allows the easy differentiated by *Methods.*electrophoretic and chromatographic. Nevertheless owing to its position in the chain which is not critique for the stability, solubility and affinity for the oxygen deals of silent or asymptomatic forms. The Hb Stanleyville II had been described before in families of black race of the Congo, Uganda, USA, Alsace and Brazil. This case represents the first case described in Spain.

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UP REGULATION OF NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN, NGAL/LCN2, IN β THALASSEMIA PATIENTS

M. Habibi Roudkenar,[1] R. Halabian[2], M. Shokrgozar[3]

[1]Reserch Center, IBTO, TEHRAN; [2]Research center, IBTO, TEHRAN; [3]National cell bank, Pasteur Institute of Iran, TEHRAN, Iran

Background. One of the major consequences in β-thalassemia is iron overload. Oxidative statues have been reported in beta thalassemia patients by several studies. It is proved that iron plays critical role in the formation of reactive oxygen species (ROS). Recently we found induction of Lcn2/NGAL expression under oxidative stress condition. In this study it was assumed that NGAL should be up- regulated in β-thalassemia patients because of oxidative stress conditions. *Methods.* The assessment of NGAL expressions in 25 adult β-thalassemia and 8 pediatric patients were performed by semi quantitative RT-PCR, Real time RT-PCR and ELISA compared with healthy samples. *Results.* Adult β-thalassemia patients' up-regulated NGAL expression compared with the normal samples but no up regulation was observed in pediatric. *Conclusions.* The up regulation might play important role in decreasing ROS or iron in beta thalassemia patients.

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A CASE OF KLEBSIELLA PNEUMONIAE LIVER ABSCESS IN A PATIENT AFFECTED BY THALASSEMIA INTERMEDIA

A. Moscetti,[1] E. Montefusco[2], E. Conte[2], G. La Verde[2], F. Saltarelli[2], M. Pacilli[2], R. Porrini[2], B. Veggia[2], B. Monarca[2]

[1]University La Sapienza II Facoltà Az.Ospedaliera Sant'Andrea, ROMA; [2]University La Sapienza II Facoltà Az.Ospedaliera Sant'Andrea Hematology, ROMA, Italy

Background. Thalassemia intermedia belongs to the group of β-thalassemia and is characterized by a large spectrum of conditions of variable severity. We described a case of liver abscess in a adult patient affected by thalassemia intermedia. *Aims and Methods.* we observed a 43 years old male patient with past history of seminoma affected by thalassemia intermedia treated with splenectomy in childhood with persistent fever resistant to intravenous antibiotic therapy. At presentation the blood cell count showed a severe anemia (Hb 6.1 g/dL), WBC 23.5 x 103/ml, PLTS 499.000/ml with normal values of liver enzymes; all the microbiological and serological assays on serum (S.typhi, S.paratyphi, Brucella, HBV, HCV, HIV, CMV, EBV, Toxo-test), expectoration (Mycobacterium tuberculosis), blood stream colture, stool colture (Salmonella, Shigella, Yersinia enterocolitica, Clostridium difficile toxin a) and urine colture, resulted negative; chest X-ray was negative for infectious pneumonia. Because of persistent fever the patient started empiric antibiotic therapy with piperacilline/tazobactam and levofloxacine obtaining a temporary defervescence of three days; then, for fever representation the patient was treated empirically with meropenem and teicoplanin obtaining a quick and apparently stable defervescence, so he was dismissed in good clinical conditions with oral ciprofloxacine. After 15 days the patient became pyretic and was readmitted to hospital: a total body CT scan and consecutive blood stream coltures showed the presence of a liver lesion (LD 5.9 cm, TD 3.2 cm, Figure 1) referring to abscess, not present at previous CT scan performed few months before for seminoma follow-up, and the positivity for Klebsiella pneumoniae ssp pneumoniae sensible to great part of carbapenems, aminoglycosides and cephalosporins, respectively. Therefore the patient started an intravenous antimicrobial therapy with meropenem and amikacine and was soon referred to emergency radiologists to perform a percutaneous CT-guided catheter drainage of the liver abscess. The microbiological assay performed on drainage (25 mL of brown viscous fluid) confirmed the presence of Klebsiella pneumoniae ssp pneumoniae.

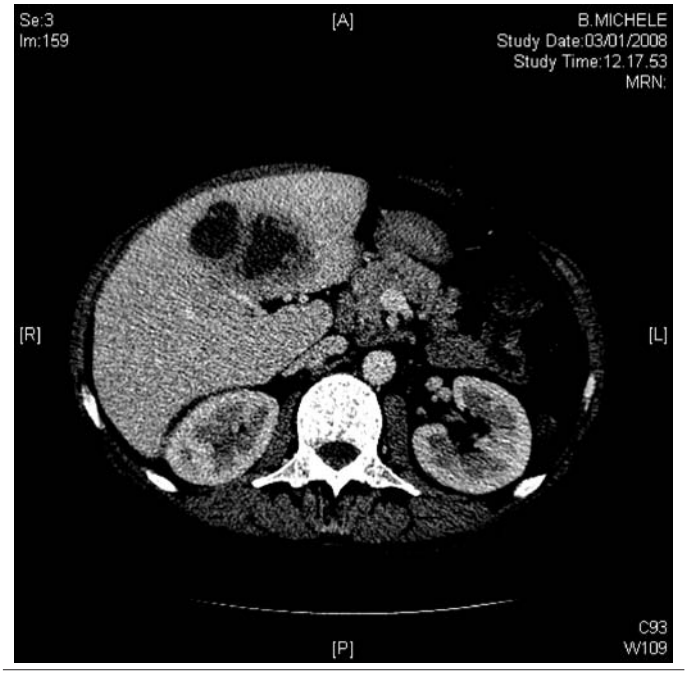

Figure 1.

Results. The patient obtained a quick and stable defervescence within few hours from the percutaneous drainage without complications related to procedure. The ultrasonography showed a reduction in dimensions of liver abscess (LD and TD 1.3 cm). Therefore the intravenous antimicrobial therapy was continued for a total of 14 days and then the patient, in good clinical conditions and persistently apyretic, was dismissed with oral ciprofloxacine maintained for 1 month. *Conclusions.* The infectious risk in thalassemia is often underrated but should be always considered for the correct management of this cathegory of patients.

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OXIDATIVE STRESS IN MALE PATIENTS WITH β-THALASSSEMIA MINOR: PRELIMINARY RESULTS

T. Cetin, O. Nevruz

Gulhane medical academy, ANKARA, Turkey

Bacground and *Aims.* In our previous studies, dysfunction of some organs was demonstrated in patients with beta-thalassemia minor (BTm). The impact of various factors, such as chronic hypoxia, oxidative stress, hemolysis or various biochemical alterations on organ dysfunction have not been studied yet. The aim of this study was to investigate the effects of oxidative stress in patients with BTm. *Methods.* Twenty-five male patients with BTm and fifteen healthy male controls were enrolled in the study. Serum glutathione peroxydase (GPx), superoxide dismutase (SPD) and catalase (CAT) were studied. *Results.* Serum GPx and CAT activities were significantly higher in BTm subjects than in controls (p<0,001 for both). SPD levels were not correlated between the BTM and control groups (p<0,847). *Conclusions.* Oxidative stress is increased in patients with BTm. Although there is no study on patients with BTm, these results support those of previous studies carried out in patients with β-thalasssemia major.

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THE IMPLICATIONS OF THE NEUTROPHIL GRANULOCYTE IN MUSCULAR ACTIVITY

M.L.A. Balea,[1] M.A. Popescu[2]

[1]Colentina Clinical Hospital, BUCHAREST; [2]GRAL Laboratory, Clinical Hospital Colentina., BUCHAREST, Romania

Starting from the clinical observations that correlates maximum muscular asthenia in patients with agranulocytosis, with the existence of a parallel between the intensity of the asthenia and the severity of neutropenia, we issued the hypothesis that the muscular fiber is using the products resulted from the apoptosis of the neutrophil granulocyte. We have initially researched the results of EMG evaluations, done random-

ly on subjects with RA on methotrexate therapy, and we determined a decrease of up to 50% of the amplitude of the maximal contraction in the episodes of neutropenia induced by the therapy. Afterwards we did an experiment by inserting a catheter into the left radial artery and into the drain vein of the muscular area irrigated by the artery, evaluating the hematological parameters of the arterial and vein blood, before and after medium, high and very high muscular effort performed by the musculature of the hand. For each subject we have done 5 measurements. We have determined: a decrease by 9.25% in the number of granulocytes after medium effort, while the other parameters remained constant: hemoglobin, erythrocyte count, hematocrit, platelets count, monocytes, eosinophils and basophils. Paradoxically we found and increase of 9.22%, respectively 5.69% in the number of neutrophils in the vein blood compared to the arterial one, after high and respectively maximal effort. This aspect can be explained by the mobilization of the marginal neutrophils pool, also demonstrated by the 15% increase in their number in the vein blood and of only 5% within the arterial blood, further proving our hypothesis with additional evaluations.

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SUPPORTING RITUXIMAB THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA KIROV SCIENTIFIC RESEARCH INSTITUTE OF HEMATOLOGY AND BLOOD TRANSFUSION OF ROSMEDTECHNOLOGIES, KIROV

T.P. zagoskina, O.V. malykh, A.V. Kudryavtseva, M.E. Golubeva, I.V. Grishina

Kirov Scientific Research Institute of Hematology and Blood Transfusion, KIROV, Russian Federation

Background. Now there is burning problem of perfection of existing programs of chronic lymphocytic leukemia (CLL) treatment. With occurrence of purine analogues and monoclonal antibodies to antigene CD20 achievement of proof and durable remissions became the therapy purpose. By results of our previous researches, efficiency of a combination rituximab, fludarabine, cyclophosphamide (RFC) was more than 70% at previously treated patients and in 96% during application as the first line of therapy. However in a number of patients it is not possible to achieve long relapse-free period. For improvement of results we used supporting rituximab therapy in CLL patients after inductioning-consolidating therapy RFC. *Aims.* The purpose of the given work was to study efficiency of supporting rituximab therapy in CLL. *Methods.*and results. Program RFC included: rituximab 375 mg/m^2 i/v in 1 day, fludarabine 25 mg/m^2 i/v 2-4 days and cyclophosphamide 300 mg/m^2 i/v 2-4 days. Courses were applied every 4 weeks to the maximum effect. 86 CLL patients are included in research, among the patients: men 48 (56%), women 38 (44%); in a stage A - 12 (14%), in a stage B - 49 (57%), in a stage C - 23 (27%); first line CLL patients 56 (65%), patients who received chemotherapy - 30 (35%). The age of patients ranged from 32 to 76 years (a median - 58 years). All the patients received 4-8 courses RFC (a median of 6 courses). Estimating of the results of the therapy, overall efficiency was observed in 79 (92%) of the patients, complete remission (CR) was received in 58 (67%), partial remission (PR) - in 22 (25%). The median of the overall survival rate in patients was not achieved for 64 months of follow-up. The median relapse-free survival rate was 48 months. 23 CLL patients after induction RFC therapy have received supporting therapy in the following regimen: rituximab 375 mg/m^2 weekly 2 times in three months within 2 years. The survival rate without progressing at term of supervision of 29 months was the criterion of efficiency of supporting therapy. *Conclusions.* The results of the research have shown, that application rituximab as supporting therapy has lowered the risk of progressing on 53% (the relation of risks: 0,42). Thus, in CLL patients, the application of supporting rituximab therapy after effective induction therapy RFC in patients with complete and partial remissions increases survival rate without progressing.

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PRELIMINARY RESULTS FROM AN ELECTRONIC OBSERVATIONAL STUDY ON BORTEZOMIB'S EFFECTIVENESS IN ADVANCED MULTIPLE MYELOMA

M.A. Dimopoulos,[1] M. Roussou,[1] E. Katodritou[2], K. Zervas[2], M. Delforge,[3] M. Linderholm,[4] D. Sargin,[5] C. Hulin,[6] V. Poon,[7] R. Dhawan[7]

[1]University of Athens School of Medicine, ATHENS, Greece; [2]Theagenion Cancer Center, THESSALONIKI, Greece; [3]University Hospital Leuven, LEUVEN, Belgium; [4]Linköping University Hospital, LINKÖPING, Sweden; [5]Istanbul University Istanbul Medical Faculty, ISTANBUL, Turkey; [6]Centre Hospitalier Universitaire of Nancy-Brabois, NANCY, France; [7]Johnson & Johnson, RARITAN, USA

Background. Multiple myeloma (MM) is a plasma-cell malignancy with approximately three years' median survival. Bortezomib (VELCADE) is a relatively new therapy indicated for the treatment of MM in patients (pts) who have received at least 1 prior therapy. *Aims.* The electronic VELCADE Observational Study (eVOBS) is a multicenter naturalistic study designed to evaluate the clinical and outcomes benefits of bortezomib in actual clinical practice. *Methods.* This is a multi-center study with sites in Belgium, France, Greece, Russia, Spain, Sweden, and Turkey. The study enrollment period is between October 2006 and December 2008 with a 3-year follow-up. Adults are eligible for study if they are scheduled to initiate bortezomib within the approved indication. All bortezomib dosages and concomitant treatments are permitted, except investigational therapies. Due to the non-interventional nature of the study, no predefined response criteria are mandated; response criteria may include M-protein, EBMT, SWOG, or others as defined by the investigator. An interim analysis has been performed on data of the 140 patients enrolled from November 2006 until November 2007 who had at least 4 months of data. *Results.* Patient characteristics at baseline show the median patients' age to be 63 yrs. The median interval from initial diagnosis was 2.5 years. 56.4% of the patients were male. The number of previous therapies for MM was 1, 2-3, and ≥4 for 40%, 42.8%, and 9.3% of pts, respectively. 51.4% of the patients had disease stage III. Demographic and clinical characteristics of the initial participants were similar to those of the participants in the prospective controlled phase 3 APEX trial (Richardson, N Engl J Med, 2005:352; 2487-98). The patients had Overall Response Rate (ORR) of 75.4% (7.9% CR, 13.5% nCR, 41.3% PR, 12.7% MR). The median time to initial response among patients who achieved a CR, nCR or PR was 31 days, while the median time to best response (CR, nCR or PR) was 57 days. The median time to CR was 60 days. 33 patients (23.6%) discontinued bortezomib because of an adverse event. Adverse events (AEs) were reported in 114 (81.4%) pts, 51(36.4%) pts had Grade ≥3 AEs and 13 (9.3%) pts experienced Grade ≥4 AEs. The most commonly reported adverse event leading to bortezomib discontinuation, regardless of relationship to study drug, was herpes zoster in 6 patients. *Conclusions.* Overall Response Rates in this study is in line or slightly better than with those reported in previous controlled clinical studies. Overall the treatment with bortezomib has been well tolerated in this patient population. Long-term follow-up on this cohort of patients will continue and will be reported in the future.

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YTTRIUM-90-LABELLED IBRITUMOMAB TIUXETAN RADIOIMMUNOTHERAPY FOR PATIENTS WITH INDOLENT B-CELL NON-HODGKIN'S LYMPHOMA (NHL): FROM CLINICAL TRIAL TO CLINICAL PRACTICE

P. Evans, A. Fortune, S. McGuckin, E. Vandenberghe

St James Hospital, DUBLIN, Ireland

Background. Yttrium-90-Labelled Ibritumomab Tiuxetan is a novel form of targetted treatment in which a monoclonal anti-CD20 antibody is linked to a beta-radiation emitting isotope. B-Non Hodgkins Lymphomas (NHL) express the CD 20 antigen, making them an ideal tumour for (90)Y ibritumomab tiuxetan therapy. Trial data indicated that (90)Y ibritumomab tiuxetan was effective in relapsed, refractory or transformed follicular lymphoma (FL). A treatment program was initiated in 2004 and now includes 3 cohorts of patients: (I) 7 patients with relapsed, bulky disease, (II) 7 patients with stable disease after debulking chemotherapy and (III) 3 patients undergoing BEAM transplant for FL in partial remission. *Aims.* We have reviewed the clinical course and treatment outcome of patients treated with (90) Y ibritumomab tiuxetan from June 2004 until the present day. *Results.*Myelosuppression was the

only toxicty and was evaluated weekly. Transfusion support was given as required and G-CSF administered at neutrophil count <1×10^9. Treatment response was assessed at 12 weeks. The 7 patients (4M, 3 F) in group I had extensive (stage III or IV) FL. Six patients had an ECOG Score of 2 and 1 patient had an ECOG Score of 1. Four patients had no response. These 4 patients all had disease progression at 12 weeks. All have since died of their disease with a median time from treatment to death of 8^1/$_2$ months (2-11 months). Three patients had a partial response at 12 weeks. However, all 3 had disease progression at median time of 38 weeks (22-56 weeks). Two patients died, at 12 and 23 months post treatment. The third is alive with active disease at 24 months. Group II included 7 female patients whose maximum tumour size was <5cm and stable for at least 6 weeks prior to therapy. Six patients had an ECOG Score of 1 and one an ECOG Score of 2. Debulking chemotherepy varied. The lymphoma types included FL (n=3), transformed FL (n=2), mantle cell (n=1) and lymphoplasmacytoid l (n=1). At Relaspse prior to(90) Y ibritumomab tiuxetan 4 patients had Stage IVB, 2 Stage IVA and 1 had Stage IIIB disease. Following debulking Chemotherapy 2 patients had achieved a clinical remission, 1 patient had Stage IIB, 2 patients had Stage IVA and 2 patients had Stage IVB disease. All patients remain in remission with a median duration of response of 40 weeks. An earlier than predicted nadir in neutrorphil count occurred and more patients required G-CSF, and transfusion support. Group III includes 3 male patients with FL in partial remission on the (90) Y ibritumomab tiuxetan -BEAM transplant program. Efficacy remains to be evaluated in this group but no excess toxicity to the BEAM transplant was noted. *Conclusions.* (90) Y ibritumomab tiuxetan is a well tolerated addition to the management of CD20-expressing indolent NHL. It is effective if used in moderate volume, stable disease with encouraging disease control achieved in our second cohort of patients. The addition of (90) Y ibritumomab tiuxetan to BEAM conditioining was well tolerated, however efficacy remains to be evaluated.

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AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA) ASSOCIATED TO NON-HODGKIN'S LYMPHOMA (NHL)

A. Colita,[1] A.R. Lupu,[1] S. Angelescu,[1] D. Barbu[2], G. Barca[2], M. Closca[2], C. Saguna,[1] O. Ciocan,[1] A. Ciobanu[2], A.M. Ivanescu,[1] M. Oprea[2], D. Mut Popescu[2], V. Teleanu[2], A. Negulescu[2], D. Coriu,[3] A. Colita[3]

[1]Coltea Clinical Hospital/UMF Carol Davil, BUCHARES; [2]Coltea Hospital, BUCHAREST; [3]Fundeni Institute/UMF carol Davila, BUCHAREST, Romania

Background. association of autoimmune anemia (AIHA) with non-Hodgkin's lymphoma (NHL) is well documented. However, there are few studies that have analyzed the clinical course of these patients. *Patients and Methods.* we describe an retrospective analysis of 29 AIHA patients diagnosed over a 11 year period (oct. 1996 - sep. 2007), representing 1,53% of all (1889) NHL patients and 28,15% of all AIHA diagnosed cases (103); median age was 59 years (range 25-79 years); M/F: 13/16. Diagnostic was established after examination of lymph node biopsy and trephine bone biopsy. Stage was reported according to the Ann Arbor system. All patients were in advanced stage disease: 15 in stage III and 14 in stage IV. Based on immunophenotypic studies 28 patients had B-cell NHL (21 - small lymphocytic, 3 - marginal type, 2 - diffuse large cell, 2 - folicular, 1 - lymphoplamacytic) and only 1 case of T- cell NHL. AIHA with warm-reactive antibodies was present in 20 patients (68,9%), with cold-reactive antibodies in 7 cases (24,13%) while 2 patients had AIHA with mixed warm and cold reactive antibodies. In 2 cases with warm reactive antibodies there was an association with autoimmune thrombocytopenia. Monoclonal serum IgM was present in 1 case. *Results.* complete remission was achieved in 12 patients (41,37%) and partial response in 5 cases (17,24%) representing a lower response rate than in the general NHL cohort (55,56% CR). Response rate was low in patients with cold reactive antibodies (2 responses out of 7 cases) and mixed reactive antibodies (both refractory). *Conclusions.* The analysis of this cohort suggests that the association of AIHA in NHL patients has a negative prognostic impact, especially in cases with cold reactive antibodies. As a particular aspect it is to mention the rare associátion of T-NHL and AIHA in the reported cohort.

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USE OF YTTRIUM-90 IBRITUMOMAB TIUXETAN IN PATIENTS WITH CD20$^+$ NHL: SINGLE CENTRE EXPERIENCE

H. Alizadeh, J. Kristensen, A. Alam, P. Kumar, M. Mohamadiyah

Tawam Hospital in affiliation with Johns Hopkins Medicine, ALAIN, United Arab Emirates

Background. Monoclonal antibodies labeled with radionuclides (radioimmunotherapy) have become a therapeutic modality in the treatment of patients with non-Hodgkin's lymphoma (NHL). Radioimmunotherapy directed against CD20 has been established is several types of B-cell non-Hodgkin's lymphoma (NHL). The use of Ibritumomab tiuxetan is approved for patients with rituximab resistant, low-grade, follicular or transformed NHL and the Yttrium-90 Ibritumomab tiuxetan is the only approved radioimmunotherapy in the EU. *Methods.* Between February 2006 and December 2007, at our institute, the authors treated 32 patients with previously treated, indolent and aggressive B-cell NHL (22 diffuse large B-cell NHL=DLBCL, 7 follicular NHL=FL, 1 mantle cell lymphoma, 1 FL transformed to DLBCL, and 1 MALT transformed to DLBCL) using Yttrium-90 Ibritumomab tiuxetan. *Results.* At the time of administration of Ibritumomab tiuxetan, 7 were in CR I, 18 were in CR II, 5 were in nCR III, and 2 patients had active disease. Nineteen patients received Ibritumomab tiuxetan as consolidation following achievement of near complete response to 1st line systemic chemotherapy with R-CHOP regimen. At a median follow-up of 22 months and of the 29 patients who were evaluable, the estimated 2-year progression free survival rate was 75.86%, and the estimated 2-year overall survival rate was 86.20%. Seven patients developed either relapse or progression of disease after radioimmunotherapy. Two patients developed coetaneous B-cell NHL and 1 patient relapsed with T-cell coetaneous NHL after Ibritumomab tiuxetan. Three patients with tonsillar NHL did not respond to the radioimmunotherapy treatment. Two patients remain in CR after Ibritumomab tiuxetan therapy for NHL with CNS involvement and gastric NHL, respectively. Nineteen patients remain in CR almost 24 months after administration of Ibritumomab tiuxetan. Three patients were not evaluable due to lost follow-up. The Yttrium-90 Ibritumomab tiuxetan toxicity included grade 2-3 haematologic toxicities in 3 patients; the most common grade 3 toxicities were thrombocytopaenia (2 patients) and neutropaenia (1 patient) and the adverse events were graded according to the WHO criteria for toxicity. Transfusion of platelets was given to 2 patients. *Conclusions.* Treatment with Yttrium-90 Ibritumomab tiuxetan induced a clinically durable PFS in patients who achieved either PR or nCR after systemic chemotherapy. This retrospective study from single institute demonstrated the efficacy, feasibility, and tolerability of Yttrium-90 Ibritumomab tiuxetan for the treatment of patients with treated and untreated CD20$^+$ B-cell NHL.

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PROLONGED SURVIVAL OF PATIENTS WITH PERIPHERAL T-CELL LYMPHOMA AFTER FIRST-LINE INTENSIVE SEQUENTIAL CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION

V. Prochazka, T. Papajik, E. Faber, .J Vondrakova, L. Raida, M. Kucerova, K. Indrak, M. Jarosova

Teaching Hospital, OLOMOUC, Czech Republic

Background. Nodal peripheral T-cell lymphomas (PTL) are infrequent subtypes of non-Hodgkin's lymphomas. The WHO classification recognizes three subgroups of nodal PTL: peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS), anaplastic large cell lymphoma (ALCL) and angioimmunoblastic lymphoma (AIL). The clinical course is aggressive, and despite multiagent chemotherapy, the median survival is about 2 years. Optimal first-line chemotherapy is not established and the role of high-dose therapy with autologous stem cell support is still controversial. *Aims.* To analyze the long term outcome of PTL patients treated with intensive first-line chemotherapy with high-dose therapy and autologous transplant consolidation. *Methods.* Sequential chemotherapy protocol consists of 3 cycles of CHOEP-21-like regimen (PACEBO), 1 cycle of an ifosfamide and methotrexate-based regimen (IVAM) and a priming regimen with high-dose cytosine arabinoside (HAM). Consolidation is provided with myeloablative conditioning (BEAM 200) and autologous stem cell support. Eighty-four patients with aggressive high-risk lymphoma were treated with the sequential protocol from 2000 to 2007 in our institution. Here we report our experience with 18 patients with nodal PTL (10 PTCL, NOS; 3 ALCL, ALK-negative; 2 ALCL, ALK-positive; 2 ALCL, unknown ALK status; 1 AIL). The median age at diagnosis was 43 years, 17 patients underwent the pro-

tocol as first-line therapy and one as salvage therapy. Twelve patients received first-line high-dose therapy and autologous transplant consolidation; two patients were consolidated with allogeneic stem cell transplantation. *Results.* Eleven (61%) patients achieved complete remission, 3 (17%) partial remission and 4 (22%) patients failed the procedure. The overall response rate was 77.8%. After a median follow-up of 25.7 months, nine patients relapsed or progressed (6 PTCL, NOS; 2 ALCL ALK-positive; 1 ALCL ALK-negative; median 14.1 months) and four patients died (lymphoma progression). The relapse was treated with allogeneic stem transplantation in one patient. The 2-year progression-free survival (PFS) was 52% (95% CI, 0.27 to 0.76); the overall survival rate reached 71% (95%CI, 0.47 to 0.95). *Conclusions.* Our results show that intensive first-line chemotherapy with high-dose therapy and autologous transplant consolidation offers a chance for long-term survival to patients with chemosensitive PTL. Patients with partial treatment response may be saved with allogeneic stem cell transplantation.

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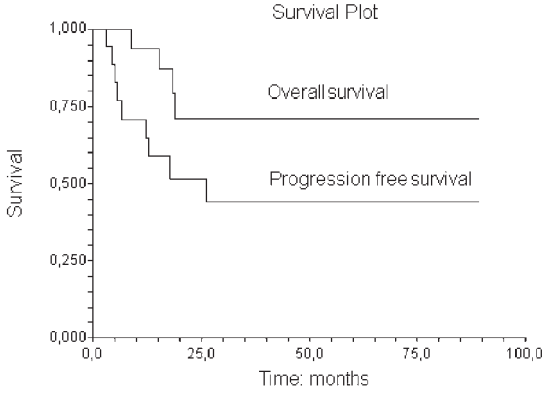

Figure 1.

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NHL-BFM-90 MODIFIED IN THE TREATMENT OF EXTRANODAL AGRESSIVE LYMPHOMAS OF ADULTS

E.E. Zvonkov Eugene,[1] A. Kremenetskaya[2], S. Kravchenko[2], E. Baryach,[1] T. Obuhova,[1] A. Morozova,[1] E. Ilushkina,[1] A. Magomedova,[1] B. Krasilnikova,[1] A. Gubkin,[1] A. Vorobjev[1]

[1]National Hematology Research Centre, MOSCOW; [2]National Hematology Research Center, MOSCOW, Russian Federation

Background. Up to 30% of all lymphoprolifirative disorders primary emerge out of hemopoetic organs, extranodally so to say. There are 70% of DLBCL (diffuse large B-cell lymphoma) and 10% of BL (Burkitt Lymphoma) among them. These tumors' prognosis is defined with tumor type, localization and intensity of polychemotherapy (PChT) given. Recently developed new intensive chemotherapy programs and ability of performing supportive accompanying treatment have reversed the surgery treatment indications for EL (extranodal lymphomas) and enable organsaving strategy to be the dominated, stopping CHOP program to be considered as a gold standard in the treatment of these tumors. *Aims.* To estimate the efficacy and toxicity NHL-BFM-90 modified program in the treatment of extranodal lymphomas in adults. *Methods.* Since 2000 till 2007 49 patients with EL (23 men and 26 women) aged 14-76 (median age 39.6) were treated with NHL-BFM-90 modified. There were 31 patients diagnosed with DLBCL and 18 with BL among them. The stomach appeared to be the most frequent primary localization (17 pts), followed by intestine (13 pts), brain (4pts), fauces (4pts), thyroid (3 pts), orbit (2pts),soft tissues (2pts), pancreas (1pts), vagina (1pts), ovary(1pts) and adrenal gland (1pts). 18 pts (10 BL and 8 DLBCL) primary received surgical treatment. The early progression was revealed within 2 months after the operation among all BL patients and 4 patients with DLBCL. The surgical complications were as follows: castration of 2 patients with ovarian BL, chronic diarrhea of 3 patients with BL and DLBCL of intestine, incontinence of urine of patient with vagina DLBCL, demping syndrom of patient with stomach DLBCL, ileus of 1 patient with stomach BL and 1 patient with adrenal gland DLBCL. NHL-BFM-90 modified consisted of 2-6 blocks of chemotherapy, including cyclophosphamide, ifosphomide, cytosar, vepesid, doxorubicin, vincristine, methotrexate, dexametasone. All courses were associated with cytopeny 4 st., according toxicity gradation of WHO. *Results.* 2 years OS and DFS was 88% and 98% in the group of EL treated with NHL-BFM-90. Early lethality -4% (2 patients). Primary resistance - 8% (4 patients). Early relapse - 2% (1 patient). The therapy response low worse among patients with brain and thyroid DLBCL. The differences in the groups of DLBCL and BL were unsignificant. 2 years OS and DFS for DLBCL - 87% and 96%, for BL - 89 and 100% correspondingly. *Conclusions.* NHL-BFM-90 modified chemotherapy program has shown high efficacy in the extranodal DLBCL and BL of adults treatment. Universal efficacy of this program has an important practical implication due to the often difficulties of differential diagnosis of extranodal DLBCL and BL. High toxicity of the therapy in adults must be associated with intensive accompanying therapy. The surgical treatment is ineffective in the treatment of EL, decreasing the quality of patients' life and confines the possibilities of intensive PChT.

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RITUXIMAB MAINTENANCE THERAPY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH NON - HODGKIN'S LYMPHOMA

M. Capponi,[1] F. Falcinelli,[1] P. Minga,[1] L. Flenghi,[1] F. Falzetti,[1] L. Marcomigni,[1] R. Ciurnelli,[1] M. Di Ianni,[1] A. Tabilio[2]

[1]Hematology and Clinical Immunology, PERUGIA; [2]Hematology, University of L'Aquila, L'AQUILA, Italy

Background and Aims. Several studies compared conventional chemotherapy and autologous stem cell transplantation (ASCT) as treatment for relapsed B cell non Hodgkin's lymphoma or as consolidation of first remission in high-risk patients with B-cell NHL. Despite the advantages of stem cell grafts, clinical outcomes still need to be improved. Whatever the approach, re-growth of malignant cells arising from transplant contamination or from residual cells in the patient underlies relapse. Outcomes after ASCT may be improved by *in vivo* purging with rituximab in an attempt to remove malignant cells from the ASC harvest. As high dose therapy (HDT) alone probably does not eliminate all residual tumour cells in the patient, eradicating minimal residual disease (MRD) after HDT may reduce the probability of relapse. Preliminary data from several studies suggested administration of Rituximab as maintenance therapy after ASCT is safe and may help to eradicate MRD, as it induced long-lasting clinical and molecular complete remission in patients with follicular and mantle lymphoma (Brugger W, Ann Oncol 2004;15:1691, Morschhauser F, Blood 2004; 104:21a, Woods AC, Blood 2004;104: 262a, Mangel J, Ann Oncol 2004;15:283). *Methods.* We analysed 17 patients (13 follicular LNH, 4 indolent B cell NLH, median age 55 years; range 42-64 years) who had undergone high dose therapy and infusion of ASCT. Eleven patients had received supra-lethal chemotherapy (8 Mitoxantrone + Melphalan; 1 Melphalan, 1 BEAM, 2 TECA) and 5 had received Total Body Irradiation + Thiotepa. 14 patients received peripheral blood stem cells and 2, who were poor mobilizers, bone marrow stem cells. At a minimum of 4 months after ASCT (median 5 months; range 4-15 months) maintenance therapy with Rituximab was started in 15 patients who had achieved complete remission and in 2 in partial remission. Eleven patients received 375 mg/mq Rituximab every 3 months. Six patients received 375 mg/mq/wk for 4 weeks which, after 6 months, was followed by the 3-month schedule. Interferon - alpha (3 million units) on alternate days was also administered to 2 patients for one year. Patients did not receive antibiotic prophylaxis before Rituximab therapy.

Table 1.

Patient	Infection	Blood test
P.B.	fungal pneumonia	grade 2 leucopenia
M.B.	viral (?) interstinal pneumonia	grade 3 leucopenia, severe hypogammaglobulinemia
M.F.	cutaneous varicella zoster virus	normal values
B.G.	no infection	grade 3 leucopenia, severe hypogammaglobulinemia

Results. At a median follow-up of 22 months (range 2-54 months) hematological toxicity was satisfactory Three patients had Grade 1 (WHO) iatrogenic leucopenia but did not need support therapy; 1 patient had Grade 2 (WHO) leucopenia and 2 patients who had received bone marrow stem cells, had Grade 3. All responded to G - CSF therapy. Markedly low gamma-globulin counts developed in 5 patients. Infectious episodes are listed in the Table 1. Complete remission was maintained in 15/ 17 patients and partial response was maintained in the other 2 who had the maximum follow-up of 54 months. Conclusions. In patients with indolent NHL maintenance therapy with RITUXIMAB after ASCT is feasible and seems to consolidate complete or partial remissions.

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IRON-DEFICIENT ANEMIA AS AN UNUSUAL PRESENTATION OF BERNARD-SOULIER DISEASE

F. Cuellar-Ambrosi, C.I. Saldarriaga-Giraldo, W. Maya-Salazar, J. Cuervo-Sierra, M. Gil-Murillo, L. Alavarez-Pelaez

Universidad de Antioquia, MEDELLIN, Colombia

First described in 1948, Bernard-Soulier disease (Giant Platelet Syndrome) is an uncommon autosomic recessive disorder of platelet function, prevalence 1/1.000.000, where there is absence of Gp Ib/IX-V complex, the von Willebrand receptor, characterized by giant platelets and greater than expected bleeding for the degree of thrombocytopenia. Very few cases have been reported in literature especially in Latin-American. This is 29-year-old Hispanic female who frequently complaints of easy bleeding since she was 2-year-old. She had been admitted several times to other hospitals for epistaxis requiring multiple blood transfusions. In 1980 a CBC revealed mild thrombocytopenia, 80K, and microcytic and hypochromic anemia with normal WBC. A bone marrow biopsy showed a hyperplasic megakaryocytic line and idiopathic thrombocytopenic purpura (ITP) was considered. Physical exam was normal and viral and autoimmune tests were negative. She was started on prednisone but despite of this she presented new episodes of epistaxis with mild thrombocytopenia, then a splenectomy supported with platelet transfusions was performed in 1986. Her clinical picture reached a steady state, with no bleeding until the menarche when again she started severe metrorrhagia and mild thrombocytopenia. In 1999 she had an uneventfully pregnancy outcome undergoing elective cesarean section at 40 weeks with platelet transfusions since the thrombocytopenia of 20K apparently related with her ITP antecedent. Thereafter she presented with persistent iron-deficient anemia (IDA) related with severe metrorrhagia and continuous iron supplementation was prescribed because of her low ferritin levels. In 2007, in our Hematology Department, she related that her parents had a remote grade of consanguinity and then a coagulation study was done revealing long bleeding time (>20 minutes, control 3 to 6 minutes) with normal PT, APTT and von Willebrand factor was 120%. Platelet aggregation displayed normal response to ADP, collagen and epinephrine but a lack of platelet response to ristocetin. This results plus the finding of giant platelets with a high medium platelet volume of 14.4 fL confirmed the diagnosis of Bernard-Soulier disease. In this patient, without family story of bleeding, ITP was first considered since the macro-thrombocytopenia as expected with high platelet turnover pictures responded to prednisone and splenectomy suggesting perhaps that there was a confusing similarity with ITP in the manifestation of her clinical picture. In this background the patient had a successful pregnancy outcome supported with platelet transfusions as been reported by others. Diagnostic difficulties of inherited thrombocytopenias derive not only from the availability of specialized laboratory investigations but the clinical and molecular features of these rare disorders are not always well defined. This woman presented all typical clues of ITP but finally the story did the diagnosis of Bernard Soulier-disease. Type 2B von Willebrand disease sometimes can present with giant platelets but FVIIIC and vWF were normals. This case suggest that although IDA in the third world countries has a multifactorial etiology like malnutrition, parasitic intestinal infestation and occult bleeding, subtle inherited hemostatic disorders must be take on mind as a possible additional factor in the study of persistent IDA.

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PARANEOPLASTIC BLEEDENG DISORDER DUE TO ISOLATED HYPOFIBRINOGENEMIA-CASE REPORT

C.Z. Cvetkovic, C. Celeketic, B. Cvetkovic, V. Libek

Clinical Hospital Center Zemun-Belgrade, BELGRADE, Serbia

Background. Various types of thrombotic or hemorrhagic events may bee seen in patients with solid tumors. The most frequent are disseminated intravascular coagulation /DIC/, antiphospholipid syndrome, impared fibrinolysis, presence of acquired inhibitors of clotthing factors or due the decreased biosynthesis of the vitamin K dependent blood clotthing proteins. Hyperfibrinogenemia or dysfibrinogenemia are also seen, but isolated hypofibrinogenemia without signs of impared liver synthetic function is extremly rare. *Aims.* To present a rare case of isolated hypofibrinogenemia as acquired hypocoagulable state in patient with prostate carcinoma /CaP/. *Case report. Methods and Results.* An 80 years old man was admitted in our hospital because of signs of massive hematomaS in the left forearm and chest and abdomen wall and symptoms of intensive back pain. Immediate laboratory evaluation was performed and showed anaemia and mild thombocytopenia (Hb 77g/L, Hct 0,28, MCV 89, Leu 5, Neutr 71%, Plt 103) and elevated lactate dehidrogenase /LDH/level (1551 IU/L) and alcaline phosphatase /AP/level (1016 U) with normal concentrations of all other biochemical parameters including total proteins, albumine, alanin-and aspartate aminotransferase, gama glutamil transpeptidase and total bilirubin. In coagulation screeneng test TT was prolonged (31,9'), with normal PT(INR) and APTT. D-dimer was slightly positive, fibrinogen was low (0,98 g/L) but FDP was negative as well as Lupus anticoagulant. All examined coagulation factors (II,V, VII, VIII, IX) and AT III were in normal ranges. CT scans of head, chast and abdomen showed as the only pathologic finding enlarged infiltrative prostate and osteolytic bone lessions in vertebres L5-S1. Prostate specific antigen /PSA/ was very high (218 ng/mL) with high free PSA (>25 ng/mL). that indicated that in patient exist prostate carcinoma/CaP/ with metastases invertebral columns that resulted in elevated AP and LDH. There were no signs of liver involvement and impared hepatic synthetic function. *Summary and Conclusions.* According to prformed tests and given results we concluded that the cause of bleeding disorder in our patient was acquired hypofibrinogenemia which is very rare paraneoplastic phenomena. Patient was treated with daily transfusions of cryoprecipitate with no long-time improvement. Than specific anti-tumor therapy was initiated (ciproteron acetate) and in a two weeks after its initiation fibrinogen concentration and TT turned to normal ranges. That was the clinical proof of our statement.

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SEQUENTIAL THERAPY WITH AMPHOTERICIN B (AMB) AND POSACONAZOLE COMBINED WITH SURGERY AS TREATMENT OF RHINOSINUS AND PALATAL ZYGOMYCOSIS IN UNRELATED ALLOGENEIC STEM CELL TRANSPLANTATION RECIPIENT

E. Simeone,[1] A. Candoni,[1] F. Patriarca,[1] F. Costa[2], M. Robiony[2], R. Fanin[1]

[1]Division of Hematology, UDINE; [22] Department of Maxillofacial Surgery, University Hospital, UDINE, Italy

A 46-year-old man was diagnosed with follicular lymphoma. The patient showed partial remission (PR) after 6 cycles of CHOP-rituximab, autologous stem cell transplantation, and radiotherapy. In July 2006, the patient relapsed with multiple pulmonary nodules and he was treated with Y90-Ibritumomab tiuxetan achieving a second PR. In October 2006, he underwent HSCT from a fully matched UD with a RIC regimen. Cya and MTX were administered as GVHD prophylaxis. He was isolated from day -7 before HSCT and received prophylactic anti-fungal treatment with Fluconazole iv 400 mg/day. On day +6 he developed a blackened area of the left posterior maxillary mucosa, fever and local pain. On day +8, the blackened area had involved on both palatal and vestibular side. A facial CT scan showed thickness of the whole left maxillary sinus and of part of the right maxillary sinus without bone erosion. The biopsy showed infiltration of the mucosa by non septate hyphae that were morfologically compatible with Zygomycosis. Fluco was discontinued and 5 mg/kg/day Liposomal AmB was started. Fever and pain improved and a PMN count above 1×10^6/L was reached on day +11. The patient underwent a left hemimaxillectomy on day +21. The histological examination showed large areas of necrosis involving the bone, muscular, fat, and mucosal tissues and invasion by hyphae; the resection borders were infiltrated. When the patient was able to initiate oral feeding, AmB was withdrawn (total dose10.5 g) and Posaconazole oral suspension was started 800 mg/day (for 5 months). At present the patient was in CR, without clinical signs of chronic GVHD and no evidence of fungal disease recurrence. This case is interesting because: a) The IFI occurred early post HSCT and pts was probably previously colonized; b) Posaconazole after AmB allowed a long-lasting and well-tolerated therapy able to eradicate the infection. c) The new antimycotic drugs combined with surgery have improve the prognosis of these severe mycotic infections. d) These infections require a prompt and multidisciplinary approach.

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CORRELATION OF ACUTE PHASE PROTEINS (CRP AND FERRITIN) TO THE WHITE BLOOD CELL NUMBER AND SEDIMATATION RATE

I. Passalidou, P. Karapavlidou, M. Chasios, A. Outas

General Hospital Kastoria, KASTORIA, Greece

Aims. The purpose of this study was to examine the correlation of CRP and ferritin as acute phase proteins with the white blood cell (WBC) number and the sedimentation rate. *Methods.* They were examined 356

incidences during the first semester of 2005, with either CRP or ferritin levels elevated and their values were collated to the WBC count and the sedimatation rate. CRP and ferritin were determined with latex immunoturbidimetry, WBC count with an automated haematology analyzer (Sysmex, Roche) and the sedimatation rate with the Westergreen method. *Results.* From the 356 samples examined 99 (27.9%) had both CRP and ferritin levels elevated. From these samples, 37 (10.5%) had both WBC and sedimentation rate values elevated, 17 samples (4.8%) had only WBC values elevated and 32 samples (9%) only sedimentation rate values elevated. In 13 samples (3.6%) both WBC and sedimentation rate were within the normal ranges. From the 356 samples examined, 235 samples (65.9%) had only CRP elevated whereas ferritin was within the normal range. From these samples, 61 samples (17%) had both WBC and sedimentation rate elevated, 33 samples (9.3%) had only WBC count elevated and 64 samples (18%) had only sedimentation rate elevated. In 77 samples (21.6%) both WBC count and sedimentation rate values were within normal ranges. Finally, from the 356 samples examined only 22 samples (6.2%) had elevated ferritin levels with CRP within the normal range. *Conclusions.* The results indicate that CRP is increased in 94% of the incidences examined. CRP increases before the sedimentation rate and decreases back to normal more rapidly. Increase in the sedimentation rate is more often than the relevant WBC increase. It has been also noticed that in a great number of incidences CRP was increases whereas WBC and sedimentation rate were not. Also, increase in ferritin levels not accompanied by increase in CRP levels was noticed in 6% of the cases, especially in indivividuals with chronic renal failure undergoing renal analysis.

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ROTHIA MUCILAGINOSA BACTEREMIA IN A PATIENT WITH ACUTE MONOBLASTIC LEUKEMIA

M.C. Mateos, J.M. Arguiñano, L. Torroba, M.A. Ardaiz, Y. Burguete, M.A. Goñi, M. Echeveste, M.J. Paloma, M. Redondo, M.A. Labaca, I. Ezpeleta, F.J. Oyarzábal

Hospital Virgen del Camino, PAMPLONA, Spain

Febrile neutropenia is a common problem for hematologists when treating malignancies such as acute leukaemia. Empirical broad spectrum antibiotic therapy is the cornerstone of management, but cultures must be taken in order to clarify the etiology. Roughly 20% of febrile episodes in neutropenic patients render positive blood cultures, but isolation and identification of pathogens is crucial. Thus centers know pathogens usually causing these episodes and are aware of those so-called emerging pathogens. These cause severe disease in immunocompromised hosts and are likely to become more important as more aggressive therapies are used for malignancies. We present the case of a 30 year old female diagnosed with acute monoblastic leukaemia with the t(9; 11)(p22; q13). Chemotherapy comprised idarubicin and cytarabine as well as intrathecal therapy. A first initial episode of fever in aplastic phase required cefepime, amikacin and vancomicin as well as liposomal amphotericin B. Teicoplanine substituted for vancomicin due to skin rash. Bad response to initial chemotherapy prompted reinduction therapy with idarubicin, cytarabine and etoposide. This caused severe mucositis and eleven days after chemotherapy a new episode of fever and neutropenia started. When Rothia mucilaginosa was isolated in our laboratory teicoplanin was associated to initial empirical therapy with cefepime. Later on, cefepime was substituted by meropenem, due to fever persistence. This eventually resolved febrile episode. *Comments.* This is the first case of Rothia mucilaginosa bacteremia detected in a patient with febrile neutropenia in our center. Rothia mucilaginosa, formely known as Stomatococcus mucilaginosus is a gram positive coccus which usually is part of the normal flora of mouth and upper respiratory tract. It is considered as an emerging grampositive pathogen causing disease in immunocompromised hosts, mostly in those with mucositis. In other studies this pathogen comprises about 5,9% of positive blood cultures in febrile neutropenic patients. All cases of documented bacteremia have one or more risk factors. Most common is profound neutropenia (less than 100 cells per cubic milimeter) and others are chemotherapy related mucositis, gingivitis and other infections. All of them favor barrier crossing and entering the bloodstream mainly through oral mucosa. Anti H2 therapy, widely used in these patients, allows extension of oral flora to stomach and intestine, thus allowing new ways of invasion. Furthermore, this pathogen can be selected by ciprofloxacin or trimetoprim-sulfametoxazole antibiotic prophylaxis. Most patients (67%) carry indwelling vascular devices in the moment of bacteremia. Most Rothia mucilaginosa strains are susceptible to antibiotics affecting gram positive cocci, mainly vancomicin and some betalactamics. Resistance to penicillin, meticilin, aminoglucosides and trimetoprim-sulfametoxazole are variable. Defervescence of fever is slow, taking 3 to 12 days, but prognosis of infection is good if vancomicin or an efficacious betalactamic is used. Our patient had the typical risk factors except for antibiotic prophylaxis, but broad spectrum antibiotics had been used in a previous febrile episode. Disappearance of fever took several days, as expected with these grampositive cocci infections.

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CENTRAL VENOUS CATHETER-RELATED INFECTION IN CHILDREN WITH ONCOLOGY/HEMATOLOGICAL DISEASES

O. Muratovska

Pediatric Clinic, SKOPJE, Macedonia

Beckround. Central venous catheter (CVC) is an essential component of multidrug chemotherapy. The placement of CVC devices not only permits the dilivery of these copmlex therapeutic regimens be also drastically improves patient's quality of life. Device-related infection is thought to be greated for tunneled external catheters (double lumen Hickman Broviac-DL-HB) as compared to subcutaneous inplanted port (SP). Infection rates differ significantly with respect to patient characteristic, device maintenance scedules and diagnostic criteria for defining a device-related infection. *Aims.* To evaluate device-related infections in children with malignant diseases in children with tunneled external catheters and subcutaneous inplanted ports. *Methods.* The study was conducted between October 2005 and January 2008. During the study period 55 central venous catheters (30 subcutaneous inplanted ports and 25 double lumen Hickman Broviac catheters) were monitoring for present of catheter- related infection. Results. During the study period 55 placed venous catheters (30 SP and 25 DL-HB catheters) were consecutively inserted in 51 children (26 male, 24 female) with hemato-oncological diseases. Four of them had more than one CVC insertion. Among the study patients 15 had solid tumor, 31 had acute leukemia and 5 had lymphoma. Patient's age at CVC insertion ranged between 1 year to 19 years. The overall length of observation range between 30 days and 520 days. There were 21 (38%) bloodstream infections and 16 of them had gram-positive cocci (13 had coagulase-negative staphylococci, 1 had Streptococcus pneumoniae, 1 had Streptococcus viridans) Six patients had gram-negative bacilli (Enterobacteraceae, Escherichia coli and Pseudomonas species). There was not fungal infection. Eleven (42%) patients had symptoms of sepsis and all of them were in neutropenic phase. Seven patients (63%) of them were younger than 6 years and all of them had SP. Seven patients had colonisation of the catheter and three patients had site infection in our study, there was not statistically significant increased risk of infection between SP and DL-HB catheters. Maintenance procedures were performed by trained pediatric nurses in the Oncology department. *Conclusions.* To define CVC- related infection in children for the purpose of prevention criteria which are necessary for increasing practical use. Newer antimicrobial and antiseptis devices and maximal sterile barrier precautions should be continue to develop.

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MEROPENEM THERAPY IN HEMATOLOGICAL PATIENTS WITH NEUTROPENIA

L. Ocroteala, N. Fraticiu, G. Gaman

Municipal Hospital Filantropia Craiova, CRAIOVA, Romania

Background. Because many chemotherapeutic agents work indiscriminately on dividing cells, one of the major toxicities of chemotherapy is myelosuppression. In fact, myelosuppression is often the dose-limiting toxicity of chemotherapy. All three blood cell lineages can be affected, resulting in neutropenia, thrombocytopenia and anemia. Neutropenia, within the hematological diseases, imposes ultra-broad spectrum antibiotic administration. Feverish neutropenia is define as neutrophils number under 1000/mm^3 (with a possible decrease under 500/mm^3) and a body temperature above 38,3°C. Meropenem, a carbapenem antibiotic, has a bactericide action by interfering with the bacterial wall synthesis. *Aims.* to evaluate the efficiency and adverse events due to the Meropenem in hematological patients with neutropenia. *Methods.* The study included 38 patients (7 acute leukemia, 11 chronic lymphatic leukemia, 9 Non-Hodgkin malignant lymphoma, 1 aplastic anemia), with the mean age 57 years, hospitalized for 90 days in the Hematology Department, Municipal Hospital Filantropia Craiova. During posttherapeutic pancitopenia, they received Meropenem 1g at every 8 hours, for 7-10 days. *Results.* The neutropenia episodes were classified as high, moderate or low risk depending on their duration (>14 days, 8-14 days or<7days):7,

25 and 6 respectively. In 7 patients (18,4%), infection was not microbiologically documented, but in 31 patients (81,5%) the biological tests included venous blood (leukocyte formula, hepatic and renal damage markers, C reactive protein)), pharyngeal exudates, urine and catheter fragment tests. In all patients we had chest (pulmonary) radiograph. The most frequent initial clinical focus were in lung (41,9%), oropharynx (35,4%), skin (6,4%), digestive (6,4%) and catheter access sites (9,6%). Gram-negative bacilli was the most frequently isolated pathogen (61,2%), followed by Gram-positive bacteria (35,4%) and fungus (6,4%). The most common Gram-negative pathogens were *E.Coli* (73,6%), Klebsiela pneumonie (15,7%), pseudomonas aeruginosa (10,5%) and Gram-positive pathogenes were staphylococcus epidermidis (60%), staphylococcus haemolyticus(20%), staphylococcus aureus (10%) and Streptoccocus viridans(10%). Evolution was favorable in all patients, without hepatic or renal damage(normal hepatic and renal probes), with the high body temperature period significantly lower than in other antibiotic administration (p<0,05). *Conclusions.* The high tolerability of Meropenem, in post chemotherapeutic neutropenia patients, as well as the limited side effects are recommending it as a first intention antibiotic therapy in these patients.

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QUALITY OF LIFE OF THALASSAEMIA PATIENTS: EVALYATION BY SF36 (SHORT FORM HEALTH SURVEY) TEST

F.K. Farmaki, Ch. Pappa, Ch. Trompoukis, I. Tzoumari

General Hospital of Corinth, CORINTH, Greece

Background. Aristode interrelate *well being* with will and virtue. Quality of life is the result of a number of dimensions relating to both physical and mental capacity. The measurement of Health-Related-Quality of Life (HRQoL) is recognized worldwide as the cornerstone of planning and valuation of healthcare therapeutic interventions. *Aims.* To validate the quality of life of patients followed in our Thalassaemia Unit Methods. The SF-36 (Short-Form-Health-Survey) is an international health utility which is applied as well in the general population, as in patients suffering from a chronic disease. The SF-36 questionnaire was translated in Greek by the doctors of Thalassaemia Unit and was filled out during personal interviews with the patients in order to reinforce question's understanding and diminish the missing value rate. The 36 questions are concerning physical and mental functioning scales. The score of the scales were converted to percentage per centum ratio, where 0 represent the reserve and one hundred per cent the fine fettle. Patients: 53 thalassaemic patients participated to the study, 25 men and 28 women of mean age 35,3+3,6 years. The 38% were alumni of higher learning. The 24,5% were married and 15% of them had children. *Results.* 1) Physical functioning scales: Physical Functionality (PF): average=86,6% (men=85,2%, women=87,9%); Physical Role (RP): average=76,8% (men=74%, women=79,5%); Bodily Pain (BP): average=78,6% (men=83%, women=74,1%); General Health (GH): average=58,5% (men=56,6%, women=60,2%). 2) Mental functioning scales: Vitality (VT): average=69,3% (men=68,8%, women=69,8%); Social Functionality (SF): average=76,9% (men=77,7%, women=76%); Emotional Role (RE): average=74,9% (men=76%, women=73,8%); Mental Health (MH): average=69,5% (men=69,9%, women=69%). *Conclusions.* Although β-Thalassaemia major is a chronic disease, 17% of our patients considered their health exceptional and in general their score is greater in physical than in mental functioning. Women reveal better are general health than men, in contrast to respective studies on general population. Age is an important factor which reflects as much physical as mental health which decline progressively. Married patients had higher score in contrast with bachelors and married patients with children.

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RARE GROSS DELETION IN TCIRG1 GENE IN IRANIAN FAMILY WITH INFANTILE MALIGNANT OSTEOPETROSIS

A. Modaresi, M.R. Abbaszadegan, E. Dadkhah, F. Khadivi-Zand, A. Velayati

Mashhad University of Medical Sciences, MASHHAD, Iran

Background. Infantile malignant osteopetrosis (OMIM: 259700) is an autosomal recessive disorder, manifests by severe osteosclerosis within the first decade of life. Mutations in TCIRG1 (T-cell immune regulator 1) gene encoding osteoclast-spesific 116-kD subunit of H+-ATPase named as a3 subunit were found as the cause of infantile malignant osteopetrosis type.

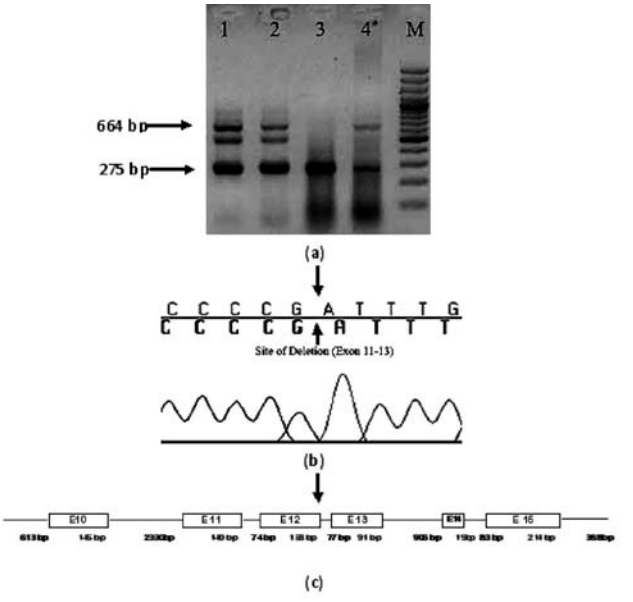

Figure 1: (a) The result of RT-PCR amplification.

Recent research found that mutations in TCIRG1 gene are responsible for about 50% of patients. We found the first Iranian patient with a rare gross deletion identified in this gene. *Case presentation*. Z.A. was a 5 year old girl referred to hematology department of Dr Sheikh pediatric hospital with macrocephaly, facial dysmorphism, blindness, mental retardation and hepatosplenomegaly. Laboratory investigations revealed pancytopenia. Radiological images showed osteosclerotic changes in skull and limb. With these findings she was referred for molecular analysis of osteopetrosis disease. Molecular analysis was performed using RT-PCR for exon 10-19 of TCIRG1 gene followed by whole gene sequencing using an ABI 3730 capillary system automated sequencer. The patient showed a 275bp unexpected amplified segment in PCR experiment. Sequencing of the PCR product revealed a gross deletion in exon 10-15 transcript region of TCIRG1. This deletion affected codon 389 to 518 including entire exon 11 to 13 of the gene. *Conclusions.* Various types of mutations in the TCIRG1 gene in infantile malignant osteopetrosis have been reported in different populations; however, gross deletions are reported rarely. This gross deletion of exon 11-13 in infantile malignant osteopetrosis is the first mutation reported among Iranian patients in this gene. This deletion is also the largest deletion of TCIRG1 gene reported until now.

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BORTEZOMIB PLUS CONVENTIONAL CHEMOTHERAPY IN POOR RISK ACUTE MYELOID LEUKAEMIA: A PILOT STUDY

F. Leoni, S. Ciolli, R. Fanci, A. Bosi

Haematology Unit, FLORENCE, Italy

Background. as relapsing AML patients develop resistance to standard chemotherapy and new therapeutic strategies which may overcome drug resistance through other cellular pathways are needed. Bortezomib, a proteasome inhibitor, has an anti-proliferative and pro-apoptotic effect either in rapidly proliferating leukemic cell lines or primary AML cells. Noteworthy, leukemic stem cells are significantly more sensitive to proteasome inhibition than normal progenitor cells thus resulting in a favourable therapeutic index. However, when bortezomib was applied as a single agent in AML, a modest and transient anti-leukemic activity was observed and no CR was reached. *In vitro*, proteasome inhibition may increase the sensitivity of leukemic cells to traditional anticancer agents. The addition of bortezomib to anthracyclines results in synergistic citotoxicity to leukemia cells and, specifically, to leukemia stem cells by targeting the aberrantly activated NF-kB. Moreover, bortezomib is more active than anthracyclines on immature CD34 positive blast cells, suggesting it may overcome the drug resistance associated with a stem cell phenotype. Several mechanisms of chemoresistance are affected by bortezomib (bcl-2, P-glycoprotein and DNA repair pathways) resulting in chemosensitization of tumour cells to DNA damaging agents. At same time, anthracyclines may enhance bortezomib activity by suppressing the bortezomib-mediated induction of the heat shock response

pathways that may limit its effectiveness. Thus, although off label in AML, we added bortezomib to conventional chemotherapy in advanced AML. *Aims of the study.* To investigate safety and efficacy of bortezomib plus standard chemotherapy in relapsed/refractory AML. *Methods.* patients with advanced disease (primary refractory or relapsed early after an allogeneic transplant) with an adequate renal, hepatic and cardiac function who were eligible for Ida-FLAG or FLAG plus Mylotarg re-induction. A single dose of bortezomib 1.5 mg/m^2 was delivered 30' before the first infusion of idarubicin or mylotarg. Responding patients were deigned to receive a second cycle as consolidation. They all provided written informed consent and agreed to use contraception; women were required to have a confirmed negative pregnancy test result before enrolment. *Results.* From October 2007, six patients entered the study: 3 males and 3 females , age 43-65 years. Four had relapsed after an allogeneic transplant, 1 was primary refractory and 1, relapsed after an autologous transplant, had failed a re-induction attempt. According to age, chemotherapy consisted of bortezomib plus Ida-FLAG (3 pts) or FLAG/Mylotarg (3 pts). All patients experienced prolonged myelosuppression. Mean time to recovery of granulocytes >1.0/μL and platelets >20×10^9/L was 41 and 32 days, respectively. Two patients had Gram-positive sepsis and two had FUO. Symptomatic decline of LVEF was recorded in one patient who previously received a total of 108 mg/m^2 of idarubicin. Neurotoxicity was not recorded. Three patients achieved CR and 3 obtained the clearance of bone marrow blasts but with incomplete recovery of blood counts (CRp). *Conclusions.* the results we achieved demonstrates the addition of bortezomib to standard AML chemotherapy is feasible, efficacious and devoid of significant additive toxicity. Further studies are warranted to explore the potential beneficial effect of adding bortezomib to anthracycline in AML therapy.

1398

THE EXPRESSION OF MULTIDRUG RESISTANCE GENES (MDR1, MRP1, BCRP, LRP) IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

M. Cioch, J. Kocki, M. Jarosz, A. Dmoszynska

Medical University, LUBLIN, Poland

Introduction. Refractory and relapsed Acute Myeloid Leukemia (AML) is associated with very bad prognosis. One of the major reason of chemoresistance is the expression of genes and proteins responsible for multidrug resistance: Multidrug Resistance 1 (MDR1), Multidrug Resistance-Related Protein 1 (MRP1), Breast Cancer Resistance Protein (BCRP) and Lung Cancer Related Protein (LRP). The aim of the study was the evaluation of the influence of MDR genes expression on results of chemotherapy in patients (pts) with AML. *Material and Methods.* The study was performed in 19 pts (11 females and 8 males; ranged from 21 to 61 years - mean 48.5) with newly diagnosed AML. There were following types acc. FAB classification: M1-1, M2-4, M4-13, M5-1 and cytogenetic risk groups: favorable-4, intermediate-13 and unfavorable-2 pts. The expression of MDR1 protein measured in flow cytometer by labeling leukemic cells with the UIC2 monoclonal antibody was low (<20%). The genes expression in leukemic cells was detected by RT-PCR method. All pts were treated with induction therapy acc. Polish Adult Leukemia Group (PALG). *Results.* The expression of MDR1 gene was detected in 9, MRP1 in 4, BCRP in 2 and LRP in 5 pts. The mean expresssion of MDR1 protein was significantly higher in the group with expression of MDR1 gene than in the group without such expression. When expression of MDR1 gene was single, complete remission (CR) was obtained in 75% pts, but when the expression of MDR1 gene was associated with the expression of BCRP and LRP, CR was never obtained. In all 5 pts with complex genetic changes (3 MDR1+MRP1, 1 MDR1+BCRP+LRP, 1 MDR1+MRP1+BCRP+LRP) CR after one course of chemoterapy was not obtained. All these pts belonged to intermediate cytogenetic risk group, because of normal karyotype. There was significant difference between CR rate after one course of chemotherapy in pts without MDR gene expression or expression only one gene and in pts with expression of two or more genes (p=0.017). *Conclusions.* Results of studies on expression of MDR genes in pts with AML showed that expression of MDR1 gene associated with low epression of MDR1 protein was not associated with resistance on chemotherapy, but associated with other MDR genes, despite of low MDR1 protein expression influenced on chemoresistance. These findings may have important significance in defining of prognosis in AML with normal karyotype and individualization of induction and postremission therapy.

1399

SINGLE DOSE OF PEGYLATED RECOMBINANT FILGRASTIM (PEG-FILGRASTIM) AFTER CONSOLIDATION CHEMOTHERAPY FOR ACUTE MYELOID LEUKEMIA IN FIRST COMPLETE REMISSION

A. Candoni, S. Buttignol, M. Tiribelli, S. Lovato, E. Simeone, A. Michelutti, D. Damiani, R. Fanin

Division of Hematology, UDINE, Italy

Background. Clinical trials are currently expanding the experience with Peg-filgrastim in a variety of solid tumours and hematologic malignancies. Previous reports showed that Peg-filgrastim was as effective as daily Filgrastim after induction chemotherapy in AML in order to reduce the time of recovery from neutropenia. No data are available about the role of Peg-filgrastim after consolidation chemotherapy in AML in first complete remission (CR). *Aims.* Aim of our study was to evaluate the efficacy of Peg-filgrastim in a setting of patients with an expected prolonged and severe neutropenia, both in term of neutrophil recovery and of mobilization of peripheral blood stem cells (PBSC). *Methods.* A total of 32 consecutive AML patients (17 M/15 W), median age 50 years (range: 25-69), in CR after an induction course with fludarabine containing regimen (FLAI) received s.c. Peg-filgrastim (6 mg single dose) 24 hours after completion of a consolidation chemotherapy with cytarabine (2 g/sqm for 6 days) plus idarubicin (12 mg/sqm days 1-3) (26/32), or cyarabine alone (3 g/sqm BID for 4 days) (6/32). *Results.* All 32 case experienced, as expected, WHO grade IV neutropenia after consolidation chemotherapy. Median time form Peg-filgrastim to neutrophil recovery, defined as first of 2 consecutive days with ANC >0.5×10^9/L and as first day with ANC >1×10^9/L, was 15 and 17 days, respectively. The mean peak value of ANC was 6,9±6,3×10^9/L and occurred after a median of 22 days (range: 10-43) from Peg-filgrastim injection. Sixteen (50%) patients experienced infectious complications during the aplastic phase (3 pneumonias, 10 bacteraemias, 1 cystitis and 1 skin abscess) but no infection-related deaths occurred. Peg-filgrastim was well tolerated and only one patient required therapy for drug-induced bone pain. In 34% of cases, with febrile neutropenia 15 days after Peg-filgrastim injection, Filgrastim was administered (mean 3 fl/pts) in order to accelerate ANC recovery. Nine of 32 patients (28%) underwent PBSC apheresis procedures when CD34$^+$ cell count was >10 micronL (mean CD34$^+$ cell count at collection was 105±128 micronL). The mean number of harvested CD34$^+$ cells was 5.0±3.8×10^6/Kg, after a mean time from Peg-filgrastim of 15±6 days. *Conclusions.* Our experience confirms that Peg-filgrastim: 1) was well tolerated after consolidation chemotherapy in AML in first CR; 2) represent a cost-effective alternative to long-term conventional Filgrastim to overcome severe and prolonged neutropenia after consolidation therapy in AML; 3) allow PBSC mobilization and apheresis procedures in AML patients. In our experience only 28% of cases obtained CD34$^+$ cells mobilization and harvest. Nonetheless, this result is quite satisfactory, considering the underling disease (AML) and the previous induction therapy with a fludarabine-based regimen, that is well know to severely impair PBSC mobilization and collection.

1400

EFFICACY AND SAFETY OF GEMTUZUMAB OZOGAMICIN, FLUDARABINE, ARA-C, AND CYCLOSPORINE A (MFAC) AS POST-REMISSION THERAPY IN ELDERLY AML PATIENTS

D. Caramazza, S. Siragusa, R. Palazzolo, S. Maisano, I. Abbene, G. Piazza, G. Quintini

Policlinico Universitario di Palermo, PALERMO, Italy

Background. Acute Myeloid Leukemia in elderly is associated with poor response to conventional chemotherapy and limited survival; this is mainly due to the high incidence of abnormal cytogenetics with multidrug resistance and the low marrow reserve which may prevent/delay the recovery of hematopoiesis after treatment. Gemtuzumab Ozogamicin (GO) is an immunoconjugate with a humanized anti-CD33 that after internalization, releases a cytotoxic drug, calicheamicin. As single agent, GO did not shown advantages in AML patients when compared to standard therapy. However, the role of MFAC in post-remission therapy in elderly AML patients is still debated. *Aims.* To evaluate the efficacy and safety of MFAC regimen, in elderly AML patients, as post-remission therapy to inhibit multidrug resistance mechanisms and eradicate Minimal Residual Disease (MRD). *Methods.* We treated 17 consecutive patients [median age of 72.3 years (56-83)] (Table 1) during the period 2004-2007 with standard induction protocols. These included 1 or 2 courses of Fluda (FLAG, 12 patients) or non-Fluda induction regimens (daunorubicin+Ara-C, 2 patients, and idarubicin+Ara-C+etopo-

side, 3 patients), followed by 2 postremission courses of MFAC (fludarabine 15 mg/m²/bid IV on days 2-4, Ara-C 0.5 g/m²/bid IV on days 2-4, GO 4.5 mg/m²/d IV on day 1 with the addition of cyclosporine A 6 mg/kg bw IV followed by 16 mg/kg bw on days 1-2). Among 11 patients with available baseline cytogenetic data, 6 were in the poor-risk group and 5 were in the favorable-risk group. All patients evaluated had CD33-positive AML cells, analysed by immunophenotyping on bone marrow aspirate. *Results.* Complete remission (CR), characterized by i) ≤5% blast in the marrow, ii) recovery of neutrophils to ≥1.5×10⁹/L and iii) RBC and platelet transfusion-independent, was achieved in 70.6% of patients (12/17). Ten patients received 2 MFAC courses, 2 patients did not receive the second MFAC course because of disease progression (1 patient) and infection (1 patient). Subsequently, MFAC was given to 4 patients twice every 4 weeks for maintenance therapy. Ten patients (58.3%) obtained a durable CR. Median relapse free survival was 12.4 months (3-22) and median overall survival was 13.6 months (3-24). The most common non-hematological toxicities were infections; no grade 3 to 4 liver toxicity and veno-occlusive disease were observed. Median duration of neutropenia (ANC<0.5×10⁹/L) and thrombocytopenia (PLT <50×10⁹/L) after induction and consolidation regimens was 18 (13-22) and 11 (7-14) days, respectively. *Conclusions.* Our data support the use of GO in combined regimens for AML. MFAC was feasible and well tolerated thus producing an encouraging CR rates after induction regimens. These data need to be supported in properly designed clinical trials.

Table 1.

Patients Characteristics		N=17	
Age (years)		72,3 (median)	56-83(range)
Performance status	WHO	**No**	%
	0 - 1	11	64,7
	2	1	5,9
	3	5	29,4
Diagnosis			
	AML de novo	8	47,1
	Secondary AML	1	5,9
	AML developed from MDS	7	41,2
	AML in 1st relapse	1	5,9
Induction regimens			
	Fluda (FLAG)	12	70,6
	Non-Fluda (3+7, ICE)	5	29,4
Cytogenetic groups			
	"poor" risk	6	35,3
	"favourable" risk	5	29,4
	no tested	6	35,3
MFAC 1st course		12	70,6
MFAC 2nd course		10	58,8
Outcome :			
CR rate post-induction		12	70,6
OS (months)		13,6 (median)	3- 24 (range)
RFS(months)		12,4 (median)	3- 22 (range)

1401

ASSOCIATION BETWEEN CHROMOSOMAL ABNORMALITIES DETECTED BY FISH AND ATYPICAL CELL MORPHOLOGY FEATURES IN CHRONIC LYMPHOCYTIC LEUKEMIA

E. Wawrzyniak, A. Palacz, J.Z. Blonski, T. Robak

Med Univ of Lodz, LODZ, Poland

Background. Chronic lymphocytic leukemia (CLL) has been characterized by heterogeneity of clinical outcome and possibly a different molecular genetic basis. In part of CLL patients atypical morphology of leukemic cells can be observed, which is often correlated to trisomy 12. *Aims.* The aim of our study was to investigate whether an association exists among different types of chromosomal abnormalities and cytomorphology of leukemic lymphocytes. *Patients and Methods.* A total of 102 patients with CLL diagnosis were analyzed (72 males and 30 females, mean age: 64, range: 28-84 years). There were 11 patients in stage 0 according to Rai, 61 patients in stage I+II and 30 patients in stage III+IV. Interphase FISH on peripheral blood smears prior to the start of first line chemotherapy was performed. Four commercial probes were used (Vysis): probes for loci D13S319 (13q14.3), the p53 gene (17p13.1), the ATM gene (11q22.3) and centromeric probe for chromosome 12. Simultaneously May-Grunwald-Giemsa stained peripheral blood films were reviewed. Leukemia was classified as atypical (aCLL) when there were more than 10% circulating prolymphocytes or more than 10% cells had cleaved nucleus and/or lymphoplasmacytic features. *Results.* Chromosomal aberrations were observed in 73/102 (72%) and atypical morhology in 31/102 (30%) patients. The most frequent chromosomal abnormality was deletion 13q14 (48%). Typical CLL morphology was significantly associated with 13q14 deletion (55% in typical CLL *vs* 32% in aCLL, p=0.0286), especially with 13q14 deletion as a sole anomaly (32% in typical CLL *vs* 10% in aCLL, p=0.0116). Trisomy 12 was observed in 15% of all cases and demonstrated the strongest correlation to aCLL of all analyzed abnormalities (4% in typical CLL *vs* 39% in aCLL, p=0.0001). Deletion of 11q23 was detected in 23% of all patients without a significant correlation to CLL morphology (21% in typical CLL *vs* 26% in aCLL). Deletion of 17p13 occurred in 13% of all patients. This abnormality was distributed equally between the morphological subtypes of CLL (14% in typical *vs* 10% in aCLL, n.s.). The frequency of aCLL cases in the group with trisomy 12 as sole abnormality or with other chromosomal abnormalities in relation to other cytogenetic groups was shown in Table 1. *Conclusions.* We conclude that atypical morphology is usually associated with trisomy 12 (adverse or intermediate prognostic factor) and is very rarely observed in patients with sole deletion 13q14 (favourable prognostic factor). This observation confirms clinical importance of atypical morphology of CLL lymphocytes.

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Table 1.

Cytogenetic groups	n	aCLL	P value
trisomy 12 vs:	15	80%	-------
- del(13)(q14) as sole abn.	26	11%	0.0001
- del(11)(q22) as sole abn. or with del(13)(q14)	20	35%	0.0097
- del(17)(p13) as sole abn. or with del(13)(q14)	12	17%	0.0016
- normal karyotype	29	24%	0.0005

1402

IMMUNOPHENOTYPIC CHARACTERIZATION OF PERIPHERAL BLOOD CELLS FROM RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS TREATED WITH LUMILIXIMAB IN COMBINATION WITH FCR

S. Tangri,[1] A. Estrellado,[1] J. Byrd[2], S. O'Brien,[3] T. Kipps,[4] A. Cesano,[1] H. Mu,[1] S. Harris[1]

[1]Biogen Idec, SAN DIEGO; [2]The Ohio State University Comprehensive Cancer Center, COLUMBUS; [3]The University of Texas/MD Anderson Cancer Center, HOUSTON; [4]Moores Cancer Center University of California San Diego, SAN DIEGO, USA

Background. Lumiliximab is an anti-CD23 monoclonal antibody under investigation for the treatment of patients (pts) with relapsed chronic lymphocytic leukemia (CLL). The 152-30 clinical study evaluated lumiliximab in combination with fludarabine, cyclophosphamide, and rituximab (L+FCR), and achieved a high CR rate (52%). *Aims.* A comprehensive immunophenotypic characterization of CLL cells obtained from patients treated in 152-30 was performed including an analysis of frequencies of CLL cells (CD5+CD19+), and expression of CD23, CD38, CD55 and CD59 antigens on these CLL cells. *Methods.* Immunophenotypic characterization of peripheral blood cells was performed by four color flow cytometry analysis. CLL cells were identified by gating on viable CD45⁺ cells coexpressing CD5 and CD19. Antigen expression levels and their frequencies were reported in terms of mean fluorescence intensities (MFI) and fraction (%) of positive CLL cells. *Results.* The analysis of peripheral blood samples from L+FCR treated pts indicated that a substantial decrease in CLL cells in 30 of the 31 evaluable pts. A majority of patients (90%) had CD23⁺ CLL cells and clinical activity was observed in pts with both high and low levels of CD38 and CD23 expression. Finally, clinical activity of L+FCR was independent of pretreatment expression levels of CD55 and CD59 antigen and was

observed in a few pts showing increased expression of CD59 receptor post therapy (potential factors of rituximab resistance). *Conclusions*. Clinically, L+FCR is an effective regimen in decreasing tumor burden in the peripheral blood of pts with relapsed CLL. Moreover, clinical activity with this regimen is independent of the low and high levels of CD23 expression and CD38 expression (a poor prognostic factor) and thereby may provide clinical benefit to a subset of pts with a phenotype indicative of unfavorable clinical outcomes. Clinical activity was also observed in pts with CLL expressing high levels of CD59 antigen, thus, suggesting a different MOA than other mAbs active in this indication.

1403

ANGIOPOIETIN-2 MRNA EXPRESSION IS INCREASED IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA WITH UNMUTATED IGVH GENES

F. Vrbacky,[1] L. Smolej,[1] V. Vroblova,[1] S. Pekova[2], K. Sarounova,[1] M. Pecka,[1] J. Maly[1]

[1]University Hospital and Faculty of Medicine, HRADEC KRALOVE; [2]Na Homolce Hospital, PRAGUE, Czech Republic

Background. Angiogenesis is considered to play an important role in pathogenesis of chronic lymphocytic leukemia (CLL). Angiopoietin-2 (Ang-2) belongs to important cytokines regulating neovascularization. Elevated expression of Ang-2 has been reported in several hematological malignancies; however, data regarding Ang-2 in CLL are very limited. *Aims.* To quantitate Ang-2 mRNA in purified mononuclear cells of untreated CLL patients and compare transcript levels with different prognostic factors. *Methods.* Ang-2 mRNA levels were analyzed in Ficoll-purified mononuclear cells from 24 untreated CLL patients by real-time quantitative PCR and normalized for differences in RNA concentration in each sample by quantitation of transcript of Abl1 housekeeping gene. ZAP-70 and CD38 expression was analyzed by flow cytometry. IgVH mutation status was assessed according to usual methodology using cDNA and IgBLAST database. *Results.* Elevated Ang-2 mRNA concentrations were detected in 10 cases (Ang-2 to Abl1 expression ratio > $5*10^{-3}$). On the other hand, 14 patients had very low or undetectable levels of Ang-2 mRNA (Ang-2 to Abl1 expression ratio <$5*10^{-3}$ or Ang-2 Cp >35). There was a significant association between high Ang-2 mRNA levels and unmutated IgVH genes (n=21, p=0.012), but not with CD38 (p=0.057), ZAP-70 expression (p=0.410) Rai stage 0 *vs* I-II (p=0.069) or clinical course (stable *vs* progressive, p=0.39). *Conclusions.* Our pilot data show that Ang-2 mRNA is differentially expressed in mononuclear cells of patients with CLL and its increased expression is associated with unmutated IgVH genes. Further studies with larger patient cohorts are needed to confirm our findings.

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1404

THE INFLUENCE OF CELL SOURCE AND CD34-SUBTYPES ON THE STEM CELL REGENERATIVE POTENTIAL

M. Jevtic, B. Balint, G. Ostojic, D. Vojvodic, B. Gligic, R. Ilic

Military Medical Academy, BELGRADE, Serbia

Background. Stem cells (SCs) could be defined as cells having 'unlimited' self-renewal and multilineage differentiation capacity, as well as extensive proliferative potential which guarantee the homeostasis of the hematopoietic or other tissue-generating systems. The clinical use of SCs represents an unique and well-working regenerative therapeutic maneuver. *Aims.* The objective of this investigation was to establish SC-harvesting protocol with optimized cell source, CD34+ and CD133+ threshold-dose (calculed by ideal body mass), as well as to evaluate how specific immature *vs* mature CD34-subsets ratio may influence on the SC-therapy outcome. *Methods.* Patients with myocardial ischemic disease (including coronary-bypass-group) underwent to cell-based (regenerative) therapy were included in this study. SCs were collected by multiple aspirations from bone marrow (patients). SCs were mobilized applying rHuG-CSF and afterward they were harvested from peripheral blood using Gambro BCT-Spectra (control group). Total nucleated and mononuclear cell (TNC and MNC) count was determined by flow cytometry. Cell viability was measured by trypan blue exclusion test or with AAD-viability-dye test. The SC surface antigens (CD34-subsets markers and CD133) by the EPICS XL-MCL device were investigated. Cryopreservation (for potential subsequent therapeutic use), by our own controlled-rate freezing protocol (with optimized DMSO) using Planer 560-16 equipment was accomplished. *Results.* In myocardial cell-therapy setting, the quantity of TNCs, $CD45^+/CD34^+$ and $CD34^+/CD133^+$ applied was $2.4\pm6.1\times10^8$, $10.4\pm7.5\times10^6$ and $7.16\pm4.6\times10^6$, respectively. The use of large volume *vs* conventional (repetitive) harvesting resulted with significantly higher immature *vs* mature CD34-subset ratio (control group). The MNC *vs* $CD34^+/CD133^+$ ratio was significantly elevated in marrow *vs* peripheral blood harvest. Cell-therapy resulted in a improved myocardial perfusion and systolic function repair. All patients tolerated the use of intensive cell-therapy well, without any adverse effects. *Summary and Conclusions.* The use of marrow-derived SCs assured high-quality organ repair due to higher tissue-colonizing (homing) and transdifferentiation (lineage-plasticity) potential, although the ideal source and type of cells in the field of regenerative medicine (myocardial and liver setting) have not been completely defined yet.

1405

DISTURBANCE OF THE PRO-OXIDATIVE/ANTIOXIDATIVE BALANCE IN ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

I. Sari,[1] A. Cetin[2], L. Kaynar[2], R. Saraymen[2], S. Kabukcu Hacioglu,[1] A. Ozturk[2], I. Kocyigit[2], F. Altuntas[2], B. Eser[2]

[1]Pamukkale University Faculty of Medicine, DENIZLI; [2]Erciyes University Faculty of Medicine, KAYSERI, Turkey

Background. High dose chemotherapy results in increased free radical formation and depletion of tissue antioxidants. It is not clearly known whether allogeneic hematopoietic stem cell transplantation (HSCT) has an effect on oxidative stress status. *Aims.* The aims of the study were (1) to determine the effect of allogeneic HSCT on plasma concentrations of major antioxidants and oxidative stress biomarkers, and (2) to investigate their relationships with graft-*vs*-host disease (GVHD), conditioning regimens, and transplant related mortality (TRM) in patients with hematological malignancies. *Methods.* Twenty-five patients undergoing allogeneic HSCT from HLA-matched sibling donors were enrolled into the study. Plasma oxidant and antioxidant status were measured at day -1 before transplantation and 30 after HSCT. *Results.* In both myeloablative (n=14) and non-myeloablative (n=11) transplant group, the mean levels of plasma malondialdehyde (MDA) and nitric oxide (NO) decreased after allogeneic HSCT (p<0.001), whereas the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) increased compared with baseline values (p<0.001). There were statistically significant increases in the mean levels of MDA and NO after allogeneic HSCT (p<0.01). Moreover, there were statistically significant differences between the mean values of pretransplant and posttransplant oxidative stress parameters for the combined data of the entire group of 25 patients. No significant relationship were found between both pretransplant and posttransplant mean levels of oxidative stress parameters and graft-*vs*-host disease (GVHD) existence, type of conditioning regimen, and transplant related mortality (TRM). *Conclusions.* This study revealed a disturbance of the pro-oxidative/antioxidative balance in the plasma of patients undergoing allogeneic HSCT regardless of intensity of the conditioning regimen. Further studies are required in a larger number of patients to explain the biology and chemistry of oxidative stress observed after allogeneic HSCT, its relationship with GVHD, and clinical efficacy of antioxidant therapy after HSCT.

1406

BONE MARROW *vs* EXTRAMEDULLARY RELAPSE OF ACUTE LEUKEMIA AFTER HEMATOPOIETIC CELL TRANSPLANTATION: SURVIVAL AND RISK FACTOR ANALYSIS

F. al Sabty, E. Demeckova, E. Bojtarova, M. Hrubisko, M. Mistrik

University Hospital, BRATISLAVA, Slovakia

Background. A different patterns of relapse has been observed after allogeneic hematopoietic cell transplantation (HCT) for patients with acute leukemia (AL). *Aims.* We have conducted a comparsion of survival between isolated extramedullary relapse (IEMR) and bone marrow relapse (BMR) following HCT for AL. *Methods.* A total of 98 patients with AL (66 acute myeloblastic leukemia, 32 acute lymphoblastic leukemia) recieved transplants from human leukocyte antigen-matched donors, either related (n=89) or unrelated (n=9), preceeded by myeloablative regimen. Median age 33 years (15-57 year). 67 in first complete remission (CR), 15 in second CR, 14 in relapse. *Results.* The 5 year overall survival (OS) was 45%, transplant related mortality (TRM) 19.4% (n=19), overall relapse rate was 29.6% (n=29), IEMR 4.1% (n=4), BMR 25.5% (n=25). The 1 year survival after relapse was 15%. The median survival was 105 days (95%CI=42.5- 176.02) after BMR and 171 days (95%CI=8.3-391.2) after IEMR (Log Rank, p=0.46). Patient's age and sex, donor's age

and sex, conditoning regimen, source of stem cells and matched related or unrelated donor's transplant were analyzed with no significant results. Multivariant analysis showed that disease state at the time of transplantation (p=0.034) and type of disease (p=0.05) were the most important risk factors for overall relapse. *Conclusions.* The post-relapse survival after HCT is generaly poor. There is no significant difference in the survival between IEMR and BMR, however, some patients with IEMR may have a longer survival and a better prognosis than BMR only

1407

PERIPHERAL BLOOD STEM CELLS YIELD IN 57 NORMAL HEALTHY DONORS MOBILISED WITH GLYCOSYLATED GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF)

F. Benedetti, A. Andreini, S. Ledro, M. Sorio, D. De Sabata, C. Tecchio, M. Tinelli, G. Ruggeri, R. Di Bella

Bone Marrow Transplant Unit, VERONA, Italy

Background. Mobilised peripheral blood is now the main source of stem cells collected from normal donors in adult allotransplant-settings. We present our experience in mobilising and collecting 57 normal healthy donors using standardised procedures and techniques. Relatively little information is available about the factors predicting for satisfactory stem cell mobilisation and collection from healthy donors, like age, sex or body weight. Registry data can provide useful information, but it may be difficult to analyze data from different centres. *Material and Methods.* Peripheral blood stem cells (PBSC) were collected from 57 normal healthy donors between January 2005 and November 2007, 37/57 (65%) for related and 20/57 (35%) for unrelated transplant. All donors were mobilised with glycosylated G-CSF at 10 μg/Kg/die s.c. for four days and the 1st apheresis started on day +5 after 9 doses of G-CSF. Further 2 vials of G-CSF were administered when a second apheresis was necessary. Apheresis was performed with Com.Tec, Fresenius, ACD was used as anticoagulant and the target $CD34^+$ recipient cell dose was 4×10^6/Kg. *Results.* Most of the related (72%) and unrelated (71%) donors were male, while the unrelated male and female tended to be younger than related male donors with median ages of 36 and 41, respectively (p<0,001). We found a significant difference in the weight of the two groups, 83 Kg for the males and 57 Kg for the females. The $CD34^+$ cell target of 4×10^6/Kg recipient body was reached after a single apheresis in 49/57 donors (86%). One donor was a poor mobiliser (1.28×10^6/Kg $CD34^+$ cells with 2 aphereses) Even a further bone marrow harvest had a poor yield (1×10^6/Kg $CD34^+$ cells). Male donors achieved significantly greater numbers of $CD34^+$ cells than female donors, both as absolute count and as median range (386 *vs* 261×10^6 $CD34^+$ cells). Nevertheless if we correct the $CD34^+$ cell yield by median donor body weight (83 kg for the males and 57 Kg for the females), we obtain 4,6 and $4{,}5\times10^6$ $CD34^+$/Kg, respectively. *Conclusions.* This single institution study confirms the effectiveness of glycosylated G-CSF mobilisation in a large group of healthy donors for allogeneic transplantation with 86% of requested cell yield after a single apheresis and 98% with two apheresis. The best peak of $CD34^+$ cells was on day +5, after 9 doses of G-CSF. The impact of age was apparently not statistically important while sex apparently was, because $CD34^+$ absolute number and dose collected were higher in males than in females. This was mainly due to their different body weight, that in the end seems to be the best factor predictive for stem cells yield.

1408

LONG TERM FOLLOW-UP OF A MEMBRANOUS GLOMERULOPATHY AS A LATE COMPLICATION OF CHRONIC GRAFT *VS* HOST DISEASE FOLLOWING ALLOGENIC STEM CELL TRANSPLANTATION

L. Cupelli,[1] P. Niscola[2], A. Tendas,[1] T. Dentamaro[2], L. Scaramucci[2], V. Baglio,[3] D. Piccioni[2], M. Giovannini,[1] A. Perrotti[2], R. Palumbo,[3] P. de Fabritiis[1]

[1]Sant'Eugenio Hospital, Tor Vergata University, ROME; [2]Sant'Eugenio Hospital, ASL Roma C, ROME; [3]Nephrology Unit, Sant'Eugenio Hospital, ROME, Italy

Aims. Graft-*Vs*-Host Disease (GVHD) may be rarely cpmplicated by membranous glomerulopathy (MG), which diagnosis may be a challenge. Its recognition may have a critical value given the good response to therapy. *Case report.* A 25-years old man with acute myeloid leukaemia (AML, October 1997), M4 subtype, after a standard anti AML chemotherapy, resulted in a complete remission, underwent allogeneic haematopoietic stem cell transplantation (HSCT) from an HLA-compatible sibling (May 1998). The transplant was conditioned with idarubicin, cyclophosphamide and busulfan. Cyclosporine-A (CsA) and metotrexate were administered as GVHD prophylaxis. The early post HSCT was uneventful and the renal function was always normal. Five months after the allograft, a limited (grade II) chronic GVHD (cGVHD) of the skin was diagnosed and treated with CsA and steroids. About one year after the discontinuation of CsA and steroids, he presented with remarkable oedema of the lower extremities and nephrotic proteinuria without hypertension. A comprehensive laboratory work-up revealed: serum albumin 2.0 g/dL, serum total protein 4.6 g/dL, total cholesterol 350 mg/dL, triglycerides 456 mg/dL, serum creatinine 1.6 mg/dL and a 24-hour urinary albumin loss of 5 gr. Serum immunoglobulin and complement levels were also reduced. A diagnosis of nephrotic syndrome (NS) was made and a renal biopsy was performed. The light microscopy revealed mild thickening of the capillary walls, small areas of tubular atrophy, fibrosis and lymphoid infiltrates. Matrix and mesangium were normal and no features of endothelial damages were found. The immunofluorescence showed subepithelial granular deposits of IgG and IgM along the capillary walls. Electron microscopic (EM) evaluation revealed subepthelial and subendothelial Ig depots. Based on these findings, a diagnosis of MG was made. Therefore, the patient was treated with prednisone (1 mg/Kg/day) achieving a rapid improvement of the signs and symptoms of MG. Prednisone was gradually tapered after one month of treatment and then completely withdrawn after one year. The patient had complete recovery. To date, he is well and active, without any signs of renal disease, 7 years after the diagnosis of MG. Summary. In the allogeneic setting, NS has been rarely described as a late onset nephropathy after conventional HSCT; the most common pathological finding sustaining NS is MG. In our case, cGVHD could have been responsible for an immunomediated MG. In addition, the withdrawal of CsA and steroids should be also considered. Therefore, the diagnosis of MG should be suspected in allogeneic HSCT patients with hypoalbuminemia and NS developing after CsA withdrawal. The renal biopsy and the EM evaluation are essential to diagnose MG, given the several form of kidney damages observed in the HSCT setting. In conclusion, we have described an additional cases of MG outlining that this potentially devastating complication shows a good and sustained response to immunosuppressive treatment, for which a close collaboration with nephrologists and a regular renal monitoring in the follow-up of HSCT patients are mandatory in order to establish an early diagnosis and an appropriate and timely therapy.

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EVALUATION OF THE CELLULAR VITALITY IN UNITS OF PERIPHERAL BLOOD STEM CELLS (PBSC)

F. Zinno, G. Balduino, F. Landi, V. Aureli, G. Isacchi

Tor Vergata University and Bambino Gesù Pediatric Hospital, ROME, Italy

Background. From a lot of time it is in discussion the way in which the haematopoietic stem cells must be preserved before the cryopreservation; in fact some authors define that the temperature of maintenance is 4°C, while others identify the optimal temperature to 20°C. *Aims.* Our study is founded on the evaluation of the cellular vitality on 22 assembled of PBSC coming from patient affections by various oncological and hematological pathologies. The evaluation has been effected on the nucleate cells (NC) and on the $CD34^+$ cells. *Methods.* The evaluation of the vitality has been performed on two samples withdrawn on every units of PBSC, preserved respectively to 4° and 20° C and valued to distance of 24, 48 and 72 hours, both to load of NC and of $CD34^+$ cells. The percentage of $CD34^+$ cells has been calculated with the a progenμtor cell enumeration kit (ProCOUNT, Becton-Dickinson, Milan, Italy) adopting the ISHAGE protocol. We have used nucleic acid dye, phycoerythrin-conjugated antibodies anti-$CD34^+$, and antibodies anti $CD45^+$ for evaluating the cells' vitality we have used 7-aminoactinomycin D, while cell count of NC has been performed using an electronic cell counter.

Table 1.

	24 hours				48 hours				72 hours			
	20°C		4°C		20°C		4°C		20°C		4°C	
	CD34+	NC	CD34+	NC	CD34+	NC	CD34+	NC	CD34+	NC	CD34+	NC
Median	100	97	100	99	95	90	100	98	91	78	99	96
Averag	100	99	100	100	100	95	100	98	91	87	100	97
Min	95	84	100	94	20	73	100	93	0	34	80	88
Max	100	100	100	100	100	99	100	100	100	97	100	99

Results. The average count of NC was 188.790 mm^3 (range 78.110-305.120), while the average value of $CD34^+$ cells results to be 2.296 mm^3 (456-12.684). The data on the found vitality are summaries in the Table 1 (all the values are express in%). It doesn't seem to be correlation among the cellular concentration of NC and $CD34^+$ cells and the vitality. *Conclusions.* As it is deduced by the obtained results, even if the number of valued samples is small, cellular vitality mainly decreases to load of the NC, especially in to 20°C. The time spent by the collecting is the other index that seems to engrave on the integrity of the cells, in fact mortality is greater to 72 hours. We can conclude that the factors that engrave on the cellular vitality are the way of maintenance and the time spent by the collecting, although $CD34^+$ cells are more resistant to both the factors.

1410

NONMYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH RICHTER SYNDROME: A REPORT OF TWO CASES

S. Reitter,[1] U. Posch[2], M. Eibl,[1] H. Sill,[1] W. Linkesch,[1] W. Zinke-Cerwenka[1]

[1]Division of Haematology, Medical University Graz, GRAZ; [2]Department of Blood Group Serology and Transfusion Medicine, GRAZ, Austria

Background. Approximately 5% of patients with chronic lymphocytic leukemia (CLL) develop high-grade non-Hodgkin lymphoma (Richter Syndrome) with poor prognosis and an overall survival between 5 and 8 months. Allogeneic stem cell transplantation is the only curative treatment option. *Methods.* In the first patient (male, 51 years), CLL stage II according to Rai classification was diagnosed in June 2000. Due to progression of CLL in 9/2000 the patient received chemotherapy and abdominal radiation leading to partial remission. In April 2003, transformation of B-CLL to high-grade non-Hodgkin lymphoma was diagnosed. Therapy with rituximab, fludarabine, cyclophosphamide and methotrexate (R-FCM) was ineffective. By application of DexaBEAM, tumour control could be achieved, preparing the way for autologous transplantation with BEAM conditioning for further tumour reduction followed by nonmyeloablative stem cell transplantation with an HLA-identical sibling. The second patient (female, same age) showed already a high-grade non-Hodgkin lymphoma arising from B-CLL when first contacting a hematologic outpatient department in 12/06. After six cycles of R-CHOP the patient achieved a very good PR so that transplantation with the HLA-identical sibling could be performed subsequently. The preparative regimen was composed of fludarabine (150 mg/m^2) and TBI (4Gy). GVHD prophylaxis consisted of cyclosporine A and mycophenolate mofetil. *Results.* Both patients engrafted and showed a full donor chimerism on day +28. Bone marrow biopsy revealed complete remission in both cases. The first patient, a farmer, had no complications until day +270. On that day, he presented with bronchopneumonia and died on day +275 due to respiratory failure. The second patient, transplanted in June 2007, is doing well. CT scans and the last bone marrow examination show that she is still in complete remission. *Conclusions.* Allogeneic stem cell transplantation with nonmyeloablative conditioning has the potential of a curative treatment. As prognosis for patients with Richter syndrome is poor this treatment option should be taken into consideration.

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RITUXIMAB FOR ALLOGENEIC STEM CELL TRANSPLANTATION (SCT) ASSOCIATED THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

A.M. Carella, G. D'Arena, E. Merla, N. Cascavilla

IRCCS Casa Sollievo della Sofferenza, SAN GIOVANNI ROTONDO, Italy

TTP may rarely complicate allogeneic SCT. First-line therapy of TTP is plasma exchange but other therapeutic approaches, such as steroids, i.v. Ig and vincristine, are still used. Recently, the anti-CD20 monoclonal antibody rituximab has been reported to successfully cure SCT-associated thrombotyc microangiopathy. We report our experience on the use of rituximab in 2 patients with TTP developed after allogeneic SCT. Two female patients were scheduled to receive allogeneic SCT for their acute leukaemia. At days +74 and +26 after stem cell infusion, respectively, blood chemistry exams revealed hemolytic anemia. Clotting parameters were normal and both direct and indirect antiglobulin tests were found negative. ADAMTS13 levels were found low in both patients. All these features were retained consistent with the diagnosis of TTP. Cyclosporin A was discontinued and patients were treated with methyl-prednisolone, plasmapheresis with plasma exchange, defibrotide, and high dose Ig without any benefit. Rituximab was then given at 375 mg/sqm weekly for 3 and 2 doses, respectively. We observed a slow but constant increase of platelet count with amelioration of haemolytic lab parameters. In one patient a complete blood cell count returned rapidly to normal values after the third dose. Rituximab was the given monthly at the same dosage as maintenance therapy for 4 consecutive times. Patient dead of broncopneumonia 10 months later. On the contrary, despite platelet count and Hb level increased along with LDH level reduction, the second patient died because of hepatic and renal failure due to acute GVHD at +51 days after allogeneic SCT. SCT-associated TTP is probably due to endothelial injury. The consensus on specific and standardized therapies for SCT-associated TTP is far from uniform. Results to plasma exchange are poor compared with those obtained in the novo TTP and mortality rate of patients treated with this modality still remains high (>80%). There is increasing evidence that the use of the chimaeric monoclonal antibody against CD20 antigen rituximab has a role in the treatment of thrombotic microangiopathy complicating allogeneic SCT such as TTP. In fact, very recently, anecdotal cases have been published on the use of rituximab in these cases. Only 7 patients with thrombotyc microangiopathy complicating allogeneic SCT treated with rituximab have been reported to date. The present report, along with previously published experiences, suggests a role of B-lymphocytes in the pathogenesis of allogeneic SCT-associated TTP and further supports the use of rituximab in the management of this often life-threatening and severe complication. However, because of the limited number of cases treated, further studies need to be performed before to drawn definitive conclusions.

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ANTINEOPLASTIC EFFECT OF FENUGREEK (TRIGONELLA FOENUM GRAECUM) SEED EXTRACT AGAINST ACUTE MYELOBLASTIC LEUKEMIA CELL LINE (KG-1)

A. Alizadeh,[1] S. Jahanmehr[2], M. Rezaian,[1] A. Ardjmand[1]

[1]Tehran University, TEHRAN; [2]TUMS, TEHRAN, Iran

Treatment of cancer patients, using conventional chemotherapies, causes serious side effects and, at best, merely extends the patient's lifespan by a few years. The potential resides in alternative therapies may therefore be of great benefit in cancer prevention and control. The effect of trigonella foenum graecum seed extract has been reported before on some neoplastic cells and it is therefore evaluated again, in this work, using human acute myeloblastic leukemia cell lines (KG-1). The cell line was treated with various concentration of Fenugreek seeds extract, within different periods of time. Results were evaluated by cellular enumeration, viability test, staining, light microscopy and finally were analysed in term of apoptosis induction, by means of flowcytometry. Resultes show significant cytotoxic effects of Fenugreek seeds extract against this cell line such as growth inhibition, cell death and morphological changes. Apoptosis induction by this extract in these cell lines was little. Fenugreek seeds extract didn't change the count and morphology of normal lymphocytes. This strategy of selectively induced apoptosis of leukemia cells, without altering healthy cells, is a major goal for the development of the new therapeutic techniques. To our knowledge, this is the first study that suggests significant chemopreventive effects of Fenugreek seeds against these cell lines.

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COMPARISION OF THE APOPTOTIC EFFECTS ON LYMPHOBLASTS AND ON INCREASE OF MYELOID LINEAGE CELLS OF A SHORT-TIME, HIGH-DOSE METHYLPREDNISOLONE AND THE CONVENTIONAL-DOSE PREDNISOLONE TREATMENTS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

E. Erduran, Y. Tekelioglu, G. Yusuf, T. Ozdemir

Karadeniz Technical University, TRABZON, Turkey

Background. High dose methylprednisolone has been used in the treatment of acute leukemias in childhood since 1990. HDMP causes apoptosis and differentiation on lymphoblasts and myeloblasts. *Aims.*We compare the apoptotic effect on the lymphoblasts and the proliferative effect on the myeloid lineage cells of a short-course HDMP and the conventional-dose prednisolone treatments in children with acute lymphoblastic leukemia (ALL). *Methods.*The patients were divided into 2 groups. Group I (n=10) received HDMP (30 mg/kg/day for 7 days) in a single dose before 6 a.m. perorally. Group II (n=10) received prednisolone (2 mg/kg/day for 7 days) in 3 doses. The apoptotic percentages of lymphpblasts and the percentages of blasts and myeloid lineage cells were determined after performing the bone marrow aspiration (BMA) at diag-

nosis on the 0th, 3rd, and 7th days of the treatments in all patients. *Results.* The mean apoptotic percentages of the lymphoblasts on the 3rd day were significantly higher than those on the 0th and 7th days in both groups ($p<0.05$). The highest apoptosis was determined on the 3rd day in group I. The mean percentages of the blast cells on the 7th day were significantly lower than those on the 0th and the 3rd days in both groups ($p<0.05$). The lowest lymphoblast percentage was determined on the 7th day in group I. The mean percentages of the $CD13^+$ and $CD33^+$ cells on the 7th day were significantly higher than those on the 0th and the 3rd days in both groups ($p<0.05$). The highest percentages of the $CD13^+$ and $CD33^+$ cells were found on the 7th day in group I. Prednisolone and HDMP showed no proliferative effect on the $CD14^+$ cells. *Conclusions.* These findings indicate that a short-course HDMP treatment shows a more effective apoptosis on the lymphoblasts and on the increase of the myeloid lineage cells when compared to the prednisolone treatment. The authors suggest that HDMP may be used in the treatment of patients with ALL instead of prednisolone.

1414

SELECTIVE CYTOTOXICITY OF RECOMBINANT STXA1-GM-CSF PROTEIN IN HEMATOPOETIC CANCER CELLS

M. Habibi Roudkenar,[1] S. Bouzari[2], M. Oolomi[2]

[1]Reserch Center , IBTO, Tehran , Iran, TEHRAN; [2]Pasteur institute of Iran, TEHRAN, Iran

Background. Chimeric proteins are composed of a cell-targeting moiety and a cell-killing moiety.aim, in this study, a chimeric protein, stxa1-gm-csf, composed of catalytic domain of shiga toxin (a1) and granulocyte-macrophage colony-stimulating factor (gm-csf) was constructed and expressed in e. coli to targeting cancer cells bearing gmcsf receptor. *Methods.* Catalytoc domain of shiga toxin was fused to GMCSF by overlapping PCR. Fugen gene was expressed in *E.coli* and toxicity was performed by MTT assay toward HL60, U937, and K562 cell lines. Results, cytotoxicity, receptor blocking, and neutralization experiments revealed that the chimeric protein induced cytotoxic effect on different cell lines. This effect was found to be specific, due to the presence of the killing moiety (a1), which exerts its effect through a specific gm-csf-targeting domain, by binding to its receptor present on those cell lines. *Conclusions.* These initial investigations indicate that the chimeric protein was functional and might be considered as a new modality for cancer treatment; however, further analyses are required for its application

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EPIGENETIC MODULATION - A NEW THERAPEUTIC APPROACH TO LYMPHOID MALIGNANCIES

A.B. Sarmento-Ribeiro,[1] C. Costa[2], J.F. Carmo[2], P.M. Oliveira[2], A.C. Gonçalves,[3] A. Oliveira,[4] D. Moreira,[4] V. Alves[2], T. Silva[2], M. Dourado[3]

[1]Faculty of Medicine, CIMAGO and CNC, University of Coimbra, COIMBRA; [2]Faculty of Medicine, University of Coimbra, COIMBRA; [3]Faculty of Medicine and CIMAGO, University of Coimbra, COIMBRA; [4]Faculty of Sciences and Techology and CNC, University of Coimbra, COIMBRA, Portugal

The initiation and progression of cancer are controlled by genetic and epigenetic mechanisms that result into the deregulation of genes involved in differentiation, proliferation and apoptosis. The epigenetic regulation involves several processes including methylation/demethylation of CpG islands and the modification of histones by acetylation/deacetylation and methylation. These are key mechanisms in gene silencing which might play a crucial role in several types of cancer, namely hematopoietic malignancies. The treatment of several hematologic malignancies, such as Acute Lymphoblastic Leukemia (ALL), includes high dose chemotherapy and/or transplant. However, these are sub-optimal therapies with high cytotoxicity; side effects and relapses are not infrequent. The understanding of both genetic and epigenetic modulation mechanisms, and also the signaling pathways involved in these diseases, has allowed the development of new, highly specific drugs targeting the malignant cells and sparing normal cells, consequently having fewer side effects. With this project we intend to study the potential therapeutic role of demethylating agents and Histone Deacetylase (HDAC) inhibitors in T-ALL cells lines *in vitro*, both as single and in combination therapy. For this purpose, T-ALL cell lines, one derived from a patient in the initial stages of disease - MOLT-3 - and another from the relapse - MOLT-4 - were maintained in culture in the presence and absence of the demethylating agent Decitabine (5-aza-20-deoxycytidine) and/or the HDAC inhibitor Trichostatin, in several concentrations and during variable periods of time. Cell density and viability were analyzed by Trypan Blue exclusion and by Alamar Blue test. Susceptibility to cell death was measured by flow cytometry using the Anexin V/Propidium Iodide double staining. The efficacy of the epigenetic modulators was determined by both the gene expression and methylation profiles of the p15 and p16 tumor suppressor genes by real time PCR and Methylation-specific PCR (MS-PCR), respectively.Our results suggest that, as a single agent, Trichostastin has an antiproliferative effect and induces cell death by apoptosis, in a time- and concentration-dependent manner. Decitabine, however, has little effects under the tested conditions. On the other hand, a synergistic cytotoxic effect has been observed at lower concentrations than those used in monotherapy when cells were previously exposed to Trichostatin and then exposed to Decitabine. In this condition an hypomethylation effect was observed This study suggests that epigenetic modulation might constitute a new approach to the treatment of lymphoid malignancies, namely ALL-T. However, the choice of the optimal schedule of drugs administration seems to be crucial to the success of the therapy.

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USE OF RECOMBINANT HUMAN ERYTHROPOIETIN FOR MANAGEMENT OF AN ANEMIA IN PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

A.D. Pavlov,[1] M.A. Lunyakova[2], A.G. Beznoshchenko,[1] E.F. Morshchakova[1]

[1]Federal Research Center Ped Hematol, RYAZAN; [2]Regional Pediatric Hospital, RYAZAN, Russian Federation

Background. In the last decennial events due to highintensive regimens of the chemotherapy an acute lymphoblastic leukemia (ALL) in children became potentially curable pathology. However chemotherapy associates with numerous complications, one of which is anemia. The cancer-related anemia worsen the quality of life these patients, and it is also cause of therapeutic problems. To date, red blood cell (RBC) transfusion remains the traditional method of the correction anemia in children with ALL. The lacks of this method are short effect and the risk of some reactions and complications. The use of recombinant human erythropoietin (rHuEPO) is an effective and safe method to treat the anemia. *Aims.* to evaluate the effectiveness of rHuEPO in treatment of the anemia in pediatric patients with ALL undergoing chemotherapy following the protocol ALL BFM-90m. *Methods.* rHuEPO was used in 30 pediatric leukemic patients who received maintanance chemotherapy in the accordance to the protocol ALL BFM-90m. The average age of the EPO treated children was 6.6±0.97 years. Patients received rHuEPO in dosage of 200 IU/kg subcutaneusly 3 time at week or 600 IU/kg intravenously once weekly. All children got the iron sulphate in daily dose of 5 mg/kg on elementary iron. RBC transfusions were performed at haemoglobin (Hb) level below 70 g/L or at higher levels if there were symptoms of hypoxia or heavy infection. 30 children, comparable on age, baseline Hb concentration and leukemia state were historical control. *Results.* The therapeutic effect of rHuEPO became evident after completion induction chemotherapy. During the course of consolidation EPO treated patients had higher Hb levels and their need for RBC transfusions was reduced: the number of RBC transfusions pro patient was reduced from 4.6±0.63 in control group to 2.7±0.46 in EPO group ($p<0.05$). The volume of transfused RBC was reduced almost two times (39.4±5.42 mL/kg in control group *vs* 20.4±2.99 mL/kg in EPO group, $p<0.01$). *Conclusions.* Using rHuEPO in anemic pediatric patients with ALL improves Hb levels, decreases RBC transfusions requirement and can become the element of effective accompanying therapy in anemic patients receiving chemotherapy.

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VASCULAR ENDOTHELIAL GROWTH FACTOR PLAYS AN IMPORTANT ROLE IN CHRONIC MYELOGENOUS LEUKEMIA: RELATIONSHIP TO DISEASE-PROGRESSION

R. Shirasaki, H. Tashiro, M. Noguchi, T. Sugao, Y. Oka, K. Kawasugi, Y. Akiyama, N. Shirafuji

Teikyo University School of Medicine, TOKYO, Japan

Objective. We reported recently that myofibroblasts derived from chronic myelogenous leukemia (CML) clone were observed by the long term-culture of non-adherent mononuclear cells, and that the generated CML-derived myofibroblasts produced various kinds of

cytokines more than that observed in the normal clone-derived myofibroblasts. In this report we focused on vascular endothelial cell growth factor (VEGF)-system because VEGF was produced significantly in CML-derived myofibroblasts. *Materials and Methods.* Bone marrow cells were obtained from CML patients in chronic phase (CP), accelerated phase (AP) and blast phase (BC), and mononuclear cells were prepared with gravity-sedimentation method. Cells were cultured for two months in DMEM with 10% FCS in a liquid culture system, and the generated myofibroblasts were separated into sub-clones in 96 well plates and selected with nested RT-PCR method using BCR-ABL primers. The concentration of the sera, conditioned media from non-adherent mono-nuclear cells, myofibroblasts derived from normal clone and from CML clones was measured with ELISA kit. The expression of VEGF, VEGF receptor type-1 (VEGFR-1) and VEGF receptor type-2 (VEGFR-2) was determined with RT-PCR. The effect of anti-human VEGF antibody to CML BC cells was also estimated using 3H-incorporation assay system. *Results and Discussion.* The VEGF levels in sera from CML patients were elevated significantly. The expression of VEGF was demonstrated in myofibroblasts obtained in CP, AP and BC patients; however, in CP patients the expression of VEGF was not observed in non-adherent mononuclear cells. In contrast, in AP and BC patients VEGF production was observed in non-adherent mononuclear cells. The expression of VEGFR-1 and -2 was detected in non-adherent mononuclear cells from CP, AP and BC patients. When non-adherent mononuclear cells from CML BC patients were cultured on the CML-derived myofibroblasts, the activity for the proliferation of CML BC cells was significantly decreased by the addition of anti VEGF antibody in the cultures. These observations indicate that VEGF plays an important role for the proliferation of CML cells, and has a relationship to the disease-progression.

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GENETIC POLYMORPHISM OF CYP1A1 IN INDIAN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS

P. Bajpai,[1] A.K. Tripathi[2], D. Agrawal[1]

[1]Indian Institute of Toxicology Research, LUCKNO; [2]CSSMU, LUCKNOW, India

Background. Genetic polymorphism of genes encoding carcinogen-metabolizing enzymes, namely, phase I cytochromes P-450 (CYPs) have been shown to influence the risk to develop cancer. CYP1A1 is one such gene which effects individual susceptibility towards the risk for cancer from environmental agents. In addition to the role of genetic polymorphism and association to increased risk for several cancers. It has been suggested that individuals possessing a modified ability to metabolize carcinogens are at increased risk of cancer. Thus, polymorphisms in genes encoding carcinogen metabolizing enzymes may have relevance in determining susceptibility to cancer individuals carrying the more active form of an enzyme involved in the activation of carcinogens (phase I), or less efficient alleles of detoxifying enzymes(phase II), will be at greater risk of cancer. Hence the present study was designed to find out the allelic frequency of CYP1A1 gene (*2A,*2B,*4 alleles) in North Indian CML patients. *Aims.* Thus owing to the importance of CYP1A1 genetic polymorphism as risk factor in various cancers and the relative differences in the frequency of its occurrence in various ethnic populations, the present study was aimed at providing valuable evidence based data from North Indian CML patients to the knowledge of CYP1A1 polymorphism and further to evaluate the role of its allelic variants in the susceptibility to develop CML. *Methods.* DNA isolation was carried out by standard proteinase K and phenol chloroform method. The prevalence of CYP1A1 *2A,*2B,*4 alleles was examined by PCR-RFLP method (Krajinovic *et al.,* 1999) in 60 Indian individuals. PCR products were separated using 2% agarose gel. *Results.* CYP1A1 mutations T6235C (m1), A4889G (m2) and C4887A (m4) were characterized by PCR-RFLP. These mutations were then used to define 3 distinct alleles, CYP1A1 *2A (presence of m1 only), *2B (both m1 and m2) and *4 (m4 only). The frequencies of CYP1A1 alleles *2A *2B and *4 in cases were 21.8% (12/55), 18.1% (10/55), and 9% (5/55), respectively .The allelic frequencies of CYP1A1 genes (*2A,*2B and *4) in controls were 32% (24/75), 16% (12/75), and 4% (3/75), respectively. *Conclusions.* A higher frequency of CYP1A1*2A was observed in controls as compared to CML patients.Thus the study provides an evidenced based data, which indicates a reduced risk for CML in individuals carrying the mutant allele CYP1A1*2A.

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BCR-ABL TRANSCRIPT NUMBER AND VIGOROUS PHYSICAL EXERCISE IN IMATINIB TREATED CML PATIENTS

S. Jönsson, B. Olsson, S. Jacobsson, A. Ricksten, L.P. Palmqvist, H. Wadenvik

Sahlgrenska University Hospital, GOTHENBURG, Sweden

Background. Molecular analyses, e.g. quantitative real-time PCR (qRT-PCR) for quantification of BCR-ABL transcript number, are increasingly used in clinical practice to monitor the course of chronic myeloid leukaemia (CML). However, it is unclear whether this sensitive assay is of value in the management of individual patients and misinterpretation of test results could lead to potentially harmful clinical decisions.[1] Also, several technical aspects of the assay have not been standardized. Preferably and because of the variability of PCR results (up to 0.5-log even in high-quality laboratories), the test should be performed on samples from the same consistent compartment. However, peripheral blood, the commonly used specimen, is not a consistent compartment; the relative proportion of myelopoietic and lymphopoietic cells can vary significantly from time to time in the same individual. *Aims.* In the present work we explored the impact of vigorous exercise on the measurable BCR-ABL transcript number in imatinib treated CML patients.

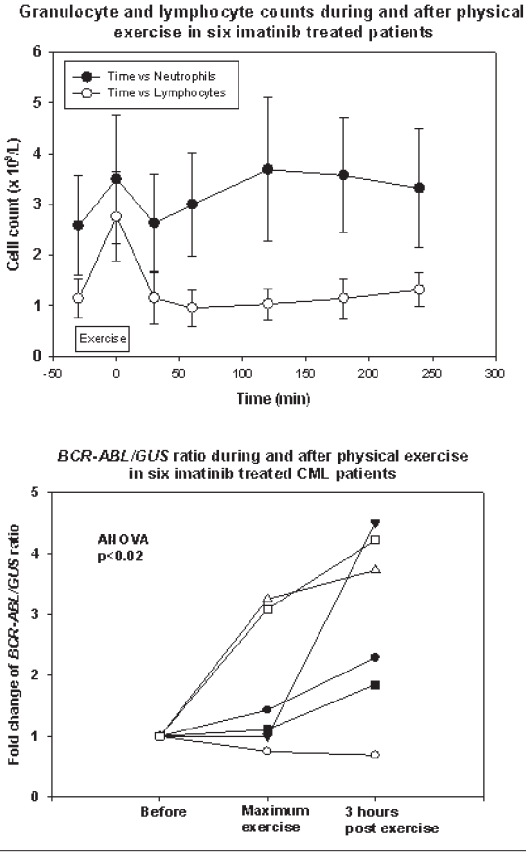

Figure 1.

Materials and Methods. Six imatinib treated CML patients in complete cytogenetic remission and with measurable BCR-ABL transcript number by qRT-PCR performed a maximal exercise test. Blood samples were withdrawn before and immediately after the test, and 30, 60, 120 and 240 min post exercise. Full blood counts were obtained using the CellDyn and FACSCanto instruments. T-lymphocytes, B-lymphocytes and granulocytes were isolated by ficoll separation and immunomagnetic cell sorting. The BCR-ABL transcript number was analyzed on the whole blood specimens and the isolated cell fractions by qRT-PCR. GUS was used as control gene. *Results.* Vigorous exercise induces significant increases in both lymphocyte and granulocyte number but with different time dependent patterns (ANOVA; $p<0.05$). The lymphocytes showed only an early and transient increase following physical exercise, while the granulocytes showed both an early and a delayed increase (Figure 1). The BCR-ABL/GUS ratio, measured on whole blood specimens, increased significantly (ANOVA; $p<0.05$) following the exercise test and up to a 4-fold increase, compared to baseline, was seen 3 hours after the exercise

test (Figure 1). *Conclusions.* Pre-analytical factors contribute to the intra-individual variability of qRT-PCR results. Up to a 4-fold increase in BCR-ABL ratio can be accounted for by such factors. Strenuous exercise should be avoided the day before collection of blood for BCR-ABL quantification.

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DASATINIB COMBINED WITH CHEMOTHERAPY IN PATIENTS WITH LYMPHOID BLAST CRYSIS OF CHRONIC MYELOID LEUKEMIA

E.S. Zakharova, A. Turkina, E. Chelysheva, N. Khoroshko

Hematologic Research Centre, MOSCOW, Russian Federation

Lymphoid blast crisis (LBC) develops approximately in 30% of patients with chronic myeloid leukemia (CML). Response to treatment differs in patients with LBC of CML and in patients with ALL. It's determined by tumor biology. Attempts to care patients with blast crisis of CML with high doses chemotherapy (CT) failed to improve survival. According to our Center's data, use of tyrosine kinases inhibitors only in LBC results in relapses occur usually after 3-4 months of treatment. Standard chemotherapy (CT) prolongs duration of hematological responces up to 7 months. The aim of this report is to evaluate the effectiveness of combined therapy including dasatinib and CT. Since 2006 we treated 2 female patients with LBC. They exhibited an immunophenotype consistent with B-cell blast phase of CML. Ph-chromosome was observed in 100% of metaphases. No mutations of ABL kinase domain prior to the start of therapy was found. Treatment plan included the induction CT with vincristine, prednisone and daunorubicin and conducted as the first line of treatment during 2-4 weeks. After bone marrow recovery the patients started to receive dasatinib 140 mg daily. Dasatinib treatment was alternated with five-day courses of CT including vincristine, prednisone, cytarabine. CNS profylaxis was conducted to all patients. First patient, female of 57 years old, received nilotitnib 800 mg daily before a CT of induction and achieved a complete hematological response (CHR) and a complete cytogenetic response (CCR). But after 2 months she relapsed. The patient started a course of first line CT for 4 weeks. After a period of agranulocytosis complicated with septicemia the patient reached a CHR and began to receive dasatinib 140 mg daily alternated with five-days CT courses. Since 1 month of the treatment a complete molecular response (CMR) was achieved. After 8 months of combined therapy CHR, CHR and CMR were maintained, but neuroleukemia developed in spite of CNS prophylaxis. The patient was treated with cytarabine 2 g/m^2 two times in a week. After 3 infusions a cytosis in cerebrospinal fluid was normalized. Duration of combined treatment is 11 months. The patient is in CHR and no Bcr-Abl transcripts are detected by molecular analysis. Second patient, female of 74 years old, firstly received a CT of induction for 2 weeks. No hematological responce was obtained. She began to receive dasatinib 140 mg daily achieved a CHR after 4 weeks of therapy. Since 3 months of combined therapy a CCR was obtained. CNS is not involved. In conclusion, a strategy of consecutive combination of induction CT with dasatinib and standard doses CT may allow to achieve and maintain complete hematological, cytogenetic and molecular responses in patients with lymphoid blast crysis of CML.

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SCREENING OF EXON 6 BCR-ABL KINASE DOMAIN MUTATIONS IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS TREATED WITH KINASE INHIBITORS USING DHPLC

C.A. De Souza,[1] C. Mascarenhas,[1] A. Cunha[2], K. Pagnano[2], R. Silveira[2], F. Costa[2], R. Pasquini,[3] N. Clementino,[4] C. De Souza[2]

[1]State University of Campinas, CAMPINAS; [2]Hematology and Hemotherapy Center, CAMPINAS; [3]Federal University of Paraná, CURITIBA; [4]Federal University of Minas Gerais, BELO HORIZONTE, Brazil

Point mutations in the BCR-ABL TK domain are a frequent mechanism for reactivation of kinase activity are the most common cause of imatinib resistance. These mutations affect amino acids involved in TK inhibitors binding or in regulatory regions. Although some mutations (Y253F/H, E255K/V, and T315I) confer a true resistant phenotype and suggest withdrawal of imatinib in favor of alternative therapeutic strategies, others (M244V/F211L and F359V) may be overcome by dose escalation. Recently the technique of D-HPLC was described for screening ABL kinase mutations. The aim of this study was to screen for mutations in exon 6 of BCR-ABL gene in patients in different phases of the disease, treated with kinase inhibitors. Genomic DNA was extracted from peripheral blood samples. After partial denaturation the PCR product was analysed by D-HPLC in WAVE® Nucleic Acid Fragment Analysis System. One wild-type sample was used as negative control and as positive control was used a sample with T315I mutation. After screening, patients samples with abnormal D-HLPC profile were submitted to automated sequencing, using specific primers. We studied 56 patients, 37 male and 19 female, the median age was 48 (51-73 years old): 50 in chronic phase, two in accelerated phase, two in blast crisis. Thirty-two patients were resistant to imatinib plus/minus dasatinib. In 16 out of 56 (28,5%) samples, D-HPLC showed an abnormal elution profile suggesting the presence of nucleotide changes. Sequencing confirmed the presence of 3 point mutations: T315I (3 patients-20%), V339L (1 patient-6,6%) and F359V (4 patients -25%). In 50% of patients that had an abnormal elution profile, the automated sequencing was not able to identify the mutation. This would be related to the sensitivity of sequencing once DHPLC is a more sensitive method (in our case the mutations were detected in amplicons with the concentrations at least 35 ng/uL). Related to T315I mutation, one of them did not achieve hematological response with dasatinib, another achieve a major cytogenetic response using imatinib loosing CyR one year after the beginning of the treatment. The third was submitted to Allo-SCT but few month after progressed to blastic crisis and died. Direct sequencing has been widely used for this purpose, but the major drawback of this method is the sensitivity for detecting mutations is only 10-20%, improved sensitivities of 1-5% could be obtained by pyrosequencing. More sensitive *Methods.*include peptide nucleic acid based PCR clamping and allele specific oligonucleotide (ASO) PCR. However these techniques are not be applied for screening of unknown mutations. D-HPLC seems to be a cheap, practical and sensitive method for routine clinical monitoring for emergence of kinase domain mutations and may be useful for optimizing therapy in CML. Early detection of emerging mutant clones may help in decision-make of alternative treatment.

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MEASUREMENT OF BCR-ABL TRANSCRIPTS IN LONG-TERM ALLOGENEIC STEM CELL TRANSPLANTATION CML PATIENTS BY REAL-TIME QUANTITATIVE PCR (RQ-PCR)

V. Scholl, R. Bitencourt, V. Pires, T.F. Padilha, R. Hassan, I.R. Zalcberg

INCA, RIO DE JANEIRO, Brazil

Background. Allogeneic-stem-cell transplantation (SCT) is still considered the only curative treatment for patients with chronic myeloid leukemia (CML). Successful long-term SCT patients without evidence of relapse may be regarded as *cured*. The mechanism underlying such *cures* is not well defined, but it is assumed that *molecular remission* achievement (negative Nested-BCR-ABL RT-PCR) would be indispensable. While a high risk of relapse is associated with early BCR-ABL detection and increasing levels of BCR-ABL, the role of minimal residual disease (MRD) in SCT long-term survivals (>5 years) is still controversial. Relapse is seen in patients more than 10 years after transplant and a cumulative incidence of 17% relapse is seen after 15 years in patients in remission at 5 years. Thus, whether long-term SCT survivals continue to harbor residual leukemia after SCT and whether *cure* is related to undetectable BCR-ABL transcripts or to low stable transcript levels is still open. An initial approach to this issue is presented. *Aims.* To assess the molecular status for BCR-ABL transcripts levels in a group of long-term SCT CML patients by RQ-PCR. Methodology and *Results.* Peripheral blood aspirates were taken from 20 CML patients submitted to SCT at CEMO-Brazil with a follow-up of at least 5 years. RNA extraction was performed with TRIzol™ after granulocytes lyses. cDNA was synthesized using random primers and Superscript™II. RQ-PCR was performed with Taqman® technology in an Abi-7000 platform. For a multiplex strategy, labeled probes and primers for both b3a2 e b2a2 transcripts were used. ABL was used as control gene. A standard curve was generated for each assay using serial dilutions of linearized plasmid containing a BCR-ABL insert. Low and High load controls were run in every assay. All assays were performed in triplicate. A baseline was constructed using the median BCR-ABL/ABL ratios obtained from testing 30 CML patients at diagnosis. BCR-ABL/ABL ratio was converted to an interna-

tional scale (IS), using our newly derived specific-laboratory correction factor of 1.33. Patients with IS values >10 were considered with none molecular response (MR); those with levels between 10-1 as minimal-MR; between 1-0,1 as minor-MR. Major Molecular Response (MMR) was scored as 3-log reduction or ≥0,1. Complete Molecular Response (CMR) was scored in association with undetectable BCR-ABL levels. Median follow-up after transplantation was of 8,2 years (5-13 years). BCR-ABL detection by multiplex followed by non-quantitative nested PCR showed 18/20 patients negative and 2/20 with still detectable BCR-ABL transcripts. In contrast, the use of RQ-PCR showed 14/20 patients with undetectable BCR-ABL and 6/20 patients where BCR-ABL was still detected. From those, 1 showed none-MR (IS:159), 1 minimal-MR (IS:1,759) and 1 minor-MR (IS:0,376). Also, 3 presented a MMR (IS:0,152; 0,028 and 0,028). Those with undetectable BCR-ABL, 14/20, were considered to have achieved CMR. *Conclusions.* Long-term SCT patients could still harbor residual leukemia cells. In our cohort, relapse was seen in one patient with high transcript level (IS>10). Longer follow-ups will be needed to better define the significance of detectable BCR-ABL aiming to draw a pattern or a threshold capable of predicting clinical behavior in long-time CML-SCT survivals.

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THE BASELINE HISTORY OF RESISTANCE AND INTOLERANCE IN PH+ CML PATIENTS FAILING TKI THERAPY

M. Ayala,[1] A. Ayala[2], A. Giuliani,[3] L. Lopez,[4] D. De Castro Lobo,[5] L. Mendes,[6] C. Woodman[6]

[1]Hospital de Especialidades, Centro Médico Nacional La Raza, IMSS, MEXICO CITY, Mexico; [2]Hospital de Especialidades, Centro Médico Nacional La Raza, MEXICO CITY, Mexico; [3]Capital Federal, BUENOS AIRES, Argentina; [4]Banco Municipal de Sangre, CARACAS, Venezuela; [5]Instituto de Hematologia, RIO DE JANEIRO, Brazil; [6]Novartis Oncology, SAO PAULO, Brazil

Background. Imatinib is the standard frontline therapy for Ph+ CML patients (pts). Although effective and well tolerated in the majority of Ph+ CML pts there is a small subset who may eventually fail imatinib (intolerance or resistance). The exact occurrence of pts who become both intolerant and resistant to tyrosine kinase inhibitors (TKI) has not been previously reported. *Aims.* This retrospective analysis evaluated the proportion of pts who were both intolerant and resistant to either imatinib or dasatinib at evaluation for nilotinib compassionate use. *Methods.* Between June 2006 and January 2008, pts with Ph+ CML (CP, AP, BC) were evaluated for nilotinib compassionate use. These pts had failed prior TKI therapies due to resistance or intolerance - imatinib alone or imatinib followed by dasatinib. The majority of pts (98%) were nilotinib naive. At the time of medical review for nilotinib compassionate use, dosing information regarding both imatinib and dasatinib was collected, including the maximum-tolerated and maximum-attempted dose. It was presumed that dose escalations above the recommended starting dose was for inadequate therapeutic responses while dose reductions were for intolerant symptoms. Recommended starting dose for imatinib was 400 mg QD and either 70 mg BID or 100 mg QD for dasatinib. If maximum-tolerated and maximum-attempted doses were equal, the pt was considered to be resistant. If maximum-tolerated dose was less then the recommended starting dose, the pt was considered to be intolerant. If the maximum-tolerated was less then the maximum-attempted dose, the pt was considered to have both resistance and intolerance. The type and severity of intolerant symptoms was not used in the determination of intolerance. *Results.* 621 were evaluated for compassionate use nilotinib. The median age was 52 yrs (range, 12-88); 16 pts (2%) were <18 yrs of age. Using the above criteria, 359 (58%) pts had CP, 152 (24%) pts had AP, and 110 (18%) pts had BC. 491 (79%) pts had failed imatinib only and 130 (21%) pts had failed both imatinib and dasatinib therapy. For the pts who had failed imatinib, 64% had resistance alone, 22% had intolerance alone and 14% had both resistance and intolerance to imatinib. For the pts who had also failed dasatinib, 34% had resistance alone, 47% had intolerance alone and 19% had both resistance and intolerance to dasatinib. Of the pts failing prior TKI therapy, 36% of pts treated with imatinib experienced intolerance while 66% of pts treated with dasatinib experienced intolerance. *Conclusions.* Our analysis suggest that intolerance frequently occurs in combination with resistance to TKI therapy and that intolerance alone or in combination with resistance is twice as common with dasatinib compared to imatinib. Minimizing intolerance due to TKI therapy is important to optimize responses in CML pts.

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ESCALATED DASATINIB DOSE UP TO 60 MG ONCE DAILY IS AS EFFECTIVE AS STANDARD DOSE OF 100 MG QD WITH A BETTER TOXICITY PROFILE IN ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN LATE CHRONIC PHASE RESISTANT TO OR INTOLERANT OF IMATINIB

F. Iuliano,[1] E. Giugliano[2], M. Di Maio,[1] S. Molica,[3] I. Infusino,[1] A. Perricelli,[1] A. Pomillo,[1] M.T. Scuro,[1] A. Serra[2], G. Saglio[2]

[1]Onco-Hematology Unit, ROSSANO CALABRO; [2]Hematology, San Luigi Gonzaga Hospital, TORINO; [3]Onco-Hematology Dpt, CATANZARO, Italy

Background. Dasatinib (SPRYCEL®, formerly BMS-354825) is a novel, oral, multi-targeted kinase inhibitor of BCR-ABL, SRC, and other kinases that is approximately 300 times more potent than Imatinib *in vitro*. Dasatinib has been shown to be effective and safe in pts with CML resistant or intolerant to Imatinib. As might be expected, older patients experienced more adverse events, both hematologic and non-hematologic with the standard dosage of 100 mg once daily. Data regarding tolerability in elderly pts are scanty. *Aims* To test if escalated dasatinib dose up to 60 mg once daily is as effective as standard dose of 100 mg QD with a better toxicity profile in elderly patients with chronic myeloid leukemia in late chronic phase resistant to or intolerant of Imatinib. *Patients and Methods.* As of July 2007, 4 eligible patients have been enrolled and treated: 4F; median age 77 y [range 73-82]; 1 imatinib-resistant, 3 imatinib-intolerant). Dasatinib was given at 20 mg once daily (QD) starting dose with dose escalation to 40 mg QD or 60 mg QD in pts tolerant or lacking response. Complete blood counts were obtained weekly for the first 12 weeks; bone marrow cytology and cytogenetics every 1 months, and molecular monitoring of BCR-ABL transcript levels by real-time qPCR every 12 weeks. The primary endpoint was toxicity. Median time from diagnosis of CML was 62 months (range 36-88). Prior therapy included interferon-alpha in 1/4. 50 percent of pts had 400 mg of prior im 25% had 600 mg 100% of pts received im for >3 yrs. Best response to prior imatinib therapy was a CHR in 100, and partial (PCyR) cytogenetic responses in 75% of pts. No BCR-ABL baseline mutations were found. *Results.*No hematologic toxicity was reported at different dasatinib dosage Dose interruptions occurred in 1/4 pts at 60 mg QD.Patients received an average daily dose of 40 mg/day (range 20-60 mg). Non-hematologic toxicity consisted mainly of grade 1 diarrhea, headache and fatigue. A patients experienced grade II muscolo-skeletal toxicity at 60 mg/day. The analyses with a median follow-up of 8 months (3-8 months) show 100% pts had a CHR, and 75% a PCyR. *Conclusions.* Dasatinib demonstrated substantial hematologic activity in elderly patients with late CP-CML. Escalated dose up to 60 mg QD gives a better toxicity profile in this particularly subset of patients when compared with the standard dasatinib dosage.

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BEST MOLECULAR RESPONSE TO STANDARD-DOSE OF IMATINIB IN CHRONIC-PHASE OF CHRONIC MYELOID LEUKEMIA (CML) ANALYZED IN 57 PATIENTS: EXPERIENCE FROM A SINGLE INSTITUTION

A.E.K.F. Esther,[1] R. De Paz Arias[2], M. Martin[2], M.A. Canales-Albendea[2], F. Hernández-Navarro[2]

[1]University Hospital La Paz, Madrid, Spain., MADRID; [2]University Hospital La Paz, MADRID, Spain

Background. Imatinib 400 mg daily is considered to be the medical standard of care for patients with chronic-phase CML. However, the risk of relapsed exist and complete cytogenetic response (CCR) was 83% at IRIS study at 6 years follow-up. Molecular monitoring of BCR-ABL transcripts using real time quantitative polymerase chain reaction (RQ-PCR) has become an integral component for management of these patients. *Aims.* we have analyzed whether to reach any molecular response (MR) is enough at dose of 400 mg imatinib. *Patients and Methods.* 57 patients diagnosed with chronic-phase CML and treated with imatinib 400 mg daily were evaluated for hematologic and cytogenetic response in a single institution; 26 patients had been previously treated. BCR-ABL transcripts in the blood samples were measured in 54 patients. *Results.* Any molecular response was achieved in 32 patients (59.2%). The best observed major molecular response (MMR) and complete molecular response (CMR) rates were 51.8% and 7.4%. Other alternative treatments were required in 22 patients without any molecular response, such an allogenic bone marrow transplants in 2 patients, increase of imatinib dose in 12 patients and new tyrosin-kinase inhibitors in 8 patients. The causes of change to alternative

treatment were progression to AP/BC in 9 patients, intolerance was seen in 3 patients, suboptimal response in 3 patients and failure treatment in 7 of them. At this time, remain at 400 mg imatinib dose 27 patients, and maintain MMR and CMR 14 (51.8%) and 3 patients (11.1%) respectively. *Conclusions.* In terms of molecular response, these results are similar to IRIS study at 6 years follow up. A higher imatinib dose or a *second generation* ABL kinase inhibitors might allow molecular responses to be achieved earlier although whether *the best* achievement of these responses is important for progression-free survival remains unknown yet.

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THE SIGNIFICANCE OF SOLUBLE SYNDECAN-1 IN DIAGNOSIS AND MONITORING OF MULTIPLE MYELOMA

J.M. Kim, J. Lee

Eulji University Hospital, DAEJEON, South-Korea

Background. Multiple myeloma (MM) is characterized by a proliferation of clonal plasma cells in the bone marrow. Syndecan-1 (CD138) is a heparan sulfate-bearing proteoglycan that is both expressed by normal and myeloma plasma cells. CD138 is shed from the surface of myeloma cells into serum and the level of soluble syndecan-1 (sCD138) has been shown to be an independent negative prognostic factor in MM in the previous studies. *Aims.* There is small number of studies about its role in prognostic classification systems. In this study, we aimed to ascertain the usefulness of sCD138 in diagnosis of MM and to prove its prognostic significance in treated MM. *Methods.* We investigated newly diagnosed 21 patients with MM (M:F=11:10, age 65.9±9.6 years) from June 2005 to December 2006 and 32 normal controls. Patients were subdivided by the Durie-Salmon staging system. For comparative analysis of MM patients, the following data were obtained: percentage of plasma cells in the bone marrow, serum M-protein concentration, β2 microglobulin, creatinine and free light chain ratio. The serum concentration of sCD138 was measured using a human syndecan-1 enzyme linked immunosorbent assay kit (Diaclone Research, France). Therapeutic response to treatment was defined as at least a 50% reduction of serum M-protein concentration and follow-up sCD138 levels were measured after 6 months of treatment. *Results.* The median of sCD138 for MM was 265 ng/mL (95% CI: 147-421) and was significantly higher than the median for normal control (83 ng/mL, 95% CI: 67-103). A significant difference was observed in median sCD138 levels according to Durie-Salmon stage (p=0.04): group I/II (n=10, 222 ng/mL) and group III (n=11, 395 ng/mL). A clear correlation was found between sCD138 and other prognostic factors such as plasma cell% in BM (r=.31), β2 microglobulin (r=.28) and M-protein concentration (r=.40). 90.5% (19/21) of patients showed abnormal for free light chain ratio (normal range: 0.26-1.65), but only 71.4% (15/21) showed elevated concentration of sCD138 (normal range : 0-166 ng/mL). Compared to non-responders (n=5), responders (n=16) had lower sCD138 level at diagnosis (p=0.10) and larger decrease at follow-up (p=0.08). *Conclusions.* Serum sCD138 has a limitation as a diagnostic tool of MM, but is a reliable prognostic marker. The level of sCD138 correlated with other disease markers and may be helpful to predict the response to treatment.

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EPIDEMIOLOGY OF PLASMA CELL DISORDERS IN A GENERAL HOSPITAL: A RETROSPECTIVE STUDY OF 130 PATIENTS

I. Ricca, G. Tamponi, C. Arno, A. Bosio, F. Cerrato, M. Coggiola, G. Epifani, F. Giacometto, G. Monaco, O. Pallisco, E. Scalabrino, L. Varvello, C.A. Raucci, A. Salomone, C. Pascale

Presidio Ospedaliero Cottolengo, TORINO, Italy

Background. Plasma cell disorders are a group of diseases characterized by the proliferation of a plasma cell clone which produces a monoclonal protein (M protein). The most common type is Monoclonal Gammopathies of Undetermined Significance (MGUS), followed by multiple myeloma (MM) and Waldenstroms Macroglobulinemia (WM). In particular, the frequency of MGUS increases with age and its rate of progression is approximately 1% per year. Because of the high prevalence and the different fields of clinical practice in which these patients are followed, it could be of great interest to know the epidemiology of these diseases out of the Hematology Units. *Aim of study.* To describe the frequency and the progression risk of plasma cell disorders in a General Hospital during more than a twenty-year period. *Methods.* We retrospectively reviewed the medical records of patients with diagnosis of MGUS, MM or WM seen at our center from 1984 through december 2006. Statistical analysis were performed using GraphPad Prism 4 (GraphPad Software, Inc). *Results.* The study included 130 patients: 97 affected by MGUS (74%), 21 by MM (16%) and 12 by WM (10%). Patients clinical features are summarized in the Table 1.

Table 1. Patients's characteristics.

Characteristic			MGUS	MM	WM
Gender		male/female	50/47	7/14	7/5
Age at diagnosis		median (range)	70 yrs (30-91)	69 yrs (45-82)	70 yrs (37-85)
Serum M protein		median (range)	1.20 g/dl (0.10-2.92)	3.96 g/dl (0.62-8.10)	3.20 g/dl (2.22-4.98)
Isotype	IgG		59	12	-
	IgA		18	9	-
	IGM		16	-	12
	bidonal		4	-	-
Immunoparesis		yes/no	27/70	21/0	11/1
Bence Jones proteinuria		pos/neg	11/86	17/4	2/10

Median follow-up was 48 months (range:12-292). Among the 107 patients with a first diagnosis of MGUS, malignant transformation occurred in 10 patients (9 MM and 1 WM). The median time from diagnosis of MGUS to diagnosis of a plasma cell proliferative disorder was 60 months (range 12-196). Median time to progression (TTP) was 174 months, as shown in Figure 1.

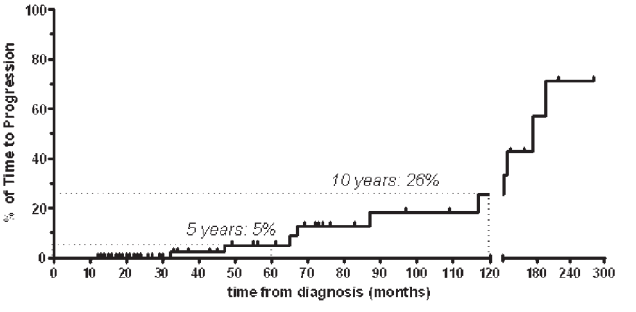

Figure 1. Time to progression curve in 107 patients with first diagnosis of MGUS.

The cumulative probability of progression was 5% at 5 years and 26% at 10 years. *Conclusions.* Unlike MM and WM which represent a rare diagnosis in Internal Medicine Units, MGUS are very common in clinical practice, accounting approximately 75% of lymphoplasma cell disorders followed at our Hospital. The premalignant nature of this condition is comfirmed by the rate of transformation in lymphoplasma cell proliferative disorders (in our study, 43% of MM and 8% of WM were preceded by MGUS). Larger studies have to be designed by Hematology Units in cohoperation with General Hospitals in order to identify clinical or biological features predictive of malignant progression.

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CLINICAL PRESENTATION OF MULTIPLE MYELOMA IN CORNWALL: NOW AND TEN YEARS AGO

B. Cowley, P. Harrison, J. Blundell

Royal Cornwall Hospital NHS Trust, TRURO, UK

Background. Multiple Myeloma (MM) is a malignant clonal neoplasm of plasma cells - the clinical presentation varies from asymptomatic individuals, to those with life threatening symptoms. Some studies have suggested the clinical presentation of MM has changed with diagnosis occurring earlier in the course of the disease process. Other studies suggest that the epidemiology of multiple myeloma has not changed over recent decades. *Aims.* The aim of this study was to compare the presenting features of MM diagnosed in the Haematology Unit of the Royal Cornwall Hospital in two cohorts of patients, ten years apart. *Methods.* Patients diagnosed with MM within the two periods, August 1994 to August 1996 and August 2004 to August 2006, (33 and 38, respectively) were evaluated. The patient demographics, clinical symptoms, any prior diagnosis of monoclonal gammopathy of undetermined significance (MGUS), and laboratory parameters were documented. *Results.* Our find-

ings showed that the patient demographics were similar and there was no significant difference in the numbers of patients with symptoms related to MM at initial presentation. There was no significant difference in the proportions of patients presenting with anaemia (21 *vs* 29%), hypercalcaemia (9 *vs* 5%), and renal failure (15 *vs* 8%) in the two groups. In both groups over 60% had an abnormal beta2microglobulin level. Similarly there was 1 patient in each cohort who had previous monoclonal gammopathy of undetermined significance. *Summary.* These data suggest that in our population there have been no significant changes in the demographics, clinical presentation or laboratory features of multiple myeloma presenting in recent years compared to 10 years ago.

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ZOLEDRONATE- ASSOCIATED FOCAL SEGMENTAL GLOMERULOSCLEROSIS IN A PATIENT SUFFERING FROM MULTIPLE MYELOMA

C. Vadikolia, A. Papalexandri, G. Bamihas, I. Batsis, L. Papaemmanouil, I. Sakellari, A. Fassas, A. Anagnostopoulos

G. Papanicolaou Hospital, THESSALONIKI, Greece

Zoledronate is a bisphosphonate analogue which is in use for the treatment of multiple myeloma but it has been associated with toxic acute tubular necrosis (ATN). To our knowledge only one patient was reported presenting focal segmental glomerulosclerosis (FSGS), and renal failure. Zoledronate- associated FSGS has been described following treatment with pamidronate, lithium, interferon-α , heroin, viral infections, malignant arterial hypertension and hereditary conditions. We report a 36 year-old female patient who developed zoledronate-associated FSGS and nephrotic syndrome. At diagnosis, she had a paravertebral plasmocytoma IgG-κ; serum electrophoresis revealed low monoclonal IgG-lambda levels and bone marrow aspiration showed no evidence of monoclonal plasmocytes. She received radiotherapy (3600cGy), and zoledronate, 4 mg over15 min for 9 months. Zoledronate was discontinued due to concerns for jaw osteonecrosis. Three months later, she was admitted with myocardial dysfunction, pericarditis, renal failure, proteinuria and extensive lytic lesions. She was treated with 2 cycles of D-PACE and autoSCT, with melphalan 200 mg/m^2 as conditioning regimen. Three months later she developed nephrotic syndrome with generalized oedema and severe hypoalbuminaemia (16 gr/24 h) . A kidney MRI scan revealed inflammatory lesions, along with a sacral mass. In renal biopsy ATN and FSGS were observed, with no evidence of amyloidosis or light chain deposition disease. Serology for HBV, HIV and parvovirus B19 was negative. Low-dose corticosteroids were administered for two months. The nephrotic syndrome gradually ameliorated. At present, the patient has no signs of hypoalbuminaemia, the urine protein excretion reaches 0,6 g/d, but the renal function remains impaired with serum creatinine at 2.2 mg/dL. The prognosis in patients developing biphosphonate associated FSGS and nephrotic syndrome remains poor. About 20% of such patients will experience some degree of recovery and almost half of them need haemodialysis.This patient is the second with zoledronate-associated FSGS and the first achieving an amelioration on low-dose corticosteroids.

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BISPHOSPHONATE-RELATED OSTEONECROSIS OF THE JAW IN PATIENTS WITH MULTIPLE MYELOMA: A SINGLE CENTRE EXPERIENCE

M. Sagristani,[1] A. Carola,[1] A. Nasuti[2], M. Ferrigno,[3] G. Vitiello,[3] F. Battista,[3] L. Mastrullo[4]

[1]P.O. San Gennaro ASL NA,[1] NAPOLI; [2]P.O. Sorrento ASL NA,[5] NAPOLI; [3]Chirurgia Maxillo facciale P.O. San Giovanni Bosco, NAPOLI; [4]P.O. San Gennaro ASL NA[1] Direttore U.O.C. Ematologia, NAPOLI, Italy

Background. Multiple myeloma (MM) is charcterized by malignant proliferation of plasma cells in bone marrow, and rarely, in extramedullary sites. The major clinical manifestation is related to loss of bone through osteolysis. Bisphosphonates are specific inhibitors of osteoclastic activity and are used to prevent bone complications. Recent published reports have documented a possible link between treatment with intravenous (IV) bisphosphonates and osteonecrosis of the jaw (ONJ), the unexpected appearance of necrotic bone in the oral cavity. Osteonecrosis can develop spontaneously but appears more frequent after invasive dental procedures. The pathophysiology is controversial; two mechanisms appear involved: the antiangiogenic effect of bisphophonates and accumulation of microdamage (observed in animals studies) when bone mineralization increases. *Aims.* Between February 2005 and October 2007, a study was performed in our 24 patients (14 male and 10 female patients; median age 65 years) affected by MM, receiving IV zoledronic acid (4 mg every 28 days) in association to anti-myeloma therapy, to determine the frequency of occurrence of ONJ when proper prevention is applicated. *Methods.* Every patient had received from a minimum 12 infusions to a maximum 18 infusions of IV zoledronic acid. Our experience in prevention of ONJ has been possible thanks to an intense collaboration between our Division of Haematology and Departments of Maxillofacial Surgery of our Institution. All these patients were informed of the potential risks and studied for dental evaluation with an accurate visit and orthopantomograms; dental treatment and other oral procedures were completed before initiating bisphosphonate therapy. Patients were instructed on maintaining of a good oral hygiene by chlorhexidine mouthwashes and subjected to frequent check-ups during and after bisphosphonate therapy. *Results.* The management of ONJ focused on maximizing oral health, conservative actions with mouth rinses, antibiotics, drugs for control of pain and avoidance of unnecessary invasive dental procedures. The median follow-up was18 months and no of these patients developed ONJ. *Conclusions.* ONJ is being increasingly reported in patients with MM and bone metastasis from a variety of solid tumors receiving IV bisphosphonates. The manegment of our patients, combined with the literature review, suggest that: (1) clinical dental examination and a panoramic jaw radiograph should be performed before patients begin bisphosphonate therapy; (2) dental treatment and other oral procedures should be completed before initiating bisphosphonate therapy; (3) for patients who develop ONJ, conservative, non-surgical treatment is strongly recommended; (4) patients should be informed and instructed on the importance of maintaining good oral hygiene and having regular dental assessment; and (5) the medical community needs to be aware of the association between bisphosphonate usage and ONJ so that unnecessary and harmful surgical procedures can be avoided. It is very important that Maxillofacial Surgeon should collaborate with physicians to minimize the risk for ONJ. The probable association of the therapeutic use of bisphosphonates and the development of jaw necrosis has to be studied in further investigations. However, the only effective treatment is so far dental prevention before starting treatment.

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ASSOCIATION OF BORTEZOMIB AND DEXAMETHASONE FOR THE TREATMENT OF PATIENTS WITH RELAPSED MULTIPLE MYELOMA

F. Saltarelli,[1] B. Veggia,[1] A. Moscetti,[1] A. Ferrari,[1] C. Tatarelli,[1] E. Conte,[1] M.C. Cox,[1] M.A. Aloe Spiriti,[1] R. Porrini,[1] M. Albanesi[2], F. Mendicino,[1] B. Monarca,[1] G. La Verde[3]

[1]Ematologia Az.Osp. Sant'Andrea, ROMA; [2]Az.Osp.Sant'Andrea, ROMA; [3]U.O.S.Discrasie plasmacellulari e amiloidosi, Az.Osp.Sant'Andrea, ROMA, Italy

Background. Bortezomib, a boronic acid dipeptide, is a proteasome inhibitor that has been shown in preclinical and phase 1 studies to have antimyeloma activity. Its efficacy may be increased by the addiction of dexamethasone. In this study we assessed the efficacy and toxicity of bortezomib in combination with dexamethasone in a series of patients with relapsed multiple myeloma pretrated with a median of 2 previous therapies (range 1-4) including steroids, alkylating agents, thalidomide, anthracyclines and autologous stem cell transplantation. *Aims and Methods.* Bortezomib 1.3 mg per square meter of body-surface area was administered intravenously in a total of 12 patients (median age 62 years, range 44-73) with relapsed multiple myeloma on day 1, 4, 8 and 11 of a 21-day cycle. Intravenous dexamethasone, 20 mg on the day of and the day after bortezomib administration, was added to the treatment. All patients received at least three to six courses of such a therapy and were prospectively followed-up including accurate monitoring of side effects. Response to bortezomib was assessed according to the European Group for Blood and Marrow Transplantation criteria. *Results.* The median follow-up time was 12 months (range 3-31). During follow-up, 5 patients died (3 due to progression, 2 due to infection). Response rate to bortezomib was 83% (10/12 patients) with a median duration of response of 2.5 months (range1-25). Of these, 4 patients showed a complete remission (3 patients had relapsed after autologous stem cell transplantation), 3 near complete remission (2 previously treated with autologous stem cell transplantation) and 3 partial response. No response was observed in 2/12 patients (17%). Despite the following side effects, grade 1-2 thrombocytopenia (100%), peripheral neuropathy (58%), gastrointestinal symptoms (50%), fatigue (41%) and herpes zoster (8%), all patients received every administration of each cycle. *Conclusions.* This study shows that bortezomib in combination with dexamethasone is an effective salvage therapy with a high response rate and manageable side effects for patients with relapsed multiple myeloma.

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BONE MARROW CELLULARITY, MYELOID/ERYTHROID RATIO AND SCATTER PATTERN EVALUATION BY CELL-DYN 4000 ANALYZER IN MULTYPLE MYELOMA AND OTHER HAEMATOLOGICAL MALIGNANCIES

R.F. de Cerqueira Barreira,[1] A. Pereira,[1] A.T. Pereira Simões,[1] S. Cardeira[2], R. Sanches[2], C.P. Silva Costa[2], S. Almeida Santos,[1] M.A. Marques,[1] A.P. Gonsalves,[1] J.R. Salvado,[1] M. Beja Duarte,[1] M.L. Ribeiro[1]

[1]Centro Hospitalar de Coimbra, COIMBRA; [2]Escola Superior de Tecnologia da Saúde de Coimbra, COIMBRA, Portugal

Introduction. Morphological microscopy is the reference method for bone marrow aspirates examination; however, in spite of allowing a good qualitative evaluation, cells quantification is difficult and not precise. Regardless of attempts to develop automatic methodologies, no precise and reliable *Methods.*are available. CELL-DYN 4000® haematological analyzer uses the MAPSS® technology, based on optical light dispersion, fluorescence and impedance, with great accuracy in differential cellular blood counts. *Study Purpose.* To analyse bone marrow cells on a CELL-DYN® 4000 and establish a correlation with the manual method, in order to determine the Cellularity, the Myeloide/Erythroid ratio and the Scatter pattern for Multiple Myeloma (MM) or/and other diseases. *Material and Methods.* 30 bone marrow samples (in K3-EDTA) were collected for diagnosis purposes amongst our Haematology patients. Bone marrow films were stained with May-Grünwald-Giemsa's; automatic analysis was performed on CELL-DYN® using the CBC(N), Extended Count(W) and Resistant(R) methodologies. Statistical analysis: Pearson's Correlation for association between automatic methodologies for erythroid lineage and lymphoid series counts; Cohen's Kappa Test for the cellularity; Pearson's correlation for M:E ratio; Fisher's Exact Test to determine whether a specific graphical pattern was associated with a given haematological disease. *Results.* By comparing manual and automatic *Methods.*we verified: 1) a good correlation (r=0,769, p>0,0001) with R methodology for erythroid lineage and lymphoid series, despite erythroblasts' interference in the lymphoid series; 2) an Excellent agreement (k>0,808) in the assessment of cellularity and an Excellent correlation (r=0,955; p<0,0001) with the R methodology in M:E ratio. We also found a Very good association (x^2=24; p<0,0001) of a specific Scatter pattern and MM. *Conclusions.* The present study shows that, M:E ratio evaluation, as well as the cellularity assessment by automatic *Methods.*is reliable. It also shows that it is possible to establish an association between graphic patterns and certain diseases. The data confirms the possibility of having a good global evaluation of bone marrow samples by automatic methods, however further standardized studies are needed. Morphological analysis is still essential for morphological diagnosis and it will probably never be dismissed.

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AUTOLOGOUS STEM CELL TRANSPLANTATION IS FEASIBLE AND LEADS TO DURABLE DISEASE CONTROL IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

E. Zamagni, P. Tacchetti, P. Tosi, M. Ceccolini, L. Pantani, G. Perrone, A. Brioli, M.C. Pallotti, A. Petrucci, M. Baccarani, M. Cavo

Istituto di Ematologia Seragnoli, BOLOGNA, Italy

High-dose therapy with autologous stem cell support (ASCT) is considered the standard of care for young newly diagnosed multiple myeloma (MM) patients with less than 65 years of age. At the opposite, controversies exist concerning the role of this procedure in elderly and fragile patients, particularly in the era of novel agents. However, a majority of patients with *intermediate* age, e.g. between 66 and 70 years, are often fit for an ASCT and may benefit as well from a single course of high-dose therapy. Twenty patients with these characteristics (e.g. aged from 66 to 70 years, without comorbidities and good performance status) were prospectively enrolled in a phase 2 clinical trial aimed at exploring the feasibility and efficacy of ASCT to support a single course of melphalan at 200 mg/m2. By study design, patients received VAD or thalidomide-dexamethasone as primary induction therapy, followed by cyclophosphamide (CTX, 4-7 g/m^2) to collect peripheral blood stem cells and, subsequently, a single ASCT. The median age of the patients was 67 years (range 66-70). Eleven patients were primarily treated with 4 monthly cycles of thalidomide and high dose dexamethasone, while the remaining 8 patients were treated with VAD. Seventeen patients received the lower dose of CTX and a single ASCT. Overall, a median of 6.4×10^6 CD34 cells/kg body weight (range 1.85-10.4) were collected. The median time from diagnosis to ASCT was 8 months (range 4-13). Median time to recovery of neutrophils and platelets was 12 days (range 10-16) and 11 days (range 9-15), respectively. Median duration of hospitalization was 18 days (range 14-23). Grade I-II gastrointestinal toxicity was recorded in 6 (30%) patients and FUO/sepsis in 7 (35%). Cumulative incidence of 100 day transplant related mortality (TRM) was 0%. On an intention to treat basis, 50% of patients achieved at least a very good partial response (VGPR), including 20% of immunofixation negative complete response (CR). With a median follow up of 32 months, the 5- year projected overall survival (OS) rate was 93% and median duration of event free survival (EFS) was 36 months. In conclusion, this study suggests that up-front ASCT was feasible and well tolerated in MM patients aged between 66 and 70 years, without an excess of TRM. The rate of at least a VGPR or CR, as well as OS and EFS, were comparable to those obtained in younger patients. ASCT can be considered an effective therapeutic option for selected elderly patients with newly diagnosed MM.

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BORTEZOMIB-BASED REGIMENS AS INDUCTION AND MAINTENANCE TREATMENT IN MULTIPLE MYELOMA (MM) PATIENTS NOT ELIGIBLE FOR AGGRESSIVE THERAPY

C. Mazzone, M. Gentile, E. Vigna, E. Lucia, C. Iorio, R. Morelli, M.G. Bisconte, C. Gentile, F. Morabito

UO di Ematologia, COSENZA, Italy

Background. Several publications reported on the safety and efficacy of bortezomib (Vel) in the treatment of relapsed/refractory multiple myeloma (MM) in controlled clinical trials. Complementary data on the experience with Vel in maintenance until progression are needed. *Aims.* The purpose of this study was to assess feasibility, tolerability and efficacy of the Vel-based regimens as induction therapy followed by Vel+Dexamethasone (Dex) maintenance in MM patients not eligible for aggressive therapy. *Methods.* From October 2006 to April 2007 11 MM patients (9 males and 2 females) either untreated (2 pts), or relapsed (5 pts) or refractory (4 pts) to prior therapy were treated with Vel based regimens. In the 9 previously treated patients the median number of prior therapy was 2 (range1-4). The median age was 71 years (range 56-81 years). The Vel-based induction regimens were: PAD (Vel+pegylated liposomial doxorubicin+Dex) in 1 case, VTD (Vel+Thalidomide+Dex) in 5 cases, VD (Vel+Dex) in 5. All patients received 1.3 mg/m^2 of Vel for 4 doses per cycle administered on days 1, 4, 8 and 11 every 3 weeks, while Dex was administered at dose of 40 mg on days 1-4. The induction therapy has been administered up to the achievement of the best response or for a maximum of 9 cycles. All patients were subsequently treated by a combination of Vel at dose of 1.3 mg/m^2 (d 1, 15) and Dex at dose of 40 mg/d (d 1-2, 15-16) every 28 days until progression. Progression free survival (PFS) were calculated from the start of induction therapy. *Results*. In the induction phase, a total of 75 courses were administered with a median number of 7. Complete Response (CR) was achieved in 7 patients (64%) and a partial response (PR) in 4 (36%) in according to the EBMT criteria. After a median number of 7 cycles of Vel/Dex maintenance therapy (range 3-15 cycles) 1 out of 5 patients (20%) in PR showed an improvement of the previous response, 3 patients required termination of study drug for disease progression and 8 patients currently continue the maintenance therapy. During the induction phase, 6 episodes of grade III/IV thrombocytopenia were observed. Other, non-dose-limiting toxicities included grade I/II toxicities fatigue (6 pts), peripheral neuropathy (4 pts), diarrhoea (2 pts), nausea (2 pts) and dizziness (2 pts) were also accounted. Interestingly, during the maintenance phase, no WHO grade III/IV episodes were recorded, while Grade I/II toxicities included fatigue (3 pts) and peripheral neuropathy (6 pts). The median PFS of the entire cohort of patients treated with this therapeutic approach was 16 months. *Conclusions*. Our data show that Vel-based regimens are effective therapies with a high response rate and manageable toxicities for patients with untreated, relapsed or refractory myeloma. Moreover, this study indicates that Vel/Dex treatment can be safely administered as a maintenance regimen until progression. Further study will determine the impact of Vel/Dex maintenance on recurrence and on life expectancy.

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FOLLOW-UP OF PATIENTS WITH MULTIPLE MYELOMA UNDERGOING AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (PBSCT) FOLLOWED BY REDUCED-INTENSITY ALLOGENEIC PBSCT

W. Zinke-Cerwenka,[1] U. Posch[2], H. Sill,[1] E. Eibl,[1] W. Linkesch[1]

[1]Division of Hematology, GRAZ; [2]Department of Blood Group Transfusion Medicine, GRAZ, Austria

Background. The strategy of autologous followed by reduced-intensity allogeneic PBSCT in patients with multiple myeloma (MM) is designed to reduce the tumour burden and eradicate residual disease through the graft *vs* myeloma effect with the advantage of less toxicity. Early transplant related mortality (TRM) could be reduced with this procedure in comparison with conventional allogeneic transplantation in MM patients. *Aims.* We evaluated the remission status and the influence of the sequential autologous/non-myeloablative allogeneic PBSCT on late complications. *Methods.* Six patients (median age: 44 years, range: 35-57) with newly diagnosed MM received single autologous PBSCT with melphalan 200 mg/m^2 followed by reduced-intensity allogeneic PBSCT between three to four months later. The allogeneic transplantations took place between September 2003 and April 2004. The conditioning regimen consisted of intravenous fludarabine 30 mg/m^2 (days -4, -3, and -2) and melphalan 100 mg/m^2 on day -1. Five patients received unmanipulated peripheral stem cells from their HLA-identical siblings, one patient from an unrelated donor with c-mismatch. GVHD prophylaxis consisted of cyclosporine A and mycophenolate mofetil. The patient with the unrelated donor additionally received antithymocyte globulin (5 mg/kg, days -4, -3, -2, -1). All patients achieved complete donor chimerism by day 28. *Results.* Five patients are alive, one died on day +210 in nCR because of cardiac arrest. Three of five are in complete remission and two in nCR. Two patients developed chronic GvHD, one of them with bronchiolitis obliterans and keratokonjunctivitis sicca, the other one with sicca syndrome. Three patients have resumed their former profession. *Conclusions.* The remission rates after autologous/allogeneic transplantation are encouraging. The next task is to minimize the incidence of late complications mainly based on cGVHD.

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Withdrawn by the authors

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CLINICAL FEATURES AND OUTCOME OF FOLLICULAR LYMPHOMA PATIENTS WITH BONE MARROW INVOLVEMENT

N. Falaleeva,[1] G. Tumyan[2], A. Pavlovskaya[2], A. Kovrigina[2], N. Probatova[2], T. Kondratieva[2], E. Sholohova[2], N. Tupitsin[2], R. Khakui[2], I. Poddubnaya[2], D. Osmanov[2]

[1]N.N. Blokhin Russian Cancer Research Center, MOSCOW; [2]N. N. Blokhin Russian Cancer Research Center, MOSCOW, Russian Federation

Background. Approximately 40-45% of patients with follicular lymphoms (FL) have bone marrow (BM) involvment. In some cases clusters of malignant cells are found next to trabeculae, in others - circulating malignant cells detectable on peripheral blood. The aim of this study is to explore the clinical features and outcomes of patients FL with different types of BM involvement. *Methods.* From 1985 to 2005, 65 patients (pts) FL with BM involvement were included in the study. In 35 pts (54%) the BM involvement was accompanied with clinical and hematological manifestation of leukemia, in 30 pts (46%) - malignant cells detected only by the morphological examination of the trephine biopsy specimens. The clinical parameters and treatment options were balanced between the two groups. There were 33 (51%) men and 32 (49%) women with the median age of 53 years (range 21-73). 30 pts (46%) had FL grade 1, 39 (68%) had other extranodal sites of disease, 15 (32%) presented B symptoms, LDH was increased in 21 (32%), 33 pts (46%) had ECOG >2. According to the Follicular Lymphoma International Prognostic Index (FLIPI), 5 pts (8%) ranked in the low risk, 26 (40%) in intermediate and 34 (52%) in high risk category. 37 pts (57%) had widespread disease at diagnosis, including abdominal lymph nodes 45 (70%), liver 15 (23%) and spleen 22 (34%) involvment. *Results.* The efficacy of the first-line treatment (R+CHOP, R+CVP) was poor: CR was achieved in 28 pts (43%), while 21 (32%) were resistant to chemotherapy. The 5-year DFS and PFS were 24% and 22% respectively. There were no significant difference in OS l and DFS of patients with two different type of BM involvement (7-year OS 58% *vs* 62%, 5-year DFS 58% *vs* 50%). LDH elevation, hypoproteinaemia, abdominal mass and more than 5 lymph nodes sites involvement is significantly associated with unfavorable prognosis. *Conclusions.* The two types of BM involvement, with or without symptoms of leukemia, occur equally, do not have prognostic significance in FL, but the BM failure at diagnosis is associated with widespread disease, poor efficacy of first-line therapy and high risk of relapse. The future studies of the FL with BM involvement can help to develop the optimal therapeutic approach of this patients.

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PREDICTIVE SIGNIFICANCE OF REVISED INTERNATIONAL PRONOSTIC INDEX (R-IPI) IN DIFFUSE LARGE CELL B LYMPHOMA (DLCBL) TREATED WITH ANTHRACYCLIN-BASED CHEMOTHERAPHY PLUS RITUXIMAB

T. Gimenez

Hospital del Mar, BARCELONA, Spain

Background. Diffuse large B cell lymphoma (DLBCL) is a heterogeneous entity with variable response to treatment and overall survival (OS). International pronostic index (IPI), based in clinical features, is the most useful tool to predict outcome in these patients. Addition of Rituximab to anthracyclin-based chemotheraphy has improved the prognostic of DLBCL. Recently, a proposal of a revised IPI has shown an improvement in predicting survival in Rituximab era. *Aims.* To assess IPI and R-IPI in DLBCL patients treated with anthracyclin-based chemotheraphy plus rituximab and to evaluate their prognostic value. *Methods.* We analysed main clinical features and outcome of 60 patients consecutively diagnosed of DLBCL CD20$^+$ treated with rituximab-anthracyclin chemotheraphy in a single institution. *Results.* A total of 60 patients were identified, 27 men and 33 women with median age at diagnosis of 62 years (range 17-89). Distribution of IPI variables: age ≥60 in 57%, ECOG ≥2 in 30%, Ann Arbor stage III or IV in 48%, elevated levels of LDH in 45% and involvement of more than one extranodal sites in 28%. Distribution according to: 1) IPI: low risk group in 42%, low-intermediate risk group in 22%, high-intermediate risk group in 18% and high risk group in 18%; 2) R-IPI: low risk group in 18%, intermediate risk group in 45% and high risk group in 37%. Types of chemotheraphy : R-CHOP in 46 patients (77%), R-CMyOP in 5 (8%), R-EPOCH in 5 (8%) and R-others in 4 (7%). With a median follow-up of 38 months (range 5-70 months) for survivors, progression free survival (PFS) and OS at four years were 80% and 75%, respectively. PFS and OS at four years according to IPI and R-IPI are shown in Table 1. Updated results will be presented. *Conclusions.*In our serie, both IPI and R-IPI are predictive for PFS and OS in patients diagnosed to DLBCL treated with anthracyclin-based chemotheraphy plus rituximab. R-IPI stratifies diferent risk groups with more precision than IPI. In addition, R-IPI identifies a low risk group with very good prognosis suggesting that less treatment could be administered in this group of patients.

Table 1.

IPI	Low risk	Low-intermediate risk	High-intermediate risk	High risk	*p*
FPS	90%	62%	68%	36%	0.01
OS	91%	73%	66%	63%	0.15
R-IPI	Low risk	Intermediate risk	High risk		*p*
FPS	100%	71%	52%		0.03
OS	100%	79%	63%		0.09

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EXPERIENCE OF EXTRANODAL NK/T CELL LYMPHOMAS IN THE WEST OF SCOTLAND

P. Gallipoli,[1] P. McKay,[1] B. Jackson[2], M. Gangopadhyay[2], M. Leach[1]

[1]Western Infirmary Glasgow, GLASGOW; [2]Glasgow Royal Infirmary, GLASGOW, UK

Background. Extranodal Natural Killer/T cell (NK/T) lymphoma is a very rare disease in Western countries. It appears most prevalent in the East Asia (up to 9% of NHL), shows aggressive clinical behaviour and often short survival. Published information on this subtype of non-Hodgkin's lymphoma in Western countries is sparse and there is no real consensus on the best treatment approach. *Aims.* To evaluate the epidemiology, pathology, clinical features and treatment outcome of extranodal NK/T cell lymphomas in the West of Scotland from 2001 to 2007. *Methods.* We retrieved all cases of extranodal NK/T cell lymphoma in the West of Scotland since 2001 from regional pathology database. Clinical information, treatment and outcome data was obtained from patient case notes. This region has a population of approx 2.5 million. *Results*. Nine cases of extranodal NK/T cell lymphoma were identified between 2001 and 2007, 7 were male and 2 were female. They comprised only 0.3% of all NHL cases registered. Histologically the cases were characterised by an intermediate or large cell infiltrate with angiocentricity and necrosis. All displayed immunoreactivity for cytoplasmic CD3 and granzyme B and positivity for EBV eber by in-situ hybridisation . Six cases were positive for CD56 [Histology, pictures 1 and 2]. The median age at presentation was 61 (range 23-90). B symptoms were present in two thirds of cases. All cases had involvement of one or more extranodal sites: skin was frequently involved [Pictures 3 and 4]. Sites of involvement are shown in the attached Table 1. Four patients had a low IPI score (0-1) while the remaining 5 had IPI of 2-3. Eight patients were treated with chemotherapy, mainly CHOP or similar; 2 received radiotherapy in addition. One elderly patient was treated with radiotherapy alone. Only 2 of 8 evaluable patients achieved a CR with initial therapy. One patient is currently being treated. Three patients progressed on treatment. All patients with a CR/PR, however, had a short lived response and progression occurred within 6 months. Median overall survival was only 6 months. A low IPI score was predictive for longer survival; IPI 0-1, 15 months; IPI 2-3, 5 months, p=0.019 [Graphs 1, 2, 3]. Patients with apparent stage 1 disease, however, survived no longer than those with stage IV, p=0.36. *Conclusions.* Our experience confirms that NK/T cell lymphoma is a rare and aggressive extranodal non Hodgkin's lymphoma. Anthracycline based therapy is largely ineffective and generates only a transient response in a proportion of patients. Traditional staging systems are problematic in that even stage 1 patients have a short survival. The IPI, however, appeared useful in predicting duration of survival in this small series. Although this is a rare lymphoma in the West the other epidemiological, pathological and outcome data in our study population are similar to those described in higher incidence areas in East Asia. The prognosis overall remains very poor and most, if not all, patients will die of the disease. New treatment approaches are much needed.

Table 1. Patient stage and extranodal sites of involvement

	Number (%)	Extranodal sites involved	Median survival (p value)
Stage 1E	6 (66.6%)	3 Nasopharynx 3 Skin	10.5 months
Stage 4	3 (33.3%)	1 Skin+Bone marrow 1 Testes+Bone marrow 1 Lung+Liver	5 months (p=0.36)

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DIFFUSE LARGE B-CELL LYMPHOMA WITH PLASMACITOID DIFFERENTIATION IN HIV INFECTED PATIENTS

I. Macarie, G. Oltean, R. Demian, V. Macarie, I. Candea, L. Dorcioman, E. Horvath, P. Mocan

Spitalul Clinic Judetean de Urgenta, TARGU MURES, Romania

Background. Lymphomas with plasmablastic features are tipically related with immunodeficiencies, especially HIV infection, but are also present in transplanted patients and in other immunodeficiences (chronic diseases, therapy related). Until recently only the third cause was frecquent in our area. *Aims.* To study the incidence of plasmacitoid differentiation in patients wth newly diagnosed diffuse B-cell lymphoma, the clinical characteristics (localization), immunophenotype of the proliferating cells and corelation with HIV positivity. *Methods.* In a retrospective study we revised the characteristics of the newly diagnosed patients admitted in our clinic between august 2006-august 2007, who received at admitance the diagnosis of diffuse large B-cell lymphoma with plasmablastic differentiation. The diagnosis was not only morphological but also was confirmed by immunophenotyping with monoclonal antibodies anti CD45, CD20, CD79a and CD138. The results were corelated with clinical localization and serology for HIV. *Results.* We studied 39 patients (17 women and 22 men) with ages between 17 and 81. Plasmablastic features were present in 3 patients, representing 7,69% of the total new cases. In one case (lymphoma of the oral cavity) the immunophenotype was caracteristic (CD20 negative, CD79a and CD138 positive) with HIV positivity also present. BCL-6 was negative. The second case (lymphoma of the pharinx) was HIV positive but also CD20 positive. CD79a and CD138 were positive. The third case was negative for HIV but the lymphoma cells expresed the tipical phenotype. In this case the tumoral cells expresed strong positivity for EBV vRNA by EBER+ (FISH). Response to treatment was very good, with complete remission in two cases with CHOP therapy, associated with HAART (highly active antiretroviral therapy) in HIV positive patient. The third case was lost for evaluation of therapy. *Conclusions.* Diffuse large B-cell lymphoma with plasmacitoid differentiation is a rare diagnosis, associated with HIV infection and localisation to oral and pharinx mucosa. In future is possible that this diagnosis it will be present in larger number of patients in our area due to long time survival of HIV infected patients and an increased number of transplanted patients.

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BOMES CHEMOTHERAPY (BCNU, VICRISTIN, METHOTRAXATE, ECTOPOSIDE AND METHYLPREDNISOLONE): AN EFFECTIVE SALVAGE REGIMEN FOR PATIENTS WITH RELAPSED AND/OR REFRACTORY NON-HODGKIN LYMPHOMA

P. Lin

National Taiwan University Hospital, TAIPEI, Taiwan

Backgrounds. Although multiagent combination chemotherapy improves the outcome of patients with non-Hodgkin lymphoma (NHL), relapsed and refractory diseases constitute to be significant problems. There is no standard salvage chemotherapy regimen for relapsed or refractory lymphoma because no specific regimen is found superior to others. The BOMES chemotherapy regimen contains BCNU, vicristin, methotraxate, ectoposide and methylprednisolone, and shows good effect in primary central nerve system lymphoma in prior pilot study (Cancer 1998; 82: 1946-51). *Aims.* This study is designed to evaluate the efficacy and safety of BOMES chemotherapy regimen for patients with relapsed and/or refractory non-Hodgkin lymphoma. *Methods.* Forty-nine patients with relapsed or refractory NHL who were treated with BOMES regimen for at least two courses were analyzed. The BOMES protocol is as followed: BCNU 65 mg/m^2 on day 1 and 2, vicristine 2 mg on day 1 and 8, methotrexate 1500 mg/m^2 on day 15, etoposide 50 mg/m^2 on day1 to 5, and methylprednisolone 200 mg on day 1 to 7. Thirty three patients (69.7%) were diagnosed as having diffuse large B-cell lymphoma; five, follicular lymphoma; two, splenic marginal zone lymphoma; and the remaining nine patients, T-cell or NK/T-cell lymphoma. Thirteen patients had central nerve system (CNS) involvement of the disease at the time BOMES was given. As to the status of the patients, thirteen patients were refractory to first-line induction chemotherapy, 22 patients were in first relapse and 14 patients, second or subsequent more relapse. All but four patients had been exposed to CHOP chemotherapy; 30 patients of them also had been treated with ESHAP chemotherapy. Thirteen patients showed some response to prior chemotherapy within three months before BOMES was given, defined as chemo-sensitive group; while others did not, defined as chemo-resistant group. *Results.* The overall response rate (ORR) was 61.2% (n=30), with a complete remission rate of 32.6% (n=15). Patients with chemo-sensitive lymphoma had a significantly higher response rate than those with chemo-resistant lymphoma (84.6% *vs* 52.8%, p=0.04). Besides, a trend of favorable response rate was observed in patients with diffuse large B-cell lymphoma than others (69.7% *vs* 43.7%, p=0.08). The response rate for patients with primary refractory disease was 53.8%, compared with 68.2% for those in first relapse and 57.1%, second or more relapse. The two-year progression-free survival rate for the responders was 35.9% and five-year progression-free survival rate, 32.3%. The overall five-year survival rate for all patients was 24.9%; 38.6% for responders (n=30) and zero for non-responders

(n=19). The difference in overall survival between responders and non-responders was statistically significant ($p<0.001$). The main adverse effect of BOMES regimen was myelosuppression; the grade 3 or 4 neutropenia and thrombocytopenia was 73.5% and 49.0%, respectively. Febrile neutropenia occurred in 48.9% of patients. However, neutropenia recovered soon after G-CSF supplement and no patients died of complications. *Conclusions.* BOMES is an effective salvage chemotherapy regimen for previously heavily treated patients with refractory and/or relapsed lymphoma.

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ADULT T-CELL LYMPHOMA HTLV -1 POSITIVE: A REPORT OF EIGHT CASES

M. Vasilica, D. Coriu, C. Butca, D. Toma, C. Dobrea, D. Colita

Fundeni Clinical Institute, BUCHAREST, Romania

Human T-lymphotropic virus (HTLV)-1 infections and their associated diseases are very rare in Europe, occurring prevalently in subjects in endemic areas. The HTLV-1-associated leukemia/lymphoma, ATLL, is a very aggressive T-cell non-Hodgkin's lymphoma which can be difficult to recognize in non-endemic areas. Here we describe eight cases of adults, with no apparent risk factors, affected by a rapid fatal ATLL. All patients with the clinicopathological diagnosis of adult T-cell leukemia-lymphoma were HTLV-1 positive. These cases had the characteristics features of adult T-cell leukemia-lymphoma: diffuse histology, often mixed cell or pleomorphic, and o high frequency of hypercalcemia, leukemic phase, diffuse visceral involvement and opportunistic infections. The median survival of these patients was short, being only 24 weeks. These entities have extremely poor prognosis with an extremely aggressive clinical course and are treated with the same paradigm as for the highest-risk groups with diffuse large B-cell lymphoma. All the patients received combination chemotherapy, but all died within a year of first noticing the symptoms.

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THE OUTCOME OF PATIENTS WITH BULKY DIFFUSE LARGE B-CELL LYMPHOMA

B. Andjelic, S. Jankovic, R. Jancic-Nedeljkov, V. Milosevic, A. Sretenovic, M. Perunicic-Jovanovic, Lj. Jakovic, M. Petrovic, B. Mihaljevic

Institute for Haematology, Clinical Centre of Serbia, BELGRADE, Serbia

Background. Recent international randomized studies confirmed the benefit of event free survival (EFS), overall response rate (ORR) and overal survival (OS) in patients (pts) with Diffuse large B-cell lymphoma (DLBCL) treated with anti CD20 monoclonal antibody-rituximab (R) plus conventional chemotherapy-CHOP and CHOP-like regimens. Still aproximately one third of pts have poor outcome. *Aims.* To investigate prognostic relevance of IPI and further clinical prognostic parameters in subgroup of patients with bulky DLBCL. Their significance was evaluated according to treatment response, progression free survival (PFS) and OS. *Methods.* We analyzed the subgroup of 35 (23 male and 12 female) newly diagnosed DLBCL pts, treated with immunochemotherapy during the last 7 years period, with bulky mass on the presentation. The bulky disease was diagnosed when tumor mass was >7 cm in the greatest diametar. Twenty four pts were treated with R+CHOP and 11 pts with R+EPOCH. International Prognostic Index (IPI) was determined in all pts. The median follow up was 36 months (range 1-84). *Results.* The patients had mean age 49.31±16.5 (range 22-78) yrs; 71.43% were <60 yrs. Performance status (PS) 0-1 had 71.43%. The distribution of pts according to Ann Arbor Clinical stadium (CS) was as follows: 42.86% had CS II, 17.14% had CS III and 40% of pts had CS IV. Low IPI had 37.14%, intermediate 45.71% and high IPI risk was recorded in 17.14% of pts. The overal response rate (CR+PR) for the whole group of pts was 68.57%, CR was achieved in 62.86% of pts. There was no statistically significant difference in overal response rate, as well as in achieving CR regarding the age of patients (<60 yrs *vs* >60 yrs), PS, therapeutic regimen (R+CHOP *vs* R+EPOCH), CS and IPI. For the whole group 3 yrs PFS was 80%. The PFS was signifficantly better in pts younger than 60 yrs (86.1% *vs* 68.6%, $p<0.05$), while there was no signifficant difference regarding the PS, therapeutic regimen, CS and IPI. For the whole group 3 yrs OS was 87.9%. There was no difference in OS regarding the age, PS, therapetic regimen, CS and IPI. *Conclusions.* According to our results the treatment of DLBCL pts with initial bulky disease by immunochemotherapy gained encouraging results. However, further analysis should be taken on the larger population of pts.

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MONO CHEMOTHERAPY WITH CHLORAMBUCIL AT PATIENTS WITH BRONCHIAL- ASSOCIATED LYMHOID TISSUE (BALT) LYMPHOMA

V. Milosevic, A. Bogdanovic, S. Jankovic, A. Sretenovic, B. Andjelic, M. Perunicic-Jovanovic, Lj. Jakovic, M. Virijevic, M. Bogunovic, D. Boskovic, M. Petrovic, B. Mihaljevic

Institute for Haematology, Clinical Centre of Serbia, BELGRADE, Serbia

Background. Bronchial associated lymphoid tissue (BALT) lymphoma is a rare subtype of low grade B cell marginal zone non Hodgkin's lymphoma. BALT represents 3,6% of all extranodal lymphomas and 0,4% of all non- Hodgkin s lymphoma. The purpose of this study was to analyzed the diagnosis and the treatment of BALT-oma. *Aims.* The aim was to investigate the prognostic profile of BALT patients treated with immunochemotherapy. *Material and Methods.* This study included six patients who had BALT lymphoma diagnosed between January 2001 and April 2007 at the Institute of hematology CCS, Belgrade. Demographic characteristics were as follow: male/ female ratio was 1:5 (16,67%:83,33%); the median age was 63 years (range, 37-72). On presentation, four patients (66,66%) had nonspecific respiratory symptoms and all of them had B symptoms. Patients were seronegative for human imunodeficiency viruses (HIV and hepatotropic viruses HCV and HBsAg). One patient had Sjogren's syndrome and one pulmonary tuberculosis. The diagnosis was based on open lung at one patient or transbronchial biopsy at 5 pts. Patohistological findings suggested lymphoma of marginal zone and immunohistological profile confirmed the diagnosis: $CD20^+/CD10^-/CD5^-$/Cyclin $D1^-/CD23^-/IgM^-$. According to the Ferraro staging clinical clasiffication four patients (66,66%) had localized disease (stage I E-II E) and two had stage III E. Three patients (50%) had ECOG performance status (PS) 0 and three patients had a PS 1. Five patients (83,33%) received chemotherapy consisting of chlorambucil alone (doses was 10 mg p.o. during ten days monthly up to 6 cycles). One patient underwent surgical resection, followed with chlorambucil. *Results.* A complete response with initial therapy was achieved in one patient (16,67%) and a partial response was obtained in five patients (83,33%). All the patients were alive during the median follow-up period of 39 months (range 6-72 months). During the follow up period one patient relapsed into other extranodal localization, but achived PR after CHOP regimen. *Conclusions.* BALT lymphoma tends to be localised disease at the time of diagnosis, responds well to surgical treatment or mono chemotherapy with chlorambucil and has a favourable prognosis.

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TREATMENT OF PRIMARY CUTANEOUS B CELL LYMPHOMA WITH PEGYLATED LIPOSOMAL DOXORUBICIN

S. Pulini,[1] S. Rupoli[2], G. Goteri,[3] N. Pimpinelli,[4] A. Tassetti,[5] A.R. Scortechini[2], A. Bettacchi,[6] P. Picardi[2], R. Alterini,[1] A. Stronati[2], S. Mulattieri[2], P. Leoni[2]

[1]Department of Hematology, PESCARA; [2]Clinic of Hematology, ANCONA; [3]Institute of Pathology, ANCONA; [4]Department of Dermatological Sciences, FIRENZE; [5]Department of Medicine, CIVITANOVA; [6]Division of Dermatology, MACERATA, Italy

Background. Current therapies of primary cutaneous B cell lymphomas (PCBCLs) consist of surgery, radiotherapy, steroids, interferon-alpha, radioimmunotherapy. Monochemotherapy may be useful (i.e. chlorambucil) in relapsing or multifocal skin lesions of marginal-zone lymphomas (PCMZLs). Refractory and relapsing indolent lymphomas and expecially the aggressive diffuse large B-cell lymphomas (PCLBCLs) are treated with polychemotherapy. Pegylated liposomal doxorubicin (Peg-Doxo) improves the therapeutic index of the free-Doxo and has a favourable pharmacokynetic, pharmacodynamic and toxicity profile. *Aims.* Recently some experiences have been described with Peg-Doxo in primary cutaneous T cell lymphomas (PCTCLs); instead it hasn't been evaluated to date in other PCLs. We evaluated Peg-Doxo monotherapy in the treatment of PCBCLs. *Methods and Results.* Four patients, affected by PCBCL, received i.v. Peg-Doxo at 20 mg/m^2 every 3-4 weeks. Informed consent was obtained. Piridossine orally was given to prevent the palmar-plantar erythrodysesthesia. All patients had multiple generalized nodular skin lesions; one of them was affected by indolent and three by aggressive PCBCL. The first was a 38-year-old woman with a relapsing PCMZL diagnosed 23 months before, treated with radiotherapy and gemcitabine. She had multiple generalized nodular skin lesions and grouped plaques. The skin biopsy confirmed the PCMZL; the association with Borrelia Burgdorferi infection was excluded through serolog-

ic and molecular tests. She was treated with Peg-Doxo monotherapy, after 4 infusions reaching a clinical complete response (CR). Yet she relapsed after 8 months and was treated with interferon and rituximab, achieving a CR, now still lasting after 46 months from Peg-Doxo. The second patient, of 55, affected by PCLBCL-LT first diagnosed 35 months before had been treated with radiotherapy and R-CVP chemotherapy with a CR. He had a relapse with multifocal plaques and tumours on the trunk and started Peg-Doxo (8 infusions), reaching a CR after 2 months and now still lasting after 63 months. The third and the fourth patients were untreated. A 75-year-old man, affected by PCLBCL-LT, with multifocal plaques and tumours of 4 cm obtained a CR after Peg-Doxo (6 cycles). The first cutaneous recurrence was treated with CBVD scheme, followed by rituximab; a second cutaneous relapse was complicated by a cerebral involvement with death. The fourth patient, a 55-year-old man, presented rapidly growing multiple diffuse nodules of the upper arms (PCLBCL-other), and was treated with 6 courses of Peg-Doxo, reaching a CR still lasting after 57 months. In all the patients Peg-Doxo was well tolerated and no patients decreased or delayed the dose; the hematological toxicity was mild with one case of grade III neutropenia. *Summary and Conclusions.* Peg-Doxo has been used in cutaneous T-cell lymphomas refractory to therapy or in advanced stages. These preliminary results constitute the first report of Peg-Doxo in PCBCL; despite the small numerousness, it emerges that single-agent Peg-Doxo is well tolerated, safe and effective in these patients. All they responded well to the therapy (CR=100%), even when pretreated and experiencing the most aggressive forms. It is noteworthy that all patients reached a clinical CR in a short period (median 2,5 months). This suggests the need of further investigations in PCBCL.

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GENETIC ANALYSIS OF HLA CLASS II ANTIGENS FOR MACEDONIAN BONE MARROW DONOR REGISTRY

A. Hristova-Dimceva, E. Velkova, T. Makarovska-Bojadzieva, R. Dukovski, K. Dimitrovski, P. Kolevski

Institute for Transfusion Medicine, SKOPJE, Macedonia

Major Histocompatibility Complex - MHC is a gene complex situated in the HLA region defining individuality of every person. Genetic profile of the Macedonian population represented by serologically defined HLA polymorphism was performed thirty years ago. The progress of tissue typing and new nomenclature caused improvement of previous data according to current knowledge and technical abilities *Aims.* Genetic analysis of HLA-DRB1, DQB1 and DPB1 alleles in the Macedonian population for Macedonian Bone Marrow Donor Registry. *Material and Methods.* 300 healthy, random individuals from Macedonian population were typed for HLA-DRB1, DQB1 and DPB1 alleles using PCR-SSP method. *Results.* Results from this study show that the most frequent HLA-DR alleles are: DRB1*1104 (14.5%), DRB1*1101 (11.1%), DRB1*1601 (10.5%) and DRB1*1501 (10.3%). The most frequent HLA-DQ alleles are: DQB1*0301 (30.3%), DQB1*0502 (14.3%), DQB1*0201 (10.6%) and DQB1*0302 (10.1%), while the most frequent HLA-DP alleles are: DPB1*0401 (30.0%), DPB1*0402 (16.8%) and DPB1*0201 (13.1%). Two-locus haplotypes which are most frequent in our population are: DRB1*1104-DRB3*0202 (12.6%), DRB1*1104-DQB1*0301 (12.8%), DRB1*1601-DQB1*0502 (10.1%), while the most frequent three-locus haplotype is DRB1*1104-DQB1*0301-DPB1*0401 (8.1%). *Conclusions.* Concerning to molecular *Methods.*used for DNA typing of HLA class II alleles, PCR-SSP method is confirmed as a precise, specific, sensitive and rapid method for HLA typing. Allele and haplotype frequencies of the HLA class II antigens in our population show no big differences in comparison with the other Balkan neighboring populations. Obtained dates are necessary for Macedonian Bone Marrow Donor Registry.

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CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA IN CHILDHOOD CLINICAL COURSE AND TREATMENT OUTCOME

G. Martinova, O. Muratovska, S. Glamocanin, Z. Antevska-Trajkova, B. Coneska-Jovanova, S. Koceva

Pediatric Clinic, SKOPJE, Macedonia

Background. Idiopathic thrombocytopenic purpura (ITP) is estimated to be one of the most common acquired bleeding disorders in childhood. It is classified as acute or chronic with the latter defined of the persistence of thrombocytopenia for more than 6 months from the initial presentation of signs and symptoms. *Aims.* The aim of this work was to review the presenting features, response to therapy and natural history of children with chronic ITP treated at the Pediatric Clinic in Skopje, which is the only institution that provides tertiary care and copes with this problem in our country. *Methods and Results.* Between 1997 and 2007, 223 children with ITP were diagnosed and of those 23 (10,3%) developed the chronic form of the disease. Chronic ITP affected older children more often than younger - median age at diagnosis was 8,65 years (range from 3 to 15 years) with females being affected more frequently than males (f:m=1,9:1). Unlike acute ITP, it does not show seasonal predilection. Bone marrow aspiration was performed in all cases and in no one altered the diagnosis. The median platelet count at presentation was $29{,}8\times10^9$/L (range 4 to 63×10^9/L). Approximately half of the patients with chronic ITP presented with mild to moderate hemorrhagic manifestations at the onset of purpura and/or later during the course of the disease: 14 (60,86%) and 12 (52,17%) respectively. Major hemorrhagic was observed in 11 (47,82%) patients: 6 experienced recurrent nosebleeds requiring nasal packing, 3 gross metrorrhagio, 1 hemathemesis and 2 ICH (one after a traffic accident and one spontaneously). Initial management consisted of glucocorticosteroids (GS) in almost all of the patients, just one remained with no treatment. During the evolution of the disease intermittent treatment with GS was applied if platelets were $<10\text{-}20\times10^9$/L, significant bleeding episodes were present regardless of the platelet count, or in those needing elective surgery (GS in 16 patients, GS and immunoglobulins (IVIG) in 6 patients and GS, IVIG and Immuran in 1 patient). IVIG were rarely used as they were expensive form of treatment. Five patients were splenectomised, 3 of them achieved CR after surgery, 2 PR (1 without therapy and 1 with intermittent GS treatment). One patient with ICH died, and the other one with ICH after serious trauma recovered from comma with neurological sequels. *Conclusions.* The annual incidence of chronic ITP in our country is 0,4/100000 children under 15 years. Serious bleedings during chronic ITP are not uncommon.

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REVIEW OF CARDIAC INVOLVEMENT IN ACUTE THROMBOTIC THROMBOCYTOPENIC PURPURA: ASSOCIATION WITH IGG ANTIBODIES TO ADAMTS13

C. Hughes,[1] M. Scully[2], N. Huntley[2], H. Cohen[2], S. Machin[2]

[1]University College London Hospitals, LONDON; [2]Department of Haematology, University College London Hospitals, LONDON, UK

Background. Evidence for cardiac involvement in thrombotic thrombocytopenic purpura (TTP) is rarely described. Case studies and small patient cohorts suggest cardiac involvement maybe more common than previously recognised. *Aims.* To identify cardiac symptoms, associated risk factors for cardiac involvement in acute TTP and the outcome of affected patients. *Methods.*We report a case study of a patient who developed biventricular cardiac failure as a presentation of relapsed TTP, treated successfully with plasma exchange (PEX) and rituximab. A subsequent retrospective review of 55 consecutive patients were analysed for clinical and laboratory evidence of cardiac TTP and histopathological review of 5 patients who died of acute TTP. *Results.* 41/55 patients had troponin T levels on admission. 35/41 had acute idiopathic TTP and 6/41 secondary TTP (4 human immunodeficiency virus, 2 pancreatitis). In 54% (22/41), troponin T ≥0.05 µg/L (normal range 0-0.01 µg/L, acute coronary syndrome ≥0.05 µg/L). Half (12/22) had associated cardiac symptoms, including chest pain, syncope, palpitations and dyspnoea. 8/22 with a raised troponin T reported chest pain. Electrocardiogram (ECG) changes were present in 62% with a raised troponin T. The majority of abnormalities were T wave changes in the anterior ECG leads. 35% with a raised troponin T had an abnormal echocardiogram. Median ADAMTS13 activity on admission was <5% (0-45%, normal range 66-120%). Median IgG antibody to ADAMTS13 was greater (66%(17-162%, normal range <4.2%)) in patients with troponin T ≥0.05 µg/L compared to patients with troponin T ≤0.04 µg/L (35%, range 9-134%). This difference was significant (p=0.01). Patients who died had raised IgG (>67%), but patients with no IgG/inhibitor (secondary TTP, n=6/6) had normal troponin T on admission and there were no deaths in this group. The number of PEX to remission in raised *vs* normal troponin T was 18.5 and 15 respectively, but not significant. Of the 5 post-mortems undertaken, 3/5 died within 24 hours of diagnosis. All demonstrated widespread myocardial microvascular thrombi. A troponin T sample was obtained in 4/5 patients and was higher than in those patients who survived (mean 0.34 µg/L compared to mean 0.11 µg/L). *Conclusions .* The index case, with biventricular failure and a left ventricular (LV) ejection fraction of 28%, normal LV function can be achieved with intensive treatment. Subsequent cases had troponin T measurements at presentation: 54% had raised levels despite clinical symptoms being present in only half and ECG and echocardiogram abnormalities

evident in 62% and 35% respectively. Patients dying of acute TTP had higher admission troponin T and raised IgG antibody (>67%) to ADAMTS13. Secondary TTP was not associated with raised troponin T levels and there were no deaths in this group. Troponin T is specific for cardiac muscle and a sensitive marker of myocardial/cardiac damage. In TTP patients, raised levels (≥0.05µg/L) are likely to signify myocardial microvascular thrombi in patients with high antibody mediated disease, requiring more intensive therapy.

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RISK OF BLOOD CANCERS AMONG PATIENTS WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

I. Bennett,[1] U. Forssen,[1] C. Enger[2], J. Nelson[1]

[1]GlaxoSmithKline, PHILADELPHIA; [2]I3 Drug Safety, ANN ARBOR, USA

Background. ITP is a disease caused by inadequate platelet production as well as increased platelet destruction. Literature on the epidemiology of ITP including blood cancer co-morbidities is currently sparse and ITP remains poorly described. *Aims.* To examine the risk of lymphoma including Non-Hodgkin's lymphoma (NHL) and leukemia including Chronic Lymphoid Leukemia (CLL) among patients with chronic ITP compared to a non ITP population. *Methods.* This was a retrospective database analysis using eligibility and medical claims data from a large U.S. health plan affiliated with i3 Drug Safety. The individuals covered by this health plan were geographically diverse across the United States. Gender distribution was similar in the plan but compared to the US population but elderly individuals were under represented. Chronic ITP patients were defined using the following criteria: a) at least two physician claims separated by at least six months with ICD-9 CM diagnosis code 287.3x for primary thrombocytopenia, b) at least 12 months of continuous enrollment prior to the date of the diagnosis code eligibility, and c) at least 18 years of age between January 1, 2000 and September 30, 2006 with follow-up continued through December 31, 2006. The non ITP reference group was selected from the same time period. Blood cancer outcomes were defined using ICD-9 CM diagnosis codes. The incidence rate ratio (IRR) and 95% Confidence Interval (CI) of occurrence of blood cancers comparing the ITP to non ITP populations was estimated using Poisson regression. *Results.* All chronic ITP patients (N=3,131) who met the above mentioned criteria formed the ITP cohort; N=119,429 non ITP patients created the reference group. History of pre-existing blood cancers was higher in the chronic ITP patients (2.3% for lymphoma, 2.1% for NHL, 2.7% for leukemia and 1.5% for CLL) whereas 0.1% or less of patients in the reference group had same blood cancers at baseline. During a median follow up of 15 months, 16 ITP patients had a diagnosis of lymphoma (incidence rate of 31 per 10,000 person years [PY]) and 18 had a diagnosis of NHL type (incidence rate of 35/10,000 PY). Leukemia was diagnosed in 18 (incidence rate of 35/10,000 PY) with 6 patients (incidence rate of 12/10,000 PY) for CLL type. In the statistical modeling, after adjusting for age, gender and other variables, the adjusted IRR for lymphoma was 9.38 (95% CI: 5.13-17.15) and 11.22 (95% CI: 6.16-20.46) for NHL type; while IRR for leukemia was 15.64 (95% CI: 8.90-27.51) and 10.06 (95% CI: 4.02-25.19) for CLL type. Although the confidence intervals were wide, all adjusted IRRs were elevated and statistically significant. *Summary.* The study found an association of an increased risk for select blood cancers for patients with chronic ITP compared to the non ITP population, hence increasing the burden of this disease. However, more research is warranted to investigate whether this risk is due to the disease or the drugs used to treat ITP since patients may receive cytotoxic chemotherapy agents that can cause blood malignancies.

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POLYMORPHISMS IN HUMAN PLATELET ANTIGEN 1, 2, 3, AND 5 IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

M. Pavkovic,[1] S. Trpkovska-Terzieva,[1] O. Karanfilski,[1] L. Cevreska,[1] B. Georgievski,[1] A. Petlichkovski[2], M. Spiroski[2], A. Stojanovic[1]

[1]Department of Hematology, SKOPJE; [2]Institute for Immunobiology and human genetics, SKOPJE, Macedonia

Background. Idiopathic thrombocytopenic purpura (ITP) is an autoimmune blood disorder caused by the presence of autoantibodies specific for platelet surface glycoproteins (GP), most often GP IIb/IIIa, GP Ib/IX and GP Ia/IIa. Rarely some other auto-antigens could be the epitopes for anti-platelet autoantibodies. Human platelet antigen (HPA) system consists of more than 12 bi-allelic antigen polymorphisms. Due to these polymorphisms, platelet-membrane glycoproteins can be recognized as alloantigens or autoantigens and can cause conditions such as fetomaternal alloimmune thrombocytopenia, post-transfusion refractoriness to platelets and post-transfusion thrombocytopenic purpura. *Aims.* We carried out genotyping of biallelic HPA-1, -2, -3, and -5 systems in patients with ITP to clarify potential associations between HPA alleles and the development of autoimmune thrombocytopenia. *Methods.* We performed genotyping of 60 patients with ITP and 120 healthy control individuals. The group of patients with ITP consists of 43 women and 17 men, with average age of 46.8±16.8. DNA was isolated from peripheral blood mononuclear cells with standard phenol-chlorophorm extraction. Genotyping of HPA -1, -2, -3, and -5 alleles were performed by PCR and RFLP methods. *Results.* Allele frequencies of HPA -1, -3, and -5 were not significantly different between patients with ITP and control group of healthy individuals. There was a significant difference in the allele frequencies for HPA -2 antigens between patients and controls (p=0.023 with Yates correction, χ^2=5.18). Allele frequencies for HPA 2a were 0.852 in controls and 0.75 in patients, and for HPA 2b 0.148 in controls and 0.25 in patients. *Summary*. This result suggests an association between the HPA-2 allele and chronic ITP. The HPA-2b allele was more frequent in patients with autoimmune ITP and this allele may be involved in the formation of ITP-specific autoepitope.

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EVALUATION OF RETICULATED PLATELETS AND PLATELET ACTIVATION IN PATIENTS WITH DYSLIPIDEMIA

Z.W. Grotto, L. Santos, A. Noronha

State University of Campinas - UNICAMP, CAMPINAS, Brazil

Introduction. Platelets play an important role in the pathogenesis of thrombosis and atherosclerosis. Activated platelets interact with endothelium and inflammatory cells by P-selectin action and contribute with thrombo-embolic ischemic events. Platelet activation leads to a rapid release and surface expression of P-selectin and to changes in platelet volume. A larger medium platelet volume (MPV) is an indicator of platelet activation and it is increased in vascular diseases as myocardial and cerebral infarction. Reticulated platelets (RP) are newly formed platelets that contain some rough endoplasmatic reticulum and m RNA. RP measurement is useful for monitoring thrombopoiesis and platelet turnover. We have studied patients with dyslipidemia and preliminary results have showed an increase in the ratio of large platelets (P-LCR) in patients with high levels of serum cholesterol and/or serum triglycerides. The objective of the present study was to evaluate alteration in platelet volume, activation degree of mature and young platelets (RP) in patients with dyslipidemia. *Patients and Methods.* 46 patients were studied: Group 1 (n= 9) - total cholesterol ≥240 mg/dL and/or LDL-cholesterol >160 mg/dl; Group 2 (n= 23) - triglycerides > 150 mg/dl and Group 3 (n=14) - total cholesterol ≥240 mg/dL and triglycerides >150 mg/dL. Control group (CG): 26 normolipidemic individuals. Platelet counting, P-LCR, MPV and PDW were determined using an automated hematology analyzer. Platelets were labeled with CD 41a PECy5. Platelet activation was evaluated by using selectin P (CD62p- PE) and RP by thiazole orange (TO). Activated platelet, RP and activated RP were expressed as percentage and mean intensity of fluorescence (MIF). *Results and Conclusions.* There was not difference in the number of platelets, but VPM, PDW and P-LCR values were significantly higher in dyslipidemic group than in the control group. When the patients were analyzed according to the lipid profile it was observed between CG and Groups 1, 2 and 3, but not among patient groups. The percentage of RP was higher in Group 1 (median 2.05; range 0.32-3.60, p=0.0102), Group 2 (median 1.55; range 0.31-4.23, p=0.0039) and Group 3 (median 2.0; range 0.19-6.69, p=0.0016) than in CG (median 0.76; range 0.15-2.51). The percentage of activated platelet was equally higher in Group 1 (median 2.72; range 0.75-9.95, p=0.0029), Group 2 (median 2.47; range 0.70-6.47, p=0.0002) and Group 3 (median 1.98; range 0.52-9.26) than in CG (0.97; range 0.44-3.04). There was not difference among patient groups. When the degree of activation of RP was evaluated (platelets positive for CD62p and TO labels), it was not observed differences between CG and dyslipidemic patients. An increased number of larger platelets, RP and activated platelets in patients with abnormalities in lipid profile suggest the excess of lipoproteins may promote cell activation and platelet hyperaggregability leading to a higher risk for atherothrombotic events.

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VARIANT CLINICAL COURSES IN CHILDREN WITH ITP; 16-YEAR EXPERIENCE OF A SINGLE MEDICAL CENTER

M. Turker, I. Durak, B. Atabay, I. Yaprak, M. Turker, E. Ozer

Tepecik Teaching and Research Hospital, IZMIR, Turkey

Aims. Idiopathic thrombocytopenic purpura (ITP) is the most common cause of acquired thrombocytopenia in children. However, single-institutional and long-term natural history data are limited. The objective of this study is to evaluate the presenting features, variations in the clinical courses, response to therapy and long-term outcome in patients with ITP treated at our Pediatric Hematolgy Division. *Methods.* Three hundred and fifty out of 491 patients with ITP between 6 months to 16 years of age, diagnosed and followed-up in the last 16 years were included in this retrospective study. Patients who had low platelet counts (<150×10^9/L) for <6 months were accepted as acute ITP; patients in whom thrombocytopenia persisted for more than 6 months after initial diagnosis were accepted as chronic ITP. Patients showing recurrences within 6 months after initial diagnosis and after a sustained remission without treatment lasted at least 3 months were accepted as recurrent ITP. Chronic ITP patients were also evaluated in *non-remission* and *late-remission* subgroups. Patients with platelet counts <20×10^9/L and/or bleeding symptoms at diagnosis were treated with high dose methylprednisolone (HDMP), intravenous immunoglobulin (IVIG) and/or combination therapy of HDMP and IVIG. Complete remission was defined as the maintenance of platelet count at ≥150×10^9/L, and partial response was defined as symptomatic improvement with an increase in platelet count to more than 50×10^9/L. Platelet counts below 50×10^9/L were defined as non-response. *Results.* Among 350 patients with ITP 186 were female. Median age at diagnosis 5 years and median initial platelet count was 9.5×10^9/L. Patients were followed-up for median 3.5 years (6 months to 14.5 years). The clinical course of the patients was found as acute, chronic and recurrent in 63.8%, 29.1% and 7.1%, respectively. Acute cases presented at a younger age than chronic and recurrent cases; 4.5, 6.2 and 6.5 years, respectively (p<0.05). Initial platelet counts in acute and recurrent ITP patients were significantly lower than in chronic ITP patients (p<0.05). Platelet count >20×10^9/L and initial diagnosis age >10 years were found to increase the probability of subsequent chronic ITP at least 2-folds. Concerning the 102 chronic ITP patients, 77.5% was found to be in the non-remission and 22.5% in the late-remission group within the follow-up period. Late-remission chronic ITP patients achieved remission in median 1.5 years. Concerning the recurrent ITP patients, median age at initial diagnosis was 6.2 years (1.8-12.5 years), these patients had undergone 1-4 recurrences. Of 102 chronic ITP patients, 29.4% (n=30) were splenectomized and 24 (80%) responded well to splenectomy. *Conclusions.* The natural course of the disease is variable. Besides acute and chronic form, the recurrent form which is about 7% of all ITP patients, and chronic ITP patients with late remission should be considered. In prediction of chronic ITP, age older than 10 years at initial diagnosis, no response to initial therapy and initial platelet count over 20×10^9/L can be considered as risk factors.

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HIGH PREVALENCE OF INTRACRANIAL HEMORRHAGE(ICHGE) IN IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP) IN CHILDREN AND ADOLESCENTS; MULTICENTER STUDY FROM 6 CENTERS FROM EGYPT

M.S. Elalfy, M.S. El ALfy, Y. Al Tonbary

Ain Sahms University, CAIRO, Egypt

Idiopathic thrombocytopenic purpura is uncommon cause of mild bleeding, however serious bleeding is extremely rare .Intracranial hemorrhage is reported in 0.1% of ITP in western studies. Data from 2440 ITP were collected from 6 Pediatric hematology centers in Egypt from 1997-2007; the median age at presentation was 6.1 years, boys were 56.3%. Petechiae and ecchymosis were the commonest presenting symptoms, 10 patients 0.44% (4 were acute ITP) suffered intracranial hemorrhage, 60% were boys, 90% had median platelet count, <10×10^9/L, they received IVIG 1-2 gm /kg with platelet transfusion, three of them died, 2 recovered with seizures and 5 without squeal, range and median time for presentation to emergency room was (4-24, 8 hours respectively). Median Platelet count for the whole group was 14.5×10^9/L and 22% were <10×10^9/L.Bone marrow aspiration was still the routine in 88% of ITP patients even with classic presentation, hospitalization was necessary in 44%. Initial management consisted of no specific drug treatment in 21%, corticosteroids in 42% high dose methyl prednisone in 8%, intravenous (iv) immunoglobulin in 12%, iv Anti D in 8%, combined therapy in 9%. Platelet transfusion was prescribed in 6% of patients with severe bleeding; only one-third was properly indicated. Chronic ITP with thrombocytopenia persistent >6 months was reported in 32%; their median age was 7.8 years, 46% boys. Serious bleeding was more prevalent among chronic ITP, menorrhagia was troubling symptom and most difficult to be treated. Splenectomy was performed in 8% of those with recurrent serious bleeding after a median of 28 months, Anti CD20 was successful(complete remission) in 3 out of 8 patients refractory ITP. Multi-drug therapy was reserved for the most difficult bleeding episodes. *Conclusions.* Intracranial hemorrhage is more prevalent in our series compared to western, outcome was poorer, either a genetic or psycho-social factors may play a role. More aggressive treatment should be tried.

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TREATMENT OF IMMUNE THROMBOCYTOPENIC PURPURA. POSTSPLENECTOMY LATE RESPONSE. A SINGLE INSTITUTION EXPERIENCE

C. Ionita,[1] R. Pacurar[2], D. Nicola[2], T. Nicola,[1] M. Cheveresan[2], M. Ionita,[1] D. Calamar,[1] H. Ionita[1]

[1]*University of Medicine and Pharmacy Victor Babes, TIMISOARA;* [2]*County Hospital, Hematology Department, TIMISOARA, Romania*

Background. Idiopathic thrombocytopenic purpura (ITP), also reffered as immune or autoimmune thrombocytopenic purpura, is an acquired disease characterized by low platelet count, normal bone marrow, usually with an increased number of megakaryocytes and the absence of any other disease.The majority of patients respond, on short term, at an initial corticosteroid therapy, which is used as first line therapy. Some times after steroid therapy patients relaps and in this case splenectomy is the second line of therapy. *Aims.* We tryed to evaluate the therapeutical results in a group of patients with ITP and the duration of their remision after splenectomy. *Methods.* From may 1990 to may 2005 were hospitalised and treated in the Hematology Clinic 165 patients with ITP. The median age of patients at diagnosis was 38,18±16,44 years (range 15-72 years). The distribution on sex was 112 females and 53 males. The mean time from diagnosis was 21,61 month. The mean platelet count before tretment was 21,252±2,336/μL with limits between 5000 and 115.000. Our patients 39% presented gastrointestinal bleedings, 47% had sclerotegumentary bleedings, 9% had bleedings in the central nervous system and 5% other bleedings. The most of the patients (78%) were treated with corticosteroids, 12% received steroids and immuno globulins and the remaning of the patients were treated with steroids and vinca alkaloids and rituximab. The patients with severe thrombocytopenia received platelet transfusions. We consider a sustained response a platelet count above 50.000/μL or above 30.000/μL without hemorrhages or only with minor purpura. A complet response was considered a platelet count above 150.000/ μL after a discontinuation of therapy. Splenectomy was consider after 3 to 6 month in patient resistent to corticosteroids or erlier at patients demand. 45% (75 pts)had a splenectomy because they relapsed after steroids or they needed very high doses of steroids for a safe number of platelets. From those patients with splenectomy, 53 were females and 22 were males. Mean age at the time of the splenectomy was 36,41±16,88, the medium time from diagnosis to splenectomy was 3,5 years (0,6-96 months). The response to splenectomy was defined as followes: complete response (CR) a number of trombocytes higher than 150.000/mms for more that 4 weeks, partial response (PR) trombocytes between 50.000-150.000/mms lastig more that 4 weeks and relapse a number of trombocytes under 50.000/mms. *Results.* The medium follow-up time was 7 years (2-10 years). The overall response was 79%, with 58% of CR and 21% PR. From 75 patients with splenectomy 15 patients relapsed and 5 of this 15 were in CR after steroid therapy following splenectomy. The long therm follow-up in CR and PR proves a good, stabil and durabil response in time for more than 7 years. Post splenectomy complications in the study group were not significant. *Conclusions.* Our study proves that patients with chronic imune thrombocytopenic purpura who failled corticotherapy get a safe and durable response in time after splenectomy.

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PRESENCE OF ANTI PLATELET GP IIB-IIIA INVESTIGATION IN DENGUE INFECTED PATIENTS SERA

A. Rachman,[1] Z. Djoerban[2], A. Harahap,[3] S. Santoso[4]

[1]Division of Hematology & Medical Oncology Dept of Internal Medicine, CIPTO MANGUNKUSUMO HOSPITAL, JAKARTA, Indonesia; [2]Division of Hematology & Medical Oncology, JAKARTA PUSAT, Indonesia; [3]Dept of Clinical Pathology/Eijkman Institute, Cipto Mangunkusumo Hospital, JAKARTA PUSAT, Indonesia; [4]Institut für Klinische Immunologie und Tranfuzionsmedizin, Justus Liebig Univers, GIESSEN, Germany

Background. Thrombocytopenia is one of the most common clinical findings in patients infected with Dengue Virus (DV). The exact mechanism responsible for the platelet destruction in these patients, however, is not known. Recently, several studies showed an elevated surface IgG on patient's platelets indicating an immune mediated mechanism. The presence of specific antibody reacted against platelet glycoprotein (GP), however, is not demonstrated. *Aims.* In this study, we investigated the relevance of antibodies against platelet specific GPIIb/IIIa (αIIbβ3 integrin, fibrinogen receptor) in sera of dengue infected patients. *Methods.* Forty patients clinically suspected of having acute Dengue virus infection according to WHO 1997 criteria were investigated in a hospital base prospective study. The presence of IgG/IgM antibodies against DV were documented using Dengue Duo Rapid Strip test. Antibodies against platelets were determined from patient's sera obtained on day 5 of fever according to the significance drop of platelet count. Following *Methods.*were applied for the characterization of platelet specific antibodies; platelet ELISA, antigen capture assay using monoclonal antibody against GPIIb/IIIa (MAIPA) and immunoprecipitation with biotinylated platelets. *Results.* Twenty three male (56.1%) and 18 female (43.9%) with age range between 13-60 years old were enrolled in this study. One patient with primary infection (2.44%) and 40 (97.56%) secondary infection. By the use of ELISA with intact platelets anti-platelet IgG was detected in 29 patients (70.7%). When these sera were analyzed by the MAIPA assay, 13/29 (42.9%) showed positive reaction with platelet GPIIb/IIIa. However, these reactions are moderate according to the current MAIPA criteria. In some sera, the glycoprotein specificity GPIIb (130 kDa) and GPIIIa (100 kDa) as target protein could be confirmed by immunoprecipitation. Analysis of platelet counts *vs* detectable platelet antibodies showed a significant correlation (χ^2=7.049; p=0.008). Similar results were observed with anti-GPIIb/IIIa (χ^2=25.512; p=0.000). *Conclusions.* These results demonstrated that some Dengue infected patients, developed platelet reactive antibodies against GPIIb/IIIa associated with platelet count indicating the involvement of immune mediated platelet destruction in this disease.

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EFFECTS OF G-CSF PLUS DEXAMETHASONE ON HEMOSTATIC PARAMETERS IN HEALTHY GRANULOCYTE DONORS: ROLE OF U-PA AND NITRIC OXIDE

H. Ekmekci,[1] O. Balci Ekmekci[2], G. Ozturk[2], H. Ekmekci[2], D. Atay[2], M. Yanasik,[3] S. Anak,[1] O. Devecioglu[1]

[1]Istanbul University, Istanbul Medical Faculty, Department of Hematology Oncology, ISTANBUL; [2]Istanbul University, Istanbul Medical Faculty, Pediatric Hematology Oncology, ISTANBUL; [3]Istanbul University, Istanbul Medical Faculty, Department of Blood Bank, ISTANBUL, Turkey

Backround. Granulocyte colony-stimulating factor (G- CSF) is widely used to reduce the risk of infection resulting from neutropenias and to mobilize and collect CD34$^+$ hematopoetic progenitor cells (HPCS) for autologous and allogenic transplantation. The safety of rhG-CSF administration in healthy donors has been investigated in several studies. However, there are limited cumulative data about the effects of rhG-CSF on hemostasis. *Aims.* We evaluated hemostatic parameters including urokinase-type plasminogen activator antigen (u-PA:Ag) and nitric oxide in 17 volunteer, healthy granulocyte apheresis donors who donated for neutropenic patients. *Methods.* Recombinant Human Granulocyte Colony-stimulating Factor (rhG - CSF; single dose, 10 microg/kg subcutaneously) and dexamethasone (8 mg, single dose oral) were given to donors 12 hours before granulocyte apheresis. Two blood samples were drawn at time 0 (T0), before rhG - CSF and dexamethasone administration; time 1 (T1), immediately before the apheresis. *Results.* We showed a statistically significant rise in coagulant factor VIII (FVIII) and von Willebrand factor (vWF), and slightly rise in u-PA:Ag after G-CSF dexamethasone administration. In addition, there were positive correlations between vWF-D-dimer and FVIII-D-dimer. We have also found a significant decrease in mean total nitric oxide (NOx), nitrit and nitrate levels after G-CSF plus dexamethasone administration. Moreover, there was a strong negative correlation between nitrite and D-dimer levels (r=-0,611; p=0.009). *Conclusions.* Even if partially compansated with u-PA and protein C, increased FVIII and vWF activity, and decreased nitric oxide levels may stil partially contribute to progress of thrombosis risk in rhG - CSF plus dexamethasone administered healthy granulocyte donors. Large numbers of healthy donors exposed to G-CSF plus dexamethasone will be needed to evaluate the risk of thrombosis in this population.

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NANOPARTICLES OF TRADITIONAL CHINESE HERBS INHIBIT THROMBOSIS *IN VIVO*

J. Yin,[1] Y.J. Shen,[1] Z.W. Zhang,[1] X.G. Luo,[1] X.F. Wang[2], H.L. Wang[2]

[1]Medical College of Shantou University, SHANTOU; [2]Shanghai Institute of Hematology, Ruijin Hospital, SHANGHAI, China

Background. Thrombosis is a major and increasing public health problem in both developed and developing countries worldwide. China's extensive experience in the use of traditional Chinese herbs (TCH) in thrombosis therapy indicates that TCH preparations are effective, with few or no side-effects. There are more than 100 traditional herbs in use for thrombosis therapy in China. Nanoparticles of TCH are helpful to improve their absorption and distribution in body, and therefore enhance their efficacies. In this article, we investigated thrombolytic effects of nanoparticles of TCH by comparing with their non-nanometer form. Aimes: Thrombolytic effects *in vivo* of nanoparticles of TCH were investigated. *Methods.* TCH including peach seed, safflower, angelica root, szechwan lovage rhizome, rehmannia root, red peony root, leech, gadfly, earth worm and ground beetle, were mixed and prepared through drying, mincing, extracting, crushing into liquid particles with ultrasonic wave, filtering and nanometerizing into nanoparticles soliquoid with nanometer collider. 30 Sprague-Dawley rats were randomly divided into 3 groups, and thrombosis *in vivo* was accomplished by stimulating rat abdominal aorta with electricity for 60 min and no blood flow displayed for 5 min. In group I, natural saline was administered intravenously 30 min after thrombosis, and in group II and III, with equal volume of non-nanometer particles of TCH and their nanometer soliquoid form instead respectively. During the experiment, recanalization of abdominal aorta was assessed with electromagnetic flowmeter, and the segment of abdominal aorta in which thrombus existed was cut into thin slices and observed under microscope after hematoxylin-eosin stain to evaluate the occlusion at the end of the experiment. *Results.* 1) Recanalization of abdominal aorta: Recanalization was defined as over 30% of basic blood flow was observed. In group III, there were 9 rats demonstrated recanalization of abdominal aorta, the recanalization start time was (79.65±5.21) min, recanalization rate was (83.6±9.5)% [p<0.05≥0.01 compared with group I and II, in group I, however, there was no rat demonstrated recanalization of abdominal aorta, the recanalization start time was longer than 180 min, recanalization rate was 0%. In group II, 5 rats displayed recanalization of abdominal aorta, the recanalization start time was (119.53±8.25) min, recanalization rate was (46.39±6.91)%, there were also significant differences between group I and II (p<0.05≥0.01)]. 2) Blood flow of abdominal aorta: Data was displayed in the table. 3) Area ratio of thrombus/lumen: The mean area ratio of thrombus/lumen was (22.36±6.95)% in group III [p<0.05 compared with group I and II, while in group I and II, the mean area ratio of that was (83.65±10.51)% and (55.74±8.83)% respectively, p<0.05 compared between group I and group II]. The maximal area ratio of thrombus/lumen was 33.25% in group III, while 93.44% and 65.18% in group I and II respectively. *Conclusions.* Nanoparticles of traditional Chinese herbs showed significant thrombolytic effects, resulting in quick recovery from arterial embolism and diminution of thrombi. The thrombolytic effects of nanoparticles of traditional Chinese herbs are much intensified than their non-nanometer form.

Table 1. Blood flow of abdominal aorta around thrombosis.

			after administration of natural saline, non-nanometer particles of traditional Chinese herbs or their nanometer soliquoid form								
groups	before thrombosis	thrombosis	20min	40min	60min	80min	100min	120min	140min	160min	180min
group I	27.33± 2.72	0.62± 0.09	0.63± 0.07	0.65± 0.09	0.61± 0.08	0.60± 0.11	0.63± 0.09	0.62± 0.06	0.59± 0.09	0.65± 0.05	0.61± 0.06
group II	26.62± 3.33	0.64± 0.06	0.66± 0.05	0.63± 0.09	0.59± 0.07	2.15± 1.09*	5.08± 1.92*	7.63± 2.26*	8.39± 2.83*	9.65± 3.31*	10.18± 3.95*
group III	25.58± 2.16	0.65± 0.08	0.64± 0.09	2.52± 0.56**	5.64± 0.83**	7.27± 1.12**	10.56± 2.30**	12.84± 2.81**	13.61± 3.10**	14.45± 3.81**	14.96 4.06**

*: p<0.01, compared with group I, *: p<0.05, compared with group II

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ESTROGEN RECEPTOR 1 GENE POLYMORPHISMS AND CORONARY ARTERY DISEASE IN THE TURKISH POPULATION

T.U. Ulutin,[1] M. Güven,[2] E. Öz,[3] B. Batar,[2] B. Karadag,[3] N. Domaniç[3]

[1]Istanbul University, ISTANBUL; [2]Cerrahpasa Medical Faculty, Department of Medical Biology, ISTANBUL; [3]Cerrahpasa Medical Faculty, Department of Cardiology, ISTANBUL, Turkey

Estrogen protects against atherosclerosis through its genomic/nongenomic effects. We examined the association of three established single nucleotide polymorphisms, intron 1 -397T>C (PvuII or IVS1-397) and -351A>G (XbaI or IVS1-351), and exon 8 G594A, in the ESR1 gene with the prevalence and severity of coronary atherosclerosis in Turkish populations. Our study population consisted of 141 patients with angiographically documented CAD and 47 controls who had normal electrocardiographic findings. Genotypes of ESR1 gene were determined by polymerase chain reaction followed by restriction enzyme digestion. When the patients with CAD were grouped according to affected coronary vessel number, the IVS1-397T>C polymorphism was associated with the extent of CAD (p=0.045). Homozygosity for the T allele of the IVS1-397T>C polymorphism was a risk factor for single-vessel CAD (OR:1.15; 95% CI=1.15-9.86; p=0.02). In addition, when comparing allele frequency of the exon 8 G/A polymorphism between CAD subgroups and control individuals, a significant difference was observed (p=0.03). The A allele of exon 8 G/A polymorphism was significantly associated with a protective factor for single-vessel CAD (OR:0.23; 95% CI=0.06-0.80; p=0.02). On the other hand, no association between IVS1-351A>G polymorphism and the presence or the severity of CAD was observed. Our results point to the importance of ESR1 genotype in relation to cardiovascular disease susceptibility.

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THE INCIDENCE OF PRIMARY ANTIPHOSPOLIPID SYNDROME AMONG PATIENTS WITH PERIPHERAL DISTURBANCES IN THE ARTERIES OF UPPER AND LOWER LIMBS

A. Lehmann-Kopydlowska,[1] A. Lehmann-Kopydlowska,[1] K. Wachal[2], E. Wojtasinska,[3] Z. Turowiecka,[3] K. Ciepluch,[3] W. Majewski[2], K. Zawilska[3]

[1]J. Strus Hospital, POZNAN; [2]Poznan University of Medical Sciences, Department of General and Vascular Surger, POZNAN; [3]Poznan University of Medical Sciences, Department of Hematology, POZNAN, Poland

Background. In the past the changes in small, peripheral limb arteries was considered as first symptoms of thrombarteritis obliterans (TAO, Buerger disease), especially if these changes were observed in young, smoking patients in their thirties or forties. Among other causes of these vessel alterations reumatical or hematological disorders were indicated. For many years thorough immunological diagnostics has enabled to recognize the primary antiphospholipid syndrome (APS) as a reason of significant disturbances in the arterial circulation of toes and fingers. In patients suffering from this syndrome the symptoms of critical limb ischemia are often present, becoming a cause of later amputations. *Aims.* The main aim was finding the incidence of primary antiphospholipid syndrome among patients with peripheral disturbances in the arteries of upper and lower limbs. *Materials and Methods.* Initially 52 patients with changes in small, peripheral arteries of upper and lower limbs were taken under observation. The accepted clinical criteria, such as: young age, sudden character of symptoms and their feature (arterial thrombosis of uknown etiology without evidence of inflammation) were met by 18 patients (4 women and 14 men) at the age of 30-58 years. These people were taken care of by University Outpatient Clinic of Vascular Surgery for 2 to 12 years. On the base of imaging studies results (angiography, angio-CT, angio-NMR, Doppler-USG) changes in peripheral arteries of limbs were confirmed and the study group of 15 patients was finally selected. In all these selected patients thrombarteritis obliterans had been suspected earlier on the base of medical history and the course of disease. Hematological assays for congenital thrombophilia and the presence of antiphospholipid antibodies according to the actual guidelines of ISTH (detection of lupus anticoagulant, anticardiolipin antibodies and anti-β2 glycoprotein-I ELISAs on two or more occasions 12 or more weeks apart) were performed in all patients. People with the suspition of connective tissue disease were excluded from the study group. *Results.* The primary antiphospholipid syndrome was confirmed in 5 patients (28%) from the study group (2 women, 3 men). *Conclusions.* The primary antiphospholipid syndrome is a disease entity recognized more and more frequently among the patients with the symptoms of changes in peripheral arteries of limbs. Therefore a complex angiologic diagnostics to detect APS is a necessity of today, especially in patients with untypical symptoms of disturbances in peripheral circulation and in patients not responding to the routine treatment.

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EXPRESSION OF TISSUE FACTOR AND TISSUE FACTOR PATHWAY INHIBITOR IN MICROPARTICLES AND SUB-CELLULAR FRACTIONS OF NORMAL AND MALIGNANT PROSTATE CELL LINES: CORRELATION WITH DIFFERENTIATION STATUS

B.A. Lwaleed,[1] L. Lam,[2] A. Cooper,[2] P. Watson[2]

[1]Southampton University Hospitals NHS Trust, SOUTHAMPTON; [2]Department of Biomedical Sciences, University of Portsmouth, PORTSMOUTH, UK

Background. The association between cancer and thrombogenesis has been recognised since 1865 and tissue factor (TF) is important at various stages in the natural history of the disease. It is involved in cancer angiogenesis, growth and metastasis. Tissue factor pathway inhibitor (TFPI), being the major physiological regulator of the TF-dependent coagulation pathway, is also up-regulated in many tumour types. *Aims.* We determined TF and TFPI levels in prostate cancer cells showing differing morphology, hormone sensitivity and growth behaviour. *Methods.* The prostate cell lines PC3, LNCaP and the virally immortalized *normal* PNT2 strain were grown in culture flasks and were harvested at >90% confluence. The cells were fractionated into cytosol, membrane and nuclei for analysis. Microparticles (MP) secreted into the culture medium were also analysed. Tissue factor and TFPI levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA). *Results.* There was an absence of TF and TFPI in nuclei of all cell lines. Counter intuitively, TF expression was higher in other sub-cellular fractions and MP of *normal* prostate cells compared to prostate cancer cells. Tissue factor expression decreased with increasing state of morphological and biochemical differentiation in prostate cancer cells. In contrast, TFPI in MP of normal prostate cells was much lower than tumour cells. In both cell lines TFPI levels showed a direct relationship with biochemically defined differentiation. *Summary.* Our results suggest a role for TFPI in prostate cancer.

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THE RELATIONSHIP OF P2Y1 RECEPTOR POLYMORPHISMS AND ISCHEMIC HEART DISEASE

E. Park,[1] H. Kim[2], J. Park[2], I. Kim[2], S. Park[2], S. Park[2], J. Kim[2], Y. Hong[2], S. Lee[1]

[1]Chung-Ang University Hospital, SEOUL; [2]Seoul National University College of Medicine, SEOUL, South-Korea

Background. The platelet ADP receptor P2Y1 plays a key role in platelet aggregation. There is marked interindividual variation in platelet response to ADP. *Aims.* We studied the possible link between the P2Y1 ADP receptor polymorphism and the risk of ischemic heart disease in a case-control study. *Methods.* We tested A1622G, C2259G, C647G sites of P2Y1 gene. We studied 525 patients with ischemic heart disease (263 patients with acute coronary syndrome, 262 patients with chronic stable angina) and 471 age and sex matched control subjects. The three-SNP block study was performed with A1622G-C2259G-C647G haplotypes. *Results.* In patients with chronic stable angina, A1622G were 128 cases, 179 controls in AA, 109 cases, 189 controls in AG, 20 cases, 60 controls in GG (OR=0.723, 0.571~0.915; p=0.007). C647G were 159 cases, 235 controls in GG, 83 cases, 169 controls in GC, 12 cases, 24 controls in CC (OR=0.728, 0.530~0.999; p=0.049). In patients with all ischemic heart disease (ACS+CSA), A1622G were 240 cases, 179 controls in AA, 233 cases, 189 controls in AG, 47 cases, 60 controls in GG (OR=0.609, 0.406~0.914; p=0.016). In patients with chronic stable angina, A-G-G blocks were 127 cases, 176 controls in haplotype homozygote, 107 cases, 186 controls in heterozygote, 19 cases, 57 controls in other homozygote (OR=1.388, 1.093~1.763; p=0.007). G-G-C blocks were 12 cases, 24 controls in haplotype homozygote, 82 cases, 164 controls in heterozygote, 159 cases, 231 controls in other homozygote (OR=0.726, 0.528~1.000; p=0.050). In patients with all ischemic heart disease (ACS+CSA), A-G-G blocks were 237 cases, 176 controls in haplotype homozygote, 229 cases, 186 controls in heterozygote, 45 cases, 57 controls in other homozygote (OR=1.631, 1.078~2.467; p=0.020). *Summary and Conclusions.* The P2Y1 platelet ADP receptor gene polymorphism, A1622G and C647G, were associated with ischemic heart disease, especially with chronic stable angina, with higher risk in carriers of the A allele in A1622G and G alleles in C647G.

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CHANGES IN SERUM PROTHROMBOTIC MARKERS INDUCED BY BEVACIZUMAB CONTAINING CHEMOTHERAPY

F. Ni Ainle, F. Crotty, C. Murphy, F. Ni Ainle, L. Smith, O.S. Breathnach, L. Grogan, P. Murphy

Beaumont Hospital, DUBLIN 9, Ireland

Background. Angiogenesis has a vital role in tumour growth and metastasis. The first angiogenesis inhibitor, bevacizumab, was approved for use in metastatic colorectal cancer in February 2004. Its use has been associated with an increase in thromboembolic events, particularly arterial events. However, the mechanism by which this increase occurs is poorly understood. *Aims.* Our aim was to prospectively measure specific plasma coagulation parameters in this setting. To do this, we studied changes during Bevacizumab-containing therapy in markers of prothrombotic and antithrombotic states and of endothelial and platelet activation in sequential patients presenting with colorectal carcinoma. *Methods.* We recruited metastatic colorectal cancer patients commencing first line or salvage chemotherapy with bevacizumab. For each patient, plasma levels of the following markers were measured at baseline and after cycle 1 and cycle 2 of chemotherapy: prothrombin time, activated partial thromboplastin time, D-Dimer, fibrinogen, factor VIII, activated protein C resistance, protein C activity, free protein S, soluble P-selectin, platelet count, von Willebrand factor antigen and von Willebrand factor activity. *Results.* Ten patients were identified, with a median age of 55 (36-74) years. One venous thromboembolic event occurred after cycle 2 of chemotherapy. There was a significant decline in Protein C activity from baseline (99.2±13.8 U/dL) to post-cycle 2 of chemotherapy (82.0±17.6 U/dL) (p=0.003) and a significant increase in Von Willebrand Factor antigen from baseline (119±50 U/dL) to post cycle 2 of chemotherapy (159±76 U/dL) (p=0.05). There was no significant change in any other marker from baseline to post cycle 2 of chemotherapy and no significant change in any marker from baseline to post cycle 1 of chemotherapy. *Summary and Conclusions.* These preliminary results suggest for the first time a trend towards reduction in Protein C activity and towards an increase in Von Willebrand Factor antigen from baseline in patients receiving Bevacizumab containing chemotherapy. Larger prospective studies will be necessary in order to confirm these trends.

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Withdrawn by the authors

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MUTATION DETECTION IN PROMOTER REGION OF COAGULATION FACTOR IX IN HEMOPHILIA B PATIENTS IN IRAN

M. Allahbakhshian Farsani,[1] G. Rastegar[2], A. Kazemi[2], F. Ala,[3] M. Mohammadi[2], S. Ravanbood,[3] A. Allahbakhshian[4]

[1]Dept. of Hematology, School of Medical Sciences, Tarbiat Modares University, TEHRAN; [2]Dept. of Hematology, School of Medicn.Iran Medical University, TEHRAN; [3]Compreasive Hemophilia Center, TEHRAN; [4]Dept. of Nursing, School of Medicine, Tabriz Medical University, TABRIZ, Iran

Hemophilia B Leyden is an X chromosome-linked bleeding disorder characterized by an altered developmental expression of blood coagulation factor IX. This form of hemophilia has been found to be associated with variety of single point mutations encompassing a 40-nucleotide region in factor IX promoter region. Mutations in promoter of factor IX gene are relatively rare (about 2% of total) but they are important because can give rise to the unique hemophilia B Leyden phenotype, where symptoms typically ameliorate at puberty from severe to asymptomatic. Our objective was to study mutation in exon-1 in 43 iranian hemophilia B patients to recognize possible cases of hemophilia B Leyden. Exon-1 of factor IX gene was amplified by PCR and then its products were studied using conformational sensitive gel electrophoresis (CSGE) to distinguish cases having mutation in this region. Two cases show bandshifts on CSGE. Exon-1 of these patients was directly sequenced. We have found two different mutations in exon-1 that are included the A/T mutation at +6 and the A/G mutation at +13. Although most patients with hemophilia B have one mutation, about 1% has double mutations. Thus the resultant of double mutations in tow different region of factor IX gene might causes hemophilia B during whole life. So we examined exon 1-8 and polyadenylation site of factor IX gene in these two patients and have not found any other mutation. By recognizing and distinguishing these patients from classic hemophilia B we could inform these patients about special kind of their disorder and their post pubertal recovery. In addition they could be advised before marriage and childbearing. Our results show hemophilia B leyden may has higher frequency in Iran.

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OCCURANCE OF HEMOPHILIA IN IRAN

M Mehdizadeh,[1] G Zamani[2]

[1]Shaheed Beheshti medical university, TEHRAN; [2]Tehran university of medical sciences, TEHRAN, Iran

*Background.*The congenital bleeding disorders hemophilia A and B are estimated to affect between 1 in 10,000and 1 in 5,000 males.*Aims.*To estimate prevalence and occurrence rates and to document the epidemiological features and disease severity of the hemophilic patients this study was conducted on hemophilic population in Tehran which includes about 1/4 of country population and also a genetic mixture of different Iranian populations. *Methods.* Age, sex, first clinical presentation,patient age at the time of diagnosis; family history of bleeding tendency,patient blood group and Rh, consanguineous marriage history in parents and most common bleeding symptoms, Platelets count , bleeding time, prothrombin time and activated partial thromboplastin time factor ïò screening were all collected from the hemophilia registry in Iran Hemophilia center . clinical records. Descriptive Statistics, t test, chi square, and analysis of variance were employed to analyze the dataset. Total population by age groups and other information about Tehran province population obtained from the statistical Center of Iran in 2006. *Results.* Of 6427 registered patients with inherited coagulation disorders 1077(59.7%) had hemophilia A and 211(11.7%) hemophilia B in Tehran.The proportion of cases with hemophilia A to B was 5:1. Patients included 96.8%males and only 3.2% females. The prevalence of hemophilia A and B in Tehran province in 2006 were 14 cases per 100,000 males and 2.5 case per 100,000 males respectively. The incidence rates were 1.91 per 1,000,000 live male birth for hemophilia A and 0.15 per 1,000,000for hemophilia B. Correlation between age group and the prevalence of inherited coagulation disorders was shown to be significant. The mean age of patients with hemophilia A was 26.4±15.6years. The mean (±SD) age of all cases was 25.92±15.19 years and half of the population was younger than 24 years of age. Compared with the overall Tehran male population, the hemophilia population had a much greater proportion of males younger than 25 years .It is also seen that severe disease is more common in patients under 40 years old and the mild and moderate disease is dominant then .Correlation between mean age and disease severity was shown to be significant.(mild disease *vs* moderate disease: p=0.004,moderate disease *vs* severe disease: p<0.0001). Mean age at time of diagnosis was also significantly lower in severe disease (p<0.0001). Factor inhibitors were found in 8.5% of patients with hemophilia A. None of patients with type B hemophilia carried factor inhibitors. The relation between age and presence of inhibitor factors was not significant. Overall, the most common first clinical presentations were ecchymosis and haematoma after trauma, post circumcision bleeding, haemarthrosis and bleeding after dental extraction. *Conclusions.* It seems that despite frequent consanguineous marriages that results in more frequent inheritance of autosomal recessive coagulation disorders in Iran the epidemiological features and disease severity of the hemophilic patients in Iran is similar to western countries.

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RITUXIMAB IN THE MANAGEMENT OF ADULT PATIENTS WITH HEMOPHILIA-A AND INHIBITORS: MARKED REDUCTION IN INHIBITOR LEVEL AND CLINICAL IMPROVEMENT IN BLEEDING BUT FAILURE TO ERADICATE THE INHIBITOR

A. Aleem, A. Saidu, H. AbdulKarim, A. Al-Sagheer, A. Al-Diab, A. Arain, A. Al-Momen

King Khalid University Hospital, RIYADH, Saudi Arabia

Background. Management of hemophilia A patients with inhibitors is difficult and costly. Standard treatment for such patients is immune tolerance induction (ITI) therapy which is successful only in 60-80% of patients. Patients who fail ITI are at a higher risk of morbidity and mortality. Rituximab is an anti-CD20 antibody which was originally developed for B-cell lymphomas and has shown excellent activity in various B-cell lymphoproliferative disorders. Apart from being effective in B-cell lymphomas, it has also shown activity in many autoimmune hematological and non-hematological disorders. *Aims.* To evaluate the effectiveness of rituximab in patients with hemophilia A and inhibitors. Patients

and *Methods.* We used (off label) rituximab in 3 patients with severe hemophilia A and inhibitors as ITI was not possible either because of patients' refusal or non-availability of funds. All patients gave informed consent. Rituximab was given at a dose of 375 mg/m^2 at weekly intervals and patients received 2 to 5 doses. Bleeding episodes were treated with recombinant activated factor VII. Results. Two patients with high titer inhibitors had marked reduction in the inhibitor level from 320 and 160 Bethesda units (BU) to 8 and one BU, while the inhibitor disappeared in the third patient with low titer inhibitor (4.5 BU). All the patients improved clinically with reduction in the bleeding episodes and a better quality of life. Inhibitor level has increased with time in these patients but the clinical benefit continues in 2 patients with high titer inhibitors initially, after a follow-up of 48 and 22 months. Third patient had a reduction in bleeding episodes for 6 months but the inhibitor reappeared and rose to near the base line level when he was challenged with plasma derived factor VIII concentrate. Patients' characteristics are shown in Table 1. One of the patients with concomitant human immunodeficiency virus (HIV) infection and a very low CD4 lymphocyte count developed severe truncal herpes zoster after the third weekly dose of rituximab. Caution is required in such patients and we recommend to avoid rituximab use in HIV infected patients with very low CD4 lymphocyte count. *Conclusions.* Rituximab is useful in reducing the inhibitor level with clinical benefit in patients with hemophilia A and inhibitors, but is not able to eradicate the inhibitors for long periods with the currently used weekly infusions of up to 5 doses.

Table 1. Patients' characteristics.

No	Age (years)	Pre treatment Inhibitor level (BU/ml)	No of rituximab doses	Lowest inhibitor level achieved (BU/ml)	Inhibitor level at last follow-up (BU)	Follow-up (months)
1	23	160	5	1	8.0	48
2	30	4.5	2	0	4.0	17
3	20	320	4	8	14	22

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THROMBIN ACTIVATABLE FIBRINOLYSIS INHIBITOR IN PEDIATRIC CYANOTIC HEART DISEASE

S. Yilmaz,[1] H. Oren,[1] F. Demircioglu,[1] F. Yuksel,[1] V. Tavli[2], T. Saritas[2], G. Sagin Saylam,[1] G. Irken[1]

[1]Dokuz Eylul University Medical School, IZMIR; [2]Dr. Behcet Uz Childrens Hospital, IZMIR, Turkey

Background. Human thrombin activatable fibrinolysis inhibitor is a basic carboxypeptidase zymogen with functional role in fibrinolysis. Patients with congenital cardiac disease, escpecially congenital cyanotic heart diseases (CCHD) are prone both to thrombosis and to bleeding. Previous were many hemostatic parameters were evaluated in this patient group. Hemostatic disturbances observed in patients with CCHD are polycytemia, thrombocytopenia, thrombocyte function defects, disseminated intravascular coagulation, decreased synthesis of coagulation factors (liver function failure, vitamin K deficiency) and primary fibrinolysis. CCHD patients are at risk for bleeding at surgery. *Aims.* In our study, considering the role of TAFI in down-regulation of fibrinolysis, we have investigated whether TAFI contributes to impaired fibrinolysis in patients with congenital cyanotic heart disease. *Methods.* Fifty-eight patients with CCHD followed by the Departments of Pediatric Cardiology at Dr. Behçet Uz Children Hospital and Dokuz Eylul University Hospital and 51 healthy children from Dokuz Eylul University Hospital were included in this study. The median age was 2 years, and the mean age was 3.1±3.41 (range 0-16 years) in the group of CCHD patients; median age was 2 years, and mean age was 2.39±2.24 (range 0-12 years) in the control group. Informed consent was obtained from all parents. TAFI antigen was determined using an ELISA kit. *Results.* There were no differences in age and sex between CCHD group and control group. Hemoglobin, hematocrit, red blood cell count and white blood cell count was higher in the CCHD group. TAFI antigen level was found as 6.54±1.24 μg/mL in the CCHD group, and 6.03±1.54 μg/mL in the control group. A statistically significant difference was not found between TAFI antigen levels of two groups (p>0.05). Among the patients with CCHD 22 patients (37.9%) had erythrocytosis (hematocrit >45%), and when the CCHD group was divided according to the presence of erythrocytosis, TAFI antigen level was found to be lower than the group without erythrocytosis, 6.03±1.31 μg/mL (range 3.69-7.82) and 6.86±1.09 μg/mL, (range 5.04-9.48) respectively (p=0.031). *Conclusions.* A lower level of TAFI was found in CCHD patients with higher blood viscosity. This may promote the fibrinolytic functions and may contribute to bleeding tendency in this group of patients.

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GRANULOCYTE TRANSFUSION ITS VALUE AND PRACTICALITY: A SINGLE CENTER EXPERIENCE

T. Owaidah, H. Al Humidan, R. Al Nounou, H. AL-Tanbal,

King Faisal Specialist Hospital & RC, RIYADH, Saudi Arabia

Backgraound. The risk of infection is increased in neutropenic patients, particularly in those with very low neutrophil counts (<500/uL). Neutropenia-associated infection remains a limiting factor in the treatment of malignancy especially hematological malignancies. Transfusion of donor neutrophils is a logical approach to these problems, however, granulocyte concentrates (GCs) is an involved process requiring recruitment, selection, and scheduling of suitable volunteer donors, pretreatment of donors with G-CSF growth factor and/or corticosteroids to increase peripheral white blood cell count/granulocyte yield. In addition the initiation of this process is time dependent. Granulocyte transfusion(GTx) is an old practice that is reviving with still controversial opinions among hematologists. *Aims.* To study local practice and out come for neutropenic patients received GTx. *Materials and Methods.* From blood bank records for the period 2003-2007,we had identified 21 patients who received GTx . All donor were ABO compatible with respective recipient, 18 donors were prepared for granulocyte harvest by both a single injection of G-CSF and oral dose of dexamethasone. We had reviewed both medical records and GTx records for each patient and Blood bank- records for each donor. Each patient had received at least 3 GTx, range from 3-18 doses, median of 10.5 doses/patient. Mean WBC at the start of GTx was 0.9×10^9/L, range from 0.01×10^9/L to 12.9×10^9/L. Data were collected in excel software and analyzed by statistical *Methods. Results.* Out of 21 patients, 12 were males and 9 females with mean age 28.3 year ranging between 12 -52 years. Severe Aplastic anemia was the most common diagnosis occurring in 9 patients (42.9%), followed by acute myeloid leukemia in 5 patients (23.8%).Three patients(14.3%) had acute lymphocytic leukemia, 2 patients (9.5%) had Fanconi"s anemia and 1 patient had biphenotypic leukemia and anther had chronic granulomatous disease . Fungal infection was the most common reason for granulocyte transfusion, 16 patients (76%) had disseminated fungal infection, followed by febrile neutropenia in 7 patients. Clinical efficacy: Twelve (57.1%) patients responded to transfusion by resolution of infections, 7 patients (33.3%) died during or immediately after GT for various reasons and 2 patients (9.5%) did not show any clinical response. *Conclusions.* We present here a clinical evidence that the use of GTx is useful in treatment of patients with neutropenia and can reduce mortality and morbidity especially in patients with hematological disorders

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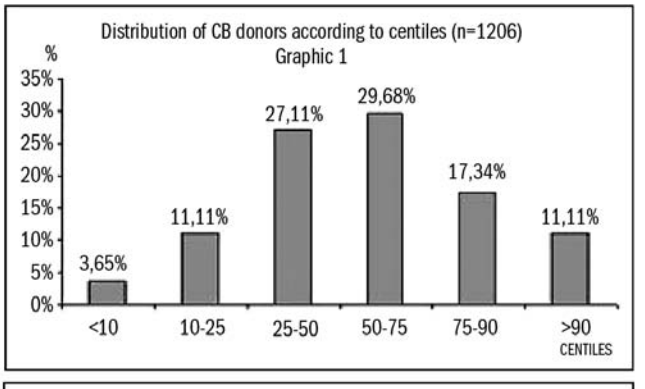
HLA-A*02;B*15 HAPLOTYPE IS ASSOCIATED WITH <10 BIRTH WEIGHT CENTILE IN CORD BLOOD DONORS

P. Bergamaschi,[1] A. Pasi[2], C. Capittini,[3] M.P. Mercati,[4] C. Tinelli,[5] M. Guarene,[6] C. Monti,[6] C. Parisi,[1] A. Marchesi,[1] C. Perotti,[1] M. Cuccia,[3] M. Martinetti,[6] L. Salvaneschi[1]

[1]Pavia CBB, Transfusion Medical Department, Fondazione IRCCS Policlinico San Matt, PAVIA; [2]HLA Laboratory, Transfusion Medical Department, Fondazione IRCCS Policlinico S, PAVIA; [3]Immunogenetics Laboratory, Department of Genetics and Microbiology, University o, PAVIA; [4]Faculty of Medicine, University of Pavia, PAVIA; [5]Statistics Department, Fondazione IRCCS Policlinic, PAVIA; [6]HLA Laboratory, Transfusion Med. Dept., Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy

The large employ of cord blood (CB) for unrelated stem cell transplantation is providing worldwide spreading of CB banks (CBBs). CBBs repositories consist of cryopreserved CB units whose characteristics are determined recorded into databases and managed for stem cell donor search and procurement. By this way, large amounts of data referring to the immunogenetic profile of healthy newborns are available for further investigation. Certain HLA alleles/haplotypes are known to be associated with protection and/or susceptibility to diseases. HLA markers are

also hypothesized to influence some physiological events. Multiple factors affect normal development of the foetus and HLA genotype might represent one of them. In this setting, birth weight (BW) represents a suitable marker of intrauterine growth. Assuming that HLA typed babies derived from a CBB represent a healthy population at birth, we review the data referring to 1206 CB donors of the Pavia CBB inventory aiming to investigate the association between some HLA markers and newborn's size. BW was corrected according to sex and gestational age by using centiles, whose distribution is depicted in Figure 1 Graphic 1. UCB donations and mothers were typed for HLA-A, B and DRB1 by molecular techniques at time of banking. All UCB and maternal haplotype frequencies were obtained by direct counting and unambiguously assessed. We calculated the alleles and haplotypes frequencies and analyzed their distribution according to the centiles. Newborns carrying the HLA-A*02;B*15 haplotype were found to accumulate in <10 centile group. In fact, the HLA-A*02;B*15 haplotype showed a statistically significant descending trend along centiles (2-tailed Fisher test, p=0.011) as shown in Figure 1 Graphic 2. Moreover the comparison between HLA-A*02;B*15 frequencies in the opposite groups (<10 and >90 centile) remained significant (2-tailed Fisher test, p=0.035). We previously reported the protective effect of HLA-B*38;DRB1*13 haplotype against low BW in Turner patients and its association with >90 BW centile in CB donors. According to these findings low-weight centiles seem to deviate from high-weight ones for the telomeric HLA markers (HLA-A and HLA-B loci), thus enforcing the observation that in HLA class I region we can find genes encoding proteins fundamental for life and governing the first impact reactions with the outside world. We can argue that low-weight babies might carry genes in linkage disequilibrium with HLA-A and HLA-B loci which are good markers for life survival. Furthermore, low-weight babies whose CB has sufficient cell content to be banked may supply a precious source of less frequent haplotypes which are rarely represented in high-weight babies. Finally, in our opinion the data derived from CBBs despite the utility for donor search may also provide information about healthy population otherwise not easily available.



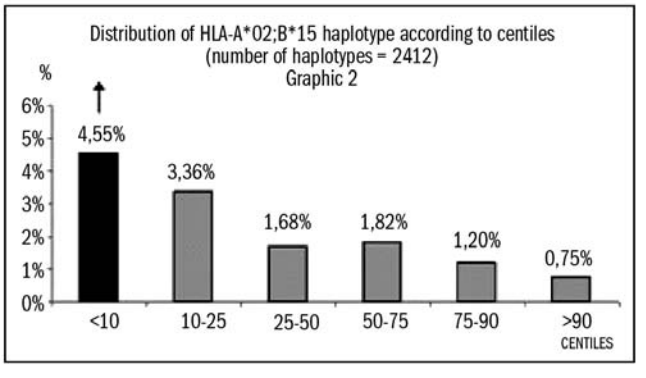


Figure 1.

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RECRUITMENT OF MONOCYTE SUBPOPULATIONS BY LEUKOCYTAPHERESIS

J. Bruegel, M. Hendelmeier, E. Strasser, R. Zimmermann, R. Eckstein

University Hospital Erlangen, ERLANGEN, Germany

Background. The separation of monocytes from peripheral blood and the vaccination with Monocyte-derived Dendritic Cells (DC) is of great value to the development of therapeutic strategies against malignancies. A major obstacle to a clinical-scale application of monocyte-derived Dendritc Cell vaccines is the requirement of a homogenous monocyte apheresis product. Monocytes are, however, a heterogenous population and consist of distinct subpopulations with different physiological roles. *Aims.* The understanding of monocyte heterogeneity and the impact of leukocytapheresis on monocyte subpopulations may have implications for the development of DC cancer vaccines. *Methods.* Blood samples of 120 blood donors were investigated before and after leukocytapheresis procedures (COM.TEC cellseparator, Fresenius HemoCare, MNC program and COBE Spectra, Gambro, PBSC program). Additionally, monocyte apheresis products were examined. Monocyte subpopulations were analyzed by flow cytometry (FACS Calibur, BD). The impact of the apheresis procedure was demonstrated by calculation of the recruitment factor (RF). Calculation Formula: RF = (postdonation cell count + cell yield) / predonation cell count. *Results.* The concentration of mononuclear cells (MNC) in the apheresis product ranged between 28×10^3 and 140×10^3 per microliter. Significant differences between pre- and postdonation cell count of $CD14^{++}CD16^-$ monocytes occurred (p<0.001), whereas $CD14^+CD16^+$ monocytes showed no significant difference between pre- and postdonation cell count. Additionally, $CD14^+CD16^+$ monocytes were enriched in the monocyte apheresis product compared to the $CD14^{++}CD16^-$ monocytes (p<0.001). Between the COM.TEC cell separator and the COBE Spectra cell separator no significant difference concerning the recruitment of $CD14^{++}CD16^-$ monocytes and $CD14^+CD16^+$ monocytes was detectable. *Summary and Conclusions.* These results suggest a different cell recruitment of $CD14^{++}CD16^-$ monocytes and $CD14^+CD16^+$ monocytes. The different recruiting pattern of these monocyte subpopulations suggest a diverse immune intervention by leukocytapheresis. This immune modulation may impact on the results of vaccination with monocyte-derived DC and may raise the potential for novel strategies in cellular therapy of malignancies.

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HAEMORHEOPHERESIS IN THE TREATMENT OF MICROCIRCULATORY DISORDERS

M. Blaha, M. Blazek, P. Zak, E. Stepanova, L. Smolej, V. Stepanova, P. Mericka, J. Maly

Medical Faculty, Charles University, HRADEC KRALOVE, Czech Republic

Background. Haemorheopheresis was specifically designed to treat microcirculatory disorders. The single rheopheresis treatment simultaneously eliminates an exactly defined spectrum of high-molecular weight rheologically relevant plasma proteins (i.e. α2-macroglobulin, fibrinogen, LDL-cholesterol, lipoprotein(a), von Willebrand factor (vWF), immunoglobulin M (IgM), fibronectin, and putatively multimeric vitronectin). This results in the immediate pulsed reduction of plasma viscosity as well whole blood viscosity, which with a series of treatments can lead to sustained microcirculatory recovery, and change significantly the natural course of a chronic disease. We describe the experience of our Hemapheresis Centre. *Methods and Patients.*In the prospective trial presented here, 28 patients were treated - severe familiar hypercholesterolemia (FH): 4 pts; non-healing lesions caused by severe ischemic diabetic foot syndrome (IDFS): 5 pts; age related macular degeneration (AMD): 13 pts; acute sensorineural hearing loss (ASHL): 3 pts; thyreoid orbital endocrinopathy (TAO): 3 pts. Our own modification of rheopheresis was used: Plasma, free from cellular elements is obtained by blood cell separator (Cobe-Spectra, Denver, USA) in high-speed centrifugation. Then it is run through the *second stage* - a rheofilter (Evaflux 4A, Kuraray) with ethylene-vinyl-alcohol hollow fibres with holes of 0,03 micrometer. Plasma flow is continuous, anticoagulation done with heparin. Basic amount of processed plasma (calculated by Cobe computer) is 1,5 of body volume. The size of holes in the filter enables to retain the above mentioned high-molecular elements. Haematological, biochemical and haemorheological parameters were measured before and after procedures and after the finishing of therapeutic series (AMD 8 procedures, IDFS 10, ASHL 3, TAO 10). *Results.* Rheological procedures were very effective and resulted in significant decreases of pathological-

ly effective substances: alfa2-macroglobulin 56,79%, fibrinogen 63,50%, IgM 60,49%, LDL-cholesterol 70,27%, apolipoprotein B 69,88%, lipoprotein(a) 62,90%. It resulted in blood and plasma viscosity decrease (10,88/15,43%). Diabetic foot ulcers were healed in 3 of 5 pts. No progression from dry to wet form of AMD has been observed during 2 years. 6,60% of side effects were observed; they were not severe, transient and easily controlled. *Conclusions.* Hemorheopheresis appears to be a method suitable adjunct therapy for diseases involving severe disturbance of microcirculation, especially when previous therapeutic options were not sufficiently effective or invasive procedures cannot be applied.

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A PHASE 1 CLINICAL TRIAL OF PRION-FILTERED RED CELL CONCENTRATES IN PATIENTS REQUIRING ALLOGENEIC BLOOD TRANSFUSION

M.R. Cahill,[1] T.M. Murphy,[1] J. Fagan[2], H. Croxon[2], S. McGrath,[1] O. Gilligan,[1] W. Murphy[2]

[1]Cork University Hospital, CORK; [2]Irish Blood Transfusion Service, DUBLIN, Ireland

Background. Variant Creutzfeldt Jakob disease (vCJD) is a fatal neurodegenerative disorder. Transmission of vCJD by blood transfusion from pre-symptomatic blood donors has occurred in 4 reported cases to date. Screening blood donors for infectivity is unlikely to be feasible for several years. Removing infectivity from blood using selective filtration may provide a useful degree of protection from transfusion transmission of the disease, particularly in the absence of donor testing. A filter that removes infectivity from red cell concentrates has been developed. Studies to date with this device have been limited to *in vitro* and animal studies of prion removal using exogenous and endogenous infectivity models, and safety, recovery and survival studies of autologous red cells in human volunteers. Prior to general introduction into clinical use, it is essential to assess safety of prion-filtered red cell concentrates in allogeneic transfusions in the clinical setting, including use in multiply transfused patients. *Aims.* We wished to establish safety and patient tolerability of transfused red cell concentrates processed using a novel prion removal filter. *Methods.* Following institutional ethical approval, fifteen patients (14 haematology patients and one cardiothoracic surgical patient), scheduled to receive transfusion in a non-emergency setting were recruited. Informed consent was obtained. A crossmatch sample, full blood count (FBC), renal and liver profile was taken from each patient prior to transfusion. Patients were observed for reactions during the transfusion with regular observations as per hospital protocol. Within twenty-four hours of transfusion an FBC, renal and liver profile were repeated. Six weeks after the transfusion a further sample was tested for red cell antibodies. *Results.* Fourteen patients each received a transfusion of one unit of prion-filtered red cells. No serious adverse events were encountered during transfusion of the prion-filtered units. No unexpected findings were noted in the 24 hour or 6 week samples. Autoantibody screen at 6 weeks showed no detectable antibodies in samples analysed to date. Follow up is ongoing. *Conclusions*. Red cell concentrates filtered through the prion removal filter seem to be well tolerated by patients, and further studies in repeat transfused, transfusion-dependent adults and children are warranted.

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PLATELET GEL APPLICATIONS IN ORAL MUCOSITIS IN CGVHD

A.S. Ferraro,[1] A. Lanti,[1] S.D. Spurio[2], M. Marinacci[2], G. Del Proposto,[1] A. Picardi,[3] R. Cerretti,[3] T. Dentamaro,[4] L. Cupelli,[4] P. De Fabritiis,[4] W. Arcese,[3] G. Adorno[2], G. Isacchi[5]

[1]Policlinico Tor Vergata, ROME; [2]Cattedra di Immunoematologia, Università Tor Vergata, ROME; [3]Cattedra di Ematologia Università Tor Vergata, ROME; [4]U.O.C. Ematologia, Osp. S. Eugenio, ROME; [5]Dip. Oncoematologia e Medicina Trasfusionale, Osp. Pediatrico Bambino Gesù, ROME, Italy

Background. Oral mucositis is among the most debilitating of side effect in patients that developed cGVHD after hematopoietic stem cell transplant (HSCT). Mucosal damage is highly correlated to myeloablative therapy and HSCT and is characterized by deep multiple variable size wounds that compromised the integrity of mucosal epithelium of mouth. Oral involvement impact upon all aspects of quality's life (physical, emotional, social and functional) by causing oral and oropharyngeal pain and by impairing communication and swallowing. *Aims.* the topic of this study was to value the efficacy of Platelet Gel (PLTs gel) to heal the mucosal barrier injury in cGVHD patients because, currently, there are no universal protocols that have been accepted as a standard for treatment. *Methods.* two patients, affected by cGVHD with oral lesions and not responsive to conventional therapy, were submitted to PLTs gel applications. The patient recruitment and the response rate were evaluated on the basis of the following wounds parameters: size, depth, pain, microbiological assessment and granulation tissue forming. Homologous haemocomponents were used to obtain PLTs gel with Vivostat System. The final product was a gel aliquot of 8 ml and the application was performed once a week. *Results.* a comparison of baseline characteristics at onset with endpoints, showed a significant reduction of oral ulcers after only 2 applications and the final healing after 5 total applications, promoting tissue regeneration and reducing the risk for severe infections. *Conclusions.* The experience with this treatment approach is very encouraging and shows that the PLTs gel is a local therapeutic option tolerated, safe and reliable in the management oral mucositis in cGVHD

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WBC REDUCTION OF BLOOD PRODUCTS MAY BE ASSOCIATED WITH A DECREASED INCIDENCE OF ALLOMINUNIZATION

G. Stuessi, R. Koeppel, U. Schanz

University Hospital Zürich, ZÜRICH, Switzerland

Alloimmunization to red blood cell (RBC) antigens is a common and potentially serious clinical problem. It has been hypothesized that white blood cell (WBC) reduction of blood products may reduce the number of alloimmunizations. Therefore, we retrospectively analyzed the the incidence of RBC antibodies (Ab) in the University hospital Zürich over the last 33 years (1973-2006). RBC Ab were detected by a 2 or 3 cell panel antibody screens (AS) and positive results were further specified by additional RBC panels. During this time, a total of 378'785 AS were performed and 4097 patients had 5340 positive AS (1.4%). The median age of the patients was 43 years (range 0-100) and 66% were female. Antibodies were most commonly directed against Rhesus (45%), Lewis (18%), and Kell (16%). The frequency against Duffy, Kidd, Lutheran, MNS, and P blood group system was below 10%. The most common Ab were anti-E (17%), anti-D (16%), anti-K (16%), and anti-Lea (12%). Of the alloimmunized patients, 86% had 1, 12% had 2 and 2% had 3 Ab. Females were more likely to have Ab against Rhesus and males against Kell, Duffy, Lutheran, and P1. Since 1990, the number of positive tests was increasing with a maximal annual incidence of 4.3%. However, after 1999 the incidence of positive AS has decreased rapidly to 1% coinciding with the introduction of general WBC reduction.

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IDENTIFICATION AND CHARACTERIZATION OF NORMAL AND ABNORMAL PLASMA CELL POPULATIONS FROM MULTIPLE MYELOMA PATIENTS: A 12 COLOR-FLOW-CYTOMETRY STUDY

N. Aouali,[1] N.H.C. Brons,[1] V. Palissot,[1] M. Bosseler,[1] S. Pierson,[1] K. Van Moer,[1] G. Berchem[2]

[1]CRP-Santé, LUXEMBOURG; [2]Centre Hospitalier de Luxembourg, LUXEMBOURG, Luxembourg

Background. Multiple myeloma (MM) is a B-cell neoplasia affecting the plasma cells. When a patient develops a multiple myeloma, the plasma cell percentage, increases in bone marrow. The last stage of the disease is defined by the passage of malignant plasma cells from bone marrow to the blood. However, when the percentage of MM plasma cell in blood reaches more than 20%, we are talking about plasma cell leukemia (PCL). *Aims.* In this study, 12 color-flow cytometry was used to identify and characterize different subpopulations of abnormal plasma cells in blood and bone marrow from patients at different stages of the MM. *Methods.* As we worked on identification of low and rare population (<0.05%), a gating strategy had to be followed. The DAPI dye had been used to gate only the nucleated cells. CD14 and CD16 markers had been used to exclude the myeloid populations which overlapped with the plasma cell populations. CD38 and CD138 markers were added to gate the plasma cell populations. CD56, CD19 had been included to discriminate the normal and malignant plasma cell populations. By using 12 color flow-cytometry, more markers have been added up (CD44, CD20). *Results.* The analysis in 10 + colors flow cytometry identified new and rare subpopulations in the bone marrow as well as in the peripheral blood and correlate their presence with stage of the disease. Indeed the use of CD19 together with CD56 is not sufficient to discriminate between normal and malignant plasma cells. Our results show that the loss of CD44 marker expression on plasma cells could be linked to the

progression of the disease. *Conclusions.* In this work we show that flow cytometry is a very powerful and very sensitive method to detect small populations of cells. The use of more than 10 colors in flow cytometry improves accuracy of population identification and allows obtaining detailed information of abnormal populations. Moreover, in complex samples such as bone marrow and/or peripheral blood from MM patients, it becomes important to increase the number of fluorochromes in order to correctly analyze multiple antigens and small abnormal subpopulations with largely unknown phenotype. As clinic diagnosis, the percentage of plasma cells is evaluated from a bone marrow smear. As a small amount of clinical material is available, flow cytometry could represent a useful method to gain more information in MM diagnosis.

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IMPACT OF FREE LIGHT CHAINS RATIO NORMALIZATION ON RESPONSE DURATION IN MULTIPLE MYELOMA (MM) PATIENTS TREATED WITH NEW DRUGS

M. Offidani,[1] C. Polloni[2], L. Corvatta,[3] S. Gentili[2], M.-N. Piersantelli[2], M. Catarini,[3] M. Brunori,[3] M. Burattini,[3] B. Amoroso,[4] P. Leoni[2]

[1]Clinica Ematologia, ANCONA; [2]Clinica Ematologia, Ospedali Riuniti Ancona, ANCONA; [3]Divisione Medicina, FABRIANO; [4]The Binding Site, ROME, Italy

Background. Several studies reported a relationship between depth of response and progression free survival (PFS) duration in MM patients, particularly post high-dose therapy followed by stem cell transplantation. The same association was hypothesized also for patients treated with new drugs combination therapies. Recently, an expert panel revised response criteria (IWMG uniform criteria) and introduced serum free light chains (sFLC) ratio as a tool to define stringent complete remission (sCR). However, there are no studies validating these new criteria particularly it has not been yet defined whether MM patients achieving sCR have a better outcome if compared whit those obtaining CR or VGPR. *Aims.* This retrospective study analysed the association between sCR and the duration of PFS in MM patients treated with new drugs in combination with dexamethasone and chemotherapy. *Methods.* MM patients tested for sFLC at the end of induction therapy, enrolled in 3 multicenter, prospective studies, were included in this analysis. Patients treatment regimens were ThaDD (Thalidomide, Dexamethasone, liposomal pegylated Doxorubicin), ThaDD-V (the same plus bortezomib) and EDA-V (Etoposide, Dexamethasone, Aracytin, bortezomib) protocols. Patients were stratified in 3 groups according to VGPR, CR, sCR in order to identify the best predictor of PFS duration. Patients obtaining VGPR were compared with those achieving CR+sCR, those with VGPR+CR were compared with patients achieving sCR and finally those obtaining CR with patients achieving sCR. *Results.* Sixty six patients met the inclusion criteria. Four patients were treated with ThaDD, 37 with EDA-V and 25 with ThaDD-V. Median age was 65 years (range 31-82). Nine patients (13.5%) obtained sCR, 11 (16.5%) CR, 17 (25.5%) VGPR, 14 (21%) PR, 12 (18%) SD and 3 (4.5%) progressed. Out of 17 patients in VGPR, 5 progressed with a 25% PFS at 2 years whereas of the 20 patients in sCR+CR, 3 progressed with a 73% PFS at 2 years (p= 0.1650). In the group of 28 patients in CR+VGPR, 8 progressed with a 28% PFS at 2 years whereas no events occurred in patients with sCR with 100% PFS at 2 years (p= 0.0433). Finally, 3 out of 11 CR patients progressed with a 36% PFS at 2 years *vs* 100% PFS at 2 years in the group of patients in sCR (p= 0.0512). *Conclusions.* This retrospective study suggests that depth of response is associated with PFS duration. Moreover, sFLC ratio normalisation at the end of induction therapy may represent the best tool to predict a longer PFS in patients with MM receiving new drugs combination therapies.

1477

RITUXIMAB PLUS CHOP IMPROVES OUTCOME IN BCL2+ DIFFUSE LARGE B CELL LYMPHOMA PATIENTS

F. Gaudio,[1] A. Giordano,[1] T. Perrone,[1] A. Guarini,[1] D. Pastore,[1] C. De Risi,[1] A. Napoli[2], R. Ricco[2], G. Specchia,[1] V. Liso[1]

[1]Hematology, BARI; [2]Pathology, BARI, Italy

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous entity and patients exhibit a wide range of outcomes. BCL2 protein expression has been associated with poor prognosis in patients with DLBCL. The addition of rituximab to CHOP chemotherapy (R-CHOP) has led to a marked improvement in survival and has cast doubt on the significance of previously recognized prognostic markers. We performed a retrospective analysis of 111 patients with *de novo* DLBCL treated at our institute between 2000 and 2005, to assess the value of BCL2 expression in the era of immunochemotherapy. Histological diagnoses were established according to the REAL-WHO classification. HIV-associated lymphomas, transformed lymphomas, cases with central nervous system involvement, primary mediastinal and primary extranodal DLBCL were excluded. Tumors were considered positive when at least 50% of tumor cells expressed the bcl-2 protein. All patients received CHOP every 3 weeks; 58 patients were treated with chemotherapy plus rituximab (R-CHOP) and 53 patients with chemotherapy alone (CHOP). There were 68 (61%) bcl-2$^+$ patients and 43 (39%) bcl-2- patients. The response rates for R-CHOP and CHOP were 71% and 59% (p<0.05) in bcl-2$^+$ patients and 74% and 72% (p=n.s.) in bcl-2- patients, respectively. At a median follow-up of 3 years, R-CHOP was significantly associated with a better overall survival than CHOP in bcl-2+ patients (79% *vs* 48%, p<0.05). In bcl-2- patients there was no statistically significant difference in terms of overall survival (75% *vs* 68%, p=n.s.). In addition, R-CHOP was associated with significantly better progression-free survival rates than CHOP in bcl-2+ patients (Figure 1.b: 65% *vs* 38%, p<0.01) but not in bcl-2- patients (60% *vs* 40%, p=n.s.). Multivariate analysis confirmed the significant benefit on survival and progression-free survival of R-CHOP in bcl-2$^+$ patients. These results suggest that the addition of rituximab to CHOP chemotherapy offsets the adverse prognostic influence of BCL-2 protein expression on progression free and overall survival in DLBCL.

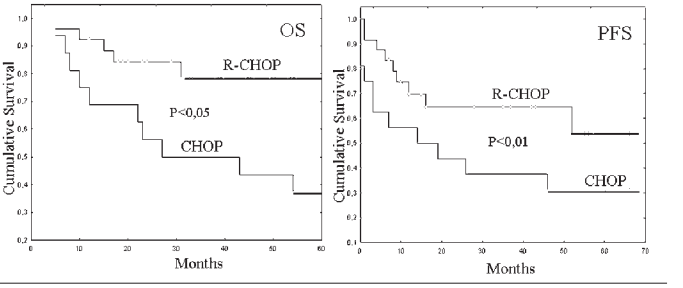

Figure 1. OS and PFS in BCL2 + patients with DLBCL.

1478

TREATMENT OF SPLENIC MARGINAL ZONE NON HODGKIN LYMPHOMA

A. Manaka, V. Kyriazi, M. Vagia, K. Liapis, F. Panitsas, F. Michelis, I. Kakkas, S. Gigantes, S. Delibasi, M. Pagoni, I. Baltathakis, J. Apostolidis, D. Karakasis, M. Bakiri, T. Karmiris, N. Harhalakis, E. Nikiforakis

Evangelismos Hospital, ATHENS, Greece

Aims. To define the clinical features and the outcome in splenic marginal zone (MZ) non-Hodgkin lymphoma (NHL). *Patients and Methods.* Forty-five patients with splenic MZ NHL were studied. Diagnosis required splenic involvement, with or without bone marrow infiltration, and the absence of excessive lymphadenopathy or evidence of extranodal (MALT) lymphoma. See Table 1.

Table 1.

Characteristics	Number of patients	%	Characteristics	Number of patients	%
Age	64yrs (23-82)		Bone marrow involvement	41/45	91%
Hb <12g/dl	30/45	67%	M component	6/45	13%
PLT <150.000/mm^3	35/45	78%	HCV serology	1/45	2%
PLT <50.000/mm^3	5/45	11%	HBV serology	6/45	13%
Neutropenia <1.000/mm^3	4/45	9%	ECOG score ≥ 2	14/45	36%
Absolute lymphocytosis	20/45	44%	Stage III-IV	42/45	93%
B symptoms	3/45	7%	Extranodal sites	1/45	2%
LDH >normal	34/45	76%	Sex (M:F)	26/19	1:1.4
Peripheral lymphadenopathy	9/45	20%	FLIPI score		
			Low	4/45	9%
			Intermediate	9/45	20%
			High	32/45	71%

Pathological/Immunophenotypic Features: Follicular or diffuse infiltration with splenic sinuses involvement, extended from splenic marginal zone to red pulp, from small or medium sized centrocyte-like or monocytoid B-cells, characterized by the immunophenotypic features of $CD20^+$, $CD79a^+$, $CD45^+$, IgM^+, $CD5^-$, $CD10^-$, $CD23^-$, Cyclin $D1^-$, and $Bcl\text{-}6^-$. Treatment: Twenty-five (56%) patients were treated at diagnosis with splenectomy alone, eight (18%) with splenectomy plus a CVP-like regimen, three (7%) with splenectomy plus Rituximab as monotherapy, and nine (20%) with Rituximab alone or watch-and-wait. *Results.* Overall response rate was 83%. Seven (16%) patients achieved complete response (CR) and thirty (67%) partial response (PR). With a median follow-up of 2.9 years (range, 0.2-16.9), the 5-year overall survival (OS) was 68% and the 5-year event-free survival (EFS) was 17.5%. According the FLIPI scoring system, the 5-year OS in the low- and intermediate-risk group was 99%, and in the high-risk group was 50%. *Summary and Conclusions.* Due to the lack of standard treatment for splenic marginal zone NHL, several treatment modalities are applied. Splenectomy has the main role with or without additional chemotherapy or Rituximab, with a good overall survival. The FLIPI scoring system may have a clinical utility to identify the high-risk group of patients.

1479

TREATMENT OF SPLENIC MARGINAL ZONE LYMPHOMA (SMZL) IN A SERIES OF 78 PATIENTS: FROM WATCH AND WAIT TO RITUXIMAB MONOTHERAPY

C. Kalpadakis,[1] G.A. Pangalis[2], T.P. Vassilakopoulos[2], M.N. Dimopoulou[2], M.C. Kyrtsonis[2], P. Korkolopoulou[2], P. Korkolopoulou[2], F.N. Kontopidou[2], M.P. Siakantaris[2], E.M. Dimitriadou[2], S.I. Kokoris[2], S. Sahanas[2], S. Dimitrakopoulos[2], H.A. Papadaki,[1] P. Panayiotidis[2], M.K. Angelopoulou[2]

[1]University of Crete, HERAKLION; [2]University of Athens, ATHENS, Greece

Background. SMZL is a well described low grade B-cell lymphoma. Treatment modalities are not standardized. *Aims.* To evaluate the treatment outcome of 78 SMZL patients (pts) diagnosed in our Unit during the last 20 years. *Methods.* Diagnosis was based on the WHO classification criteria. Treatment strategies included the watch and wait policy, splenectomy, chlorambucil (10 mg/dx10 d/mo for 12 mos), combined chemotherapy, and rituximab monotherapy (Rmono) (375 mg/m2/w x 6 ws as induction therapy (IT) and maintenance therapy (MT) every two mos for one to two years). *Results.* Pts' median age was 63 years (range 36-91) and male: female ratio 0.7. At diagnosis 24 pts did not require therapy. Among them 21 progressed at a median time of 24 mos (7-144) and submitted to therapy. So far 75 pts have been treated: 28 (36%) underwent splenectomy, 34 (44%) received Rmono, 8 chlorambucil and 5 polychemotherapy. Among splenectomized pts all but one achieved an improvement of their blood counts although circulating lymphoma cells were present. 41% of the splenectomized pts progressed at a median time of 40 months (15-101) and at a median follow up time of 57 months (3-172) 13 deaths were recorded. All 34 pts who received rituximab achieved complete clinical and hematologic remission at a median time of 5 and 4 weeks respectively. Evaluation of response 2 mos after IT showed that 47% of the pts were in CR, 19% in unconfirmed CR (CRu) and 34% in PR. Among the CRs 5 had also a complete molecular response. 15 pts have already completed maintenance therapy and 14 are in CR and 1 in CRu. Maintenance therapy sustained or even improved the response. The median follow up time of the 34 pts treated with rituximab is 26 months (2-54). During this period 4 relapses were recorded at a median time of 14 months (8-32). Among the 8 pts who received chlorambucil, 2 responded (25%). All 4 pts treated with COP did not respond (2 PD and 2 SD), while one pt who received R-COP achieved CR even at a molecular level. The median follow up time of surviving pts at the time of analysis was 40 mos (2-229). The 5- and 10-year OS and Cause Specific Survival (CSS) is 78% and 55%, and 83% and 63% respectively. Median OS is 141 mos while the median CSS has not been reached yet. Summary/*Conclusions.* The clinical course of SMZL pts was prolonged with a median survival of approximately 12 years and a 15 year CSS > 60%. Rituximab monotherapy is a highly effective treatment option for SMZL pts. Splenectomy may be reserved only for pts resistant to rituximab.

1480

RITUXIMAB IN COMBINATION WITH CHOP OR RADIOTHERAPY FOR THE TREATMENT OF AGGRESSIVE, DIFFUSE LARGE B CELL NON HODGKIN LYMPHOMAS OCCURRING AFTER SOLID ORGAN TRANSPLANTATION

A.M. Barbui, A.M. Barbui, P. Viero, A. Rossi, F. Delaini, R. Fiocchi, M.G. Lucà, A. Rambaldi

Ospedali Riuniti di Bergamo, BERGAMO, Italy

Background. Aggressive diffuse large B cell non-Hodgkin's Lymphoma (DLBCL) developing after solid organ transplantation represents a very serious complication. The incidence of DLBCL after solid organ transplant increases significantly with time being as high as 10% at ten years after heart transplantation. Late occurring DLBCL must be distinguished from relatively benign early lesions and polymorphic Post Transplant Lymphoproliferative Disorders (PTLD). DLBCL are indeed poorly responsive to discontinuation of immune suppression and they are frequently rapidly fatal because resistant to chemotherapy or the presence of significant comorbidities. *Aims.* This study reports our single center experience on the use of Rituximab in combination with CHOP chemotherapy (R-CHOP) or radiotherapy in DLBCL late occurring after heart or liver transplantation. *Methods.* All patients with a proven histological diagnosis of DLBCL occurring after at least one year from solid organ transplantation were treated with 6 cycles of R-CHOP chemotherapy administered every 21 days (stage II-IV DLBCL, according to Ann Arbor classification), or local radiotherapy and 4 weekly administration of Rituximab (stage I-II disease). *Results.* Eleven patients were diagnosed with DLBCL, of whom 8 were heart and 3 liver recipients. Median time from solid organ transplantation and DLBCL diagnosis was 58 months (range 12-232). Median age was 39 years (range 22-70). Eight out of 11 patients were treated with R-CHOP chemotherapy because of advanced stage disease at presentation, while the remaining three received local radiotherapy and Rituximab. Ten out of eleven patients achieved a complete response (CR) after treatment. The non-responding patient was a DLBCL rich in T cells occurring after 70 months after heart transplantation that rapidly progressed after 6 cycles of R-CHOP and died of disease. Following chemotherapy, neither WHO 3-4 neutropenia or infections were registered. With a median follow-up of 26 months (range 2,7-209), the overall survival at 30 months is 60%. Causes of death were related to hepatitis reactivation (one liver transplant patient) or heart failure (four heart transplant recipients), but in all cases no lymphoma relapse or cardiac rejection were documented. *Conclusions.* Our single center experience confirms that Rituximab in combination with CHOP chemotherapy or radiotherapy appears well tolerated and highly effective and should be considered the standard therapy for aggressive DLBCL late occurring after solid organ transplantation. Treatment delay must be avoided in these patients and effective immune-chemotherapy should be offered promptly.

1481

EFFICACY AND SAFETY OF MACOP-B+R AND RADIOTHERAPY IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: A REPORT ON 21 PATIENTS TREATED IN SINGLE INSTITUTION

M. Kichigina,[1] G. Tumyan[2], A. Kovrigina[2], E. Sorokin[2], O. Kolomeytsev[2], N. Tupitsin[2], O. Trofimova[2], I. Poddubnaya[2], D. Osmanov[2]

[1]N.N. Blokhin Russian Cancer Research Center, MOSCOW; [2]Russian Cancer Research Center, MOSCOW, Russian Federation

Introduction. Primary mediastinal (thymic) large B-cell lymphoma (PMLBCL) is recognized as a separate subtype of diffuse large B-cell lymphoma with unique clinical and immunopathologic characteristics and relatively favorable outcome. The standard of care for this subtype of aggressive lymphoma is not yet well established. The aim of our study was to evaluate the efficacy and safety of MACOP-B+R followed by radiotherapy (IFRT) in PMLBCL. *Methods.* Between 2004 and 2007, 21 previously untreated patients (pts) with PMLBCL were diagnosed and treated at our center. The median age was 28 years (range 18-47) and 57% (12\9) were females; 12 pts had stage II and 9 stage IV; 19 (91%) presented a bulky disease and 7 (33%) had a superior vena cava syndrome; 14 (66%) pts had B symptoms, LDH was increased in 15 (72%) pts. According to the age-adjusted IPI score 9 pts (43%) had aaIPI=0-1 and 12 pts (57%) = 2-3. The most frequent involved extranodal site was lung 12 (57%), 8 (38%) pts had pleural and 6 (29%) pericardial effusions. All patients were treated with standard MACOP-B regimen, 10 pts

received Rituximab (375 mg\m^2 - N 4-6). Mediastinal IFRT at dose 30-36 Gy was given to 16 (76%) patients. *Results.* The response rate at the completion of the programme was CR\CRu = 18 (85%), PR=2 (10%) and NR=1 (5%); 6 patients obtained a CR\CRu following IFRT. After a median follow up 16 months, the 2-years PFS and OS were 84% and 94% respectively. During the treatment twelve patients had different infectious and toxic complications: stomatitis - 8, hepatitis - 2, pneumonia - 6. These complications resulted in interruption of treatment of 6 pts, one patient died from pulmonary aspergillosis. *Conclusions.* Our data confirms that MACOP-B followed by IFRT are highly effective therapeutic regimens for patients with PMLBCL; however one should be cautioned about the significant complications. Futher studies are needed to finally define benefits of Rituximab addition to standart MACOP-B regim.

1482

NO BENEFIT OF ADDING RITUXIMAB TO CHOP REGIMEN IN PATIENTS WITH PRIMARY EXTRANODAL TYPE OF DIFFUSE LARGE B-CELL LYMPHOMA

G. Jang,[1] S.W. Kim,[1] S. Kim[2], D.H. Lee,[1] J. Huh,[1] H.J. Kim,[3] C. Suh[1]

[1]Asan Medical Center, University of Ulsan College of Medicine, SEOUL; [2]Asan Medical Center, SEOUL; [3]Hallym University Center, ANYANG, South-Korea

Background. The addition of rituximab to CHOP chemotherapy (R-CHOP) has significantly improved clinical outcome for patients with diffuse large B-cell lymphoma (DLBCL). However, new predictors of response to R-CHOP have not been established. *Aims.* We performed a retrospective analysis to evaluate the clinical impact and benefit of R-CHOP and tried to identify clinical predictors to get better benefit from R-CHOP compared with CHOP in patients with DLBCL. *Methods.* Using the population-based cancer registry for non-Hodgkin's lymphoma of Asan Medical Center, we identified eligible 177 patients who were newly diagnosed with CD20-positive DLBCL (excluding CNS lymphoma) and treated with CHOP (n=82) or R-CHOP regimen (n=95) as first-line chemotherapy from January 2001 to November 2005. We especially subgrouped all patients into either primary extranodal lymphoma (PENL, n=72) or nodal lymphoma (NL, n=105) according to the main origin of disease. PENL was defined as lymphoma which had either no or 'minor' nodal involvement along with a clinically *dominant* extranodal component after routine staging procedures. The response rate, event-free survival (EFS) and overall survival (OS) were compared between CHOP and R-CHOP group. To identify clinical predictors, subgroup analysis was performed with log-rank test and Cox regression model. *Results.* Complete response rate and overall response rate were higher in R-CHOP group than CHOP group although it didn't meet statistical significance between two groups (79% *vs* 69% p=0.164 and 97% *vs* 90% p=0.068, respectively).

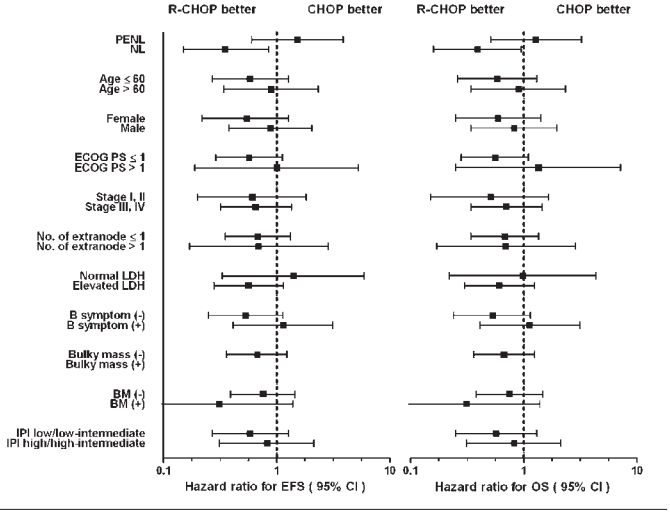

Figure 1. Hazard ratio plot for subgroup analysis without covariate according to treatment regimen. PENL, primary extranodal lymphoma; NL, nodal lymphoma; ECOGPS, Estern Clinical Oncology Group performance scale; LDH, lactate dehydrogenase; BM, bone marrow; IPI, international prognostic index; EFS, event-free survival; OS, overall survival; CI Confidance interval.

Two-year EFS rate and 2-year OS rate were higher in R-CHOP group (82% *vs* 74% p=0.223 and 83% *vs* 77% p=0.234, respectively). In subgroup analysis, patients with NL had a prominent survival benefit from R-CHOP therapy over CHOP (p=0.016 in EFS and p=0.032 in OS) but those with PENL did not (p=0.373 in EFS and p=0.608 in OS). Other factors such as age, ECOG performance status, stage, LDH and IPI showed no difference for survival outcome according to treatment regimen (Figure 1). *Summary and Conclusions.* R-CHOP regimen showed improved outcome in patients with DLBCL compared with CHOP alone, however, patients with PENL had no benefit from the addition of rituximab to CHOP chemotherapy. These patients might need other treatment strategy.

1483

EARLY 18FDG POSITON EMISSION TOMOGRAPHY (18FDG PET) NEGATIVATION IS HIGHLY PREDICTIVE OF GLOBAL SURVIVAL IN RELAPSED FOLLICULAR LYMPHOMA (FL) TREATED BY RADIOLABELLED IMMUNOTHERAPY (RIT) WITH 90Y-IBRITUMOMAB TIUXETAN (90YIT)

F. Peyrade, X. Fontana, P. Carrier, P.Y. Bondiau, N. Sapin, E. Chamorey, J. Thariat, H. Hebert, A. Thyss

Cnetre A.Lacassagne, NICE CEDEX [2], France

Background. 90YIT is indicated in relapsing CD20+ FL and appears to be a promising therapeutic. Post-therapeutics lymphoma evaluation is classically based on clinical examination, biological analysis and computerized tomography. Nevertheless, some studies have shown that metabolic imaging with 18FDG had a better prognostic value than conventional evaluation. This data has been clearly demonstrated for high grade lymphoma but there is less evidence for FL treated by 90YIT. *Aims.* Determine if 18FDG PET response is predictive of survival in FL treated by 90YIT. *Methods.* We retrospectively reviewed 10 different cases of RIT in relapsed FL treated in our institution between January 2005 and February 2007. For each case, we collected age, WHO performance status (PS), gender, histopathology, number of pre-90YIT chemotherapy lines, bone marrow (BM) involvement, blood cell count before90YIT, 90YIT dosage, grade III-IV OMS toxicities, treatment response according Cheson's criteria, event free survival (EFS) and overall survival (OS). In each case, an 18FDG PET was performed at least one month before and one month after 90YIT. All cases were retrospectively reviewed by two independent nuclear physicians. *Results.* All patients were WHO PS <3 and 9 were men. Median age was 63 years (range, 50-72). Histology was FL in all cases. Median number of pre-treatment chemotherapies was 3.1 (range, 2-5). All patients have a positive pre-therapeutics 18FDG PET. BM biopsy showed no involvement in 7 cases, 10% involvement in 2 and 20% in 1. Platelet counts were normal in 7 cases and between 100,000-150,000/mm^3 in 3. Consequently, RIT dosage was lowered to 0.3 mCi/kg in these 3 patients. The median time for complete hematologic recovery was 46 days (range, 41-53). 18-FDG PET response assessment demonstrated 6 complete responses, 2 stabilisations and 2 progressions. With a median follow-up of 20 months, 8 patients are alive, of whom 2 have no evidence of evolving disease. The median EFS is 14 months. It was 18 months in the 18FDG PET complete responder group (CRG) and 6 months in the non CRG. The median OS has not been reached and all patients with the CGR are alive. *Conclusions.* These data seem to indicate that early 18FDG PET is highly predictive of EFS in FL treated by 90YIT and consequently should became the gold standard in this indication

1484

OUTCOMES OF TREATMENT AND RISKS OF MYELOTOXICITY IN ELDERLY PATIENTS WITH NON-HODGKIN LYMPHOMA

P. Rujirojindakul, D. Kongkabpan, P. Viboonjantra, A. Lekhakula

Prince of Songkla University, SONGKHLA, Thailand

Background. The incidence of non-Hodgkin lymphoma (NHL) has been increased dramatically especially in persons over 60 years of age. When treating elderly lymphoma patients with polychemotherapy and/or radiotherapy, side effects are common and often severe. As a consequence of chemotherapy dose reductions or delays, the treatment outcome may compromise. Clearly, more data regarding NHL in the elderly are required to guide the selection of treatment regimens for this growing segment of population. *Aims.* To describe outcomes of treatment in patients with NHL, who were age 60 years or older, regarding to myelotoxicity effects, overall survival and 5-year survival. *Methods.* We analyzed the data of 256 NHL patients who were 60 years or older at diagnosis treated at Songklanagarind University Hospital from January 1998 to December 2004. The classified age groups 60-69 years (agegr1), 70-79 years (agegr2) and 80 years and older (agegr3) were analyzed in relation to the Working Formulation (WF) classification, an age-adjusted

International Prognostic Index (IPI), overall survival and 5-year survival. *Results.* Only 21 patients (8.2%) had low-grade NHL. Diffuse large cell was the most common subtype in all age groups (38.3-48.0%). According to IPI, 78 patients (55.3%), 46 patients (51.1%) and 11 patients (44%) in the age groups 60-69 years, 70-79 years and 80 years and older, respectively had high-intermediate and high risk,. Expectedly, only eight patients (32%) who were 80 years and older received standard doses and schedules of an anthracycline-containing regimen whereas 90 patients (63.8%) and 39 patients (43.3%) in both younger age groups received adequate treatment. Among 200 courses of all chemotherapy without growth factors, grade 3-4 neutropenia and lymphopenia was found in 15 and 41 percent, respectively, while grade 3-4 anemia and thrombocytopenia was found in 26.5 and 2.5 percent, respectively. Median survival for all age groups was 1.06 year and 5-year survival was 25 percent. Overall survival of each age group was shown in Figure 1. *Conclusions.* More favorable results can be achieved in the remission and survival rates of elderly NHL patients if the appropriate curative or palliative therapies, considering new and less toxic protocols such as supportive care, are chosen.

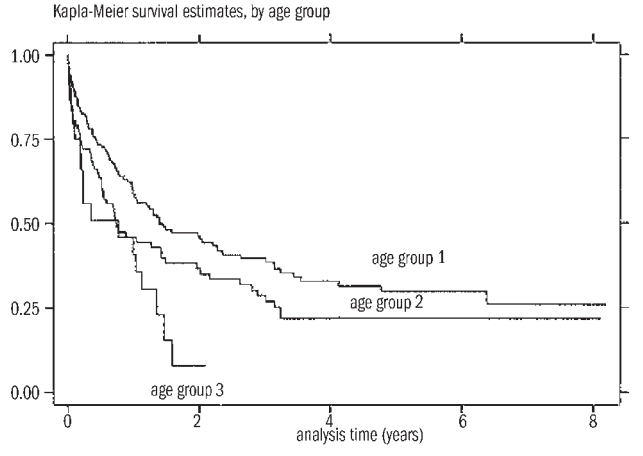

Figure 1.

1485

VINORELBINE, IFOSFAMIDE AND HIGH - DOSE CYTARABINE (VIHA) AS PRE-TRANSPLANT INDUCTION AND MOBILIZING REGIMEN IN REFRACTORY-RELAPSED AGGRESSIVE B-LYMPHOMA

A.R. Romano,[1] M. Balzarotti[2], L. Giordano[2], L. Castagna[2], M. Magagnoli[2], I. Timofeeva[2], B. Sarina[2], E. Todisco[2], R. Mazza[2], A. Anastasia[2], S. Bramanti[2], M. Demarco[2], A. Nozza[2], A. Santoro[2]

[1]Scuola Superiore di Catania, CATANIA; [2]Division of Hematology, Istituto Clinico Humanitas, ROZZANO (MILANO), Italy

Background. Response to induction chemotherapy is the main selection criterion before high-dose chemotherapy (HDCT) consolidation in eligible patients with relapsed/refractory non Hodgkin's lymphoma (NHL). Thus, identifying new regimens sharing clinical activity and mobilizing potential is of prior interest. Vinorelbine, ifosfamide, and cytarabine are active drugs usually non included in first line regimens. *Aims.* To investigate remission rate, tolerability, and mobilizing potential of the VIHA regimen in patients with aggressive large cell NHL. *Methods.* From November 1999 to August 2006, consecutive cases with aggressive relapsed/refractory NHL received 4 courses of (R)VIHA, (vinorelbine 20 mg/mq d1, ifosfamide 2500 mg/mq d1-3, ARA-C 1500 mg/mq bid d1-2, GCSF d7-12±Rituximab 375 mg/mq d -1). Non progressing patients proceeded to leukapheresis after third course and to HDT (BEAM or high-dose melphalan followed by BEAM in a tandem transplant program) after fourth course. *Results.* Sixty-four patients were accrued (M/F 33/31; median age 51 years; diffuse/follicular 42/22; relapsed/refractory 45/19; aaIPI 0-1/2-3 41/23; LDH ratio </>1 46/18; previous regimens 1/>2 23/41) . Eleven of them received R-VIHA. After the 4 induction cycles, complete remission (CR) rate was 41% and partial remission (PR) rate 18%. Fourty (66%) patients underwent HDCT , and 24 did not because of progression or inadequate $CD34^+$ mobilization. Diffuse histology, relapsed disease, low-risk sIPI, normal LDH value, and involvement of <2 extranodal sites significantly predicted the probability of CR by Fisher's exact text ($p<0.05$). Fourty-eight patients proceeded to leukapheresis, and 26 did not for the following reasons: progression 20, exitus 1, infection 2, lost at follow-up: 3.Median N^ of peripheral stem cells collected was 9.34×10^6/Kg (range 2.6-43), with 5/48 patients failing mobilization. Febrile neutropenia was the most frequent grade 3/4 non-hematological toxicity, occurring in 14% of delivered cycles. Sixty-four% and 37% of cycles required PLT and RBC transfusion, respectively. One toxic death occurred . Progression- free survival (PFS) and overall survival (OS) for the whole series were 51 and 55%, respectively. Univariate analysis of prognostic indicators for 2-year PFS and OS (log-rank test) is shown in the Table 1. *Conclusions.* So far, VIHA is a predictable and highly effective mobilization regimen in relapsed/refractory patients with aggressive NHL. It appears to induce high CR rates in patients with aggressive lymphoma even for heavily pretreated ones with poor prognostic features. Therefore, the dosage or the drugs schedule could be revised to maximize cost-effectiveness ratio.

Table 1. PFS and OS for VIHA treated patients.

		Free from progression		Overall Survival	
	Classification	No. of events (2 yrs survival %)	Log-rank p-value	No. of events (2 yrs survival %)	Log-rank p-value
All patietns		27(51)		29(55)	
Histology	DLBLC	20(43)		28(35)	<0.001
	FL	7(63)	0.129	1(94)	
BM involvement	yes	26(44)	0.044	27(50)	0.130
	no	1(86)		2(80)	
sIPI	0-1	17(56)	0.156	16(64)	0.024
	2-3	10(38)		13(34)	
Duration of last remission	< 6 months	15(56)	0.683	22(37)	0.002
	>= 6 months	12(50)		7(77)	
Response to VIHA	< CR	20(35)	0.005	22(39)	0.012
	CR	7(72)		7(77)	
HDCT performed	no	12(37)	0.002	17(22)	<0.001
	yes	14(60)		12(71)	
Overall response	< CR	6(39)	<0.001	6(28)	0.007
	CR	8(75)		6(86)	

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TUMOUR CONTAMINATION OF THE GRAFT PREDICTS POOR PROGNOSIS IN T/0-CELL NON HODGKIN LYMPHOMA AFTER AUTOLOGOUS HEMOPOIETIC STEM CELL TRANSPLANTATION. RETROSPECTIVE STUDY

S.A. Szomor,[1] T. Vidra[2], R. Csalodi[2], B. Kajtar,[3] L. Kereskai,[3] H. Losonczy,[4] M. David[4]

[1]University Pecs, PECS; [2]First Department of Medicine University Pecs, PECS; [3]Institute of Pathology, University Pecs, PECS; [4]First Department of Medicine, University Pecs, PECS, Hungary

Two hundred sixty eight patients (pts) underwent autologous hemopoietic stem cell transplantation between December 1999 and January 2008. One hundred twenty two (45,5%) had non Hodgkin lymphoma (NHL), 88 (32,8%) had multiple myeloma and 55 (20,5%) had Hodgkin lymphoma. Three pts had other disorders. Among NHL pts 25 (20,4%) had T/0 diseases. Histological subtypes: anaplastic large cell lymphoma (ALCL): 11, six of them had ALK negative, five had ALK positive, peripheral T-cell lymphoma, unspecified (PTCL-U): 10, T-lymphoblastoma (T-LB): 2, angioimmunoblastic lymphoma (AILD): 1, Lennert lymphoma: 1. Mean age of 9 female and 16 male pts was 40,0±13,5 years. Six pts were in first complete remission (3 PTCL, 2 ALK negative ALCL and 1 T-LB), 17 had chemosensitive relapse (6 ALK positive ALCL, 2 ALK negative ALCL, 6 PTCL, 1 AILD, 1 T-LB, 1 Lennert) and 2 (1-1 ALK negative ALCL and PTCL) had resistant disease at transplantation. Peripheral blood stem cell graft (PBSC) was transplanted in 20, bone marrow (BM) in 3 and mixed (both PBSC and BM) in 2 cases. The mean stem cell count was $7,6\times10^6$/kg CD34 positive cell in the graft of PBSC and $11,1\times10^5$/kg CD34 positive cell in the graft of BM. Eleven pts died (survival from transplantations: 0, 2, 3, 4, 5, 5, 8, 16 months). Eight pts had progressive disease, or relapse, three pts died in infections. Follow up time is 36 months (1-84), 10/14 pts are in complete remission (CR). We retrospectively analyzed the T-cell receptor gene (TCR) rearrangement of the transplanted grafts in 20 cases. Both two grafts of resistant pts, 8/14 of patients transplanted in chemosensitive disease and 1/6 of pts in first CR had TCR gene rearrangement positivity. TCR gene rearrangement polymerase chain reaction of grafts was positive among pts who relapsed or progressed. Complete remission proved by computed tomo-

graphic scan or PET-CT and bone marrow biopsy must be completed with TCR-PCR and flow cytometry of the blood, collected stem cell grafts to verify the real freedom of tumour cells.

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CENTRAL NERVOUS SYSTEM INVOLVEMENT IS A NEGATIVE PROGNOSTIC FACTOR IN PRIMARY CUTANEOUS B-CELL LYMPHOMA

S. Rupoli,[1] G. Goteri[2], P. Picardi,[1] A. Costagliola[2], A. Stronati,[1] D. Stramazzotti[2], S. Pulini,[3] S. Mulattieri,[1] G. Filosa,[4] G. Simonacci,[5] G. Ricotti,[6] A. Giacchetti,[6] I. Cataldi,[7] E. Grilli-Cicilioni,[5] L. Bugatti,[4] G. Brandozzi,[7] G. Mozzicafreddo,[6] M. Giangiacomi[2], G. Fabris[2], P. Leoni[1]

[1]Clinic of Hematology, ANCONA; [2]Institute of Pathology, ANCONA; [3]Department of Hematology, PESCARA; [4]Division of Dermatology, JESI; [5]Division of Dermathology, MACERATA; [6]Division of Dermathology, I.N.R.C.A.-I.R.C.C.S, ANCONA; [7]Clinic of Dermathology, ANCONA, Italy

Background. Central nervous system (CNS) involvement is mainly reported in patients with primary cutaneous T-cell lymphomas, particularly in advanced stages of MF, while in primary cutaneous B-cell lymphomas (PCBCL) seems to be a rare and unfavourable event, which accounts for 2% of cases and is related to death, according to the published data from the Dutch Cutaneous Lymphoma Registry. *Aims.* We aimed to evaluate CNS involvement incidence and prognostic impact in the series of PCBCL, recluted from January 1990 to September 2006, by our multicentric regional group. *Methods and Results.* The series included 42 patients, 25 with Follicular Cell Lymphoma (FCL), 14 with Marginal Zone Lymphoma (MZL) and 3 with Large cell Lymphoma Leg-type (LCLLT). During a median follow-up time of 41 months (range 1-109), 5 patients died, three for lymphoma (2 FCL, 1 LCLLT) and 2 for unrelated causes (both FCL, one for liver carcinoma, one for cardiovascular accident), with a median overall median survival of 83 mo.s. CNS involvement was observed in 3 male patients (7% of overall series), ranging in age from 56 to 75 yrs. Two patients were affected by FCL, one located at head and neck, treated by radiotherapy, and the other with lower limb location, treated with IFNα and PUVA. The third patient had a LCLLT with leg and trunk involvement and was treated with cyclophosphamide/pegylated liposomal doxorubicin/vincristine/prednisone and rituximab. All patients obtained a clinical complete remission, but showed subsequent skin recurrences, still responsive to chemotherapy, respectively, after 12 and 20 mo.s - the 2 FCL cases - and 12 mo.s - the LCLLT one. CNS involvement occurred after a mean time of 39 mo.s from diagnosis (range 20-76) as leptomeningeal infiltration in two cases and intra-parenchymal mass in one, and was managed with chemotherapy and radiotherapy, alone or in combination, obtaining a complete resolution in two cases. All patients died after a mean time of 23 mo.s after CNS involvement, two of them for causes related to lymphoma, respectively after 3 and 7 mo.s , whereas the third patient, still alive without disease after 59 mo.s. from CNS infiltration, unexpectedly died for a cardiovascular accident. Considering the overall series, CNS involvement significantly reduced the median overall survival to 77 mo.s (p=0.02) and also the median disease-specific survival, as 50% of patients with CNS involvement had the probability to die for lymphoma at 83 mo.s compared to 4% of patients without CNS infiltration at 109 mo. (Kaplan-Meier method: log-rank test p=0.02). *Conclusions.* Our study confirms the indolent behaviour of the majority of PCBCL and the relatively low incidence of CNS infiltration (7%), slightly higher compared to the 2% incidence observed by the Dutch Lymphoma Study Group and explainable by the different composition of the two series. In agreement with the Dutch investigators, we believe that CNS infiltration has a negative prognostic impact in PCBCL patient survival, although some patients can still achieve clinical remission with chemo- and radio-therapy.

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FLUDARABINE IN PATIENTS WITH ADVANCED MYCOSIS FUNGOIDES/SEZARY SYNDROME

L. Ocroteala, N. Fraticiu, G. Gaman

Municipal Hospital Filantropia Craiova, CRAIOVA, Romania

Background. Fludarabine is a purine (adenine) nucleoside analogue that is converted first to 9-β-D-arabino-furanosyl-2-fluoroadenine (F-ara-A) and then into the active metabolite F-ara-A triphosphate (F-ara-ATP), which interrupts DNA and RNA synthesis. Fludarabine also induces cell death through apoptosis. Fludarabine has been extensively evaluated in the treatment of a number of lymphoproliferative malignancies, including various types of cutaneous T-cell lymphoma (CTCL), like mycosis fungoides. The aim of this study was to evaluate the safety and efficacy of fludarabine (dosage 25 mg/m^2 30 min iv. infusion daily for 5 days every 4 weeks) in patients with advanced, symptomatic mycosis fungoides/ Sezary syndrome (MF/SS). *Methods.* The study included 19 patients with diagnosis of mycosis with the average age 66 years (range 54-78). Most patients had chemotherapy-refractory disease and the average number of previous anti-tumor treatment regimens was 2 (range 1-5). All patients had stage III or IV disease and most of them had severe itching due to disseminated cutaneous involvement. *Results.* The overall response rate was 83% (62% complete response and 21% partial response), but adverse events have been reported in all 19 patients (100%). The average time to treatment failure was 6 months. The most common adverse events associated with intravenously administered fludarabine treatment, as a monotherapy, in patients with mycosis fungoides/Sezary syndrome, include myelo-suppression (neutropenia, thrombocytopenia, leucopenia and anemia), nausea, vomiting, diarrhea and infections. Nausea was the most prevalent non-hematological adverse event (9 patients- 47, 3% cases). The incidence of the WHO grade 3 or 4 infection was 15, 7%. Herpes zoster was observed in 1 patient (5, 26%). Grades 3 and 4 hematological adverse event included neutropenia (26,3%), thrombocytopenia (10,5%), leucopenia (36,8%), anemia (5,2%). Severe itching was reduced or eliminated in all but 1 patient. Progression of squamous cell carcinoma of the skin was noted in 1 patient. *Conclusions.* Fludarabine shows promising clinical benefits and an acceptable safety profile in patients with advanced, heavily pretreated MF/SS, particularly in those patients with erythrodermia and severe itching.

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DIAGNOSIS AND TREATMENT OF MALIGNANT LYMPHOMAS IN PREGNANCY AND LACTATION, OUR OWN EXPERIENCES

L. Smardova, Z. Kral, I. Vasova, M. Navratil, M. Huser, M. Toskova, J. Mayer, J. Vorlicek

University Hospital and Faculty of Medicine, Masaryk University, BRNO, Czech Republic

Background. The lymphomas represent the fourth most frequent malignancy diagnosed during pregnancy, occuring in approximately 1:6000 deliveries. Considerably more often is a diagnosis of the Hodgkin's lymphoma, possibly because of the natural incidence of this disease in the reproductive age of the patients. *Aims and Methods.* Since 2001 there were diagnosed 17 patients with a lymphoma during pregnancy or lactation at our clinic. The median age of the patients was 27 years. The histological types of lymphomas were in most of the cases Hodgkin´s lymphoma (N=13), then diffuse large B-cell lymphoma (N=3) and MALT lymphoma of a eye-lid (N=1). The diagnosis was determined in the 1st (N=2), the 2nd (N=4) and the 3rd trimester (N=4) of pregnancy, at the other 7 patients during lactation (4 of them had a fever or swelling nodes aready during the pregnancy). In most of the cases the patients were diagnosed at clinical stage II (N=13), then I (N=3) and IV (N=1). *Results.* 3 of the patients diagnosed during the 1st and the 2nd trimester had an abortion on their own request, 11 patients gave birth to a healthy child naturally (N=7) or by Caesarean section (N=4). In three cases the disease was diagnosed in a critical state of the patients (superior vena cava syndrome or an obstructive ileus), requiring an abortion because of the medical indication of the mother. In the therapeutic plan a histological type of the lymphoma and a clinical stage of the disease were respected (7x ABVD, 3x BEACOPP, 2x BEACOPP+ABVD, 3x R-CHOP, 1xsalvage DHAP plus HD BEAM, 1x radiotherapy of a eye-lid. All the patients initiated the treatment after the ending of the pregnancy. The complete remission was achieved at all the patients. 4 of the patients gave birth to a healthy child after the completion of the treatment, the other 2 are currently pregnant. *Conclusions.* The cases of the diagnosis of the malignant lymphoma during pregnancy are increasing together with the tendency to the family planning in the older age of the mother. However, the pregnancy doesn't affect the overall survival as well as it doesn't influence the prognosis of the disease negatively. The diagnosis of the malignant lymphoma during pregnancy requires a specific and individual attitude while planning the staging examinations and the therapeutical programme has to take into account the wish of the patient.

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RAPID DIAGNOSIS OF BURKITT LYMPHOMA USING CYTOGENETIC FISH ANALYSIS OF DEPARAFFINISED HISTOLOGICAL TISSUE SAMPLES

L. Smith, M. Neat, M. Moonim, J. Van der Walt, P. Fields, R. Carr, B. Wilkins

Guy's & St Thomas' NHS Foundation Trust, LONDON, UK

Background. Classical Burkitt lymphoma (BL) is a highly aggressive B-cell lymphoma characterised by MYC dysregulation with clonal translocation of MYC to IGH [t(8;14)(q24;q32)] or light chain loci [2q11 or 22q11], a proliferative fraction ~ 100%, monomorphic morphology of medium-sized lymphocytes, basophilic cytoplasm with multiple, medium-sized nucleoli and a germinal centre phenotype ($CD10^+$, $bcl6^+$). Diffuse large B-cell lymphoma (DLBL) is clinically heterogeneous and DLBL and BL can share morphological and immunophenotypic features, including a high proliferative fraction, yet MYC translocations appear uncommon. In the absence of marrow disease or fluid collections it can prove difficult to analyse lymphoma samples for MYC translocations as the only diagnostic sample is often fixed, paraffin-embedded tissue. Clinicians must therefore make urgent treatment decisions between DLBL and BL solely on morphology and immunohistochemistry. *Aims.* To improve the diagnostic process for patients with high proliferative fraction (>95%), germinal centre phenotype, B cell lymphoma, tissue samples from *de novo* patients (n=11) treated at a single centre were analysed by fluorescence *in situ* hybridisation (FISH) in conjunction with morphological and immunohistochemical analyses. Deparaffinised intact, fixed tissue sections were used, facilitated by our developing experience in HER2 analysis. *Methods.* Involved areas were located by correlation with immunohistochemistry. Tissue sections were pre-treated, co-denatured and hybridised with FISH probes [IGH/MYC and IGH/BCL2 (Vysis), IGH break-apart (DAKO)] according to manufacturers' protocols. *Results.* Eleven patients (5M:6F), median age 42 years (34-67) were studied. Eight were HIV seropositive; 4 with CD4 counts <200. Seven had limited stage I/II disease; 2 had CNS disease. Median serum LDH was 619 (398 - 3161iu/l). Morphology was divided - BL (4), atypical BL (4), DLBL (3). Ki67 was >95% in 9/11; $CD10^+$ (9/11), $Bcl6^+$ (10/10). Tissue samples used were gastric (3), colon (1), abdominal mass (2), node (3), bone (1) and brain (1) biopsies. IGH/MYC translocation was seen in 4 of 9 evaluable cases with a further 2 demonstrating MYC rearrangement. IGH/BCL2 was demonstrated in 1/4 evaluable samples. Therapies used included LMB86 (2), LMB89 (8) and RCHOP14 (1). Nine achieved CR/PR; 1 TRM and 2 relapse result in 8/11 patients currently being alive. *Summary and Conclusions.* We identified MYC rearrangements in 67% of evaluable samples from a cohort of 11 patients with immunohistochemical/morphological features of BL and high remission rates if treated with BL protocols. Such techniques were rapid and easily reproducible. While 5-10% of DLBL may have MYC translocations, MYC analysis added additional information in our patients with probable BL. Intensive therapies offer a favourable prognosis in BL, even for advanced stage disease, whereas standard therapies used for DLBL prove inadequate. Gene expression profiling has identified a molecular BL signature which may supersede current methodologies, however, work has identified that cases with MYC breakpoints have adverse survival and that the nature of such a rearrangement is significant {NEJM 354:2419}. Therefore, MYC analysis by FISH on tissue sections may become a crucial tool to the clinician.

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THE CLINICAL FEATURES OF PRIMARY INTRAOCULAR LYMPHOMA: THE SINGLE CENTER RETROSPECTIVE ANALYSIS IN JAPAN

A. Arai,[1] O. Miura[2], H. Takase,[3] Y. Iwanaga,[3] H. Takahashi,[3] Y. Sugamoto,[3] S. Sugita,[3] M. Mochizuki[3]

[1]Tokyo Medical and Dental University, TOKYO; [2]Department of Hematology, Tokyo Medical and Dental University, TOKYO; [3]Department of Ophthalmology, Tokyo Medical and Dental University, TOKYO, Japan

Background. Primary intraocular lymphoma (PIOL) is a rare non-Hodgkin lymphoma which arises in the globe of eye. Because of its rareness and difficulty to diagnose, few clinical analysis has been reported especially none from Asian countries. The optimal treatment remains undetermined and its prognosis has been very poor. Most patients develop the central nervous system (CNS) involvement. *Aims.* The aims of this report are to analyze the clinical features of PIOL in Japan and to evaluate the effects of focal treatments, especially intravitreal methotrexate infusion (IMI). *Methods.* We retrospectively analyzed the clinical features of the patients diagnosed as PIOL at Tokyo Medical and Dental University. The analysis was approved by the institutional review board and the written informed consents were obtained from the patients. *Results.* Five patients were diagnosed PIOL from 1999 to 2006. One was male, and 4 were female. Median age was 68.8 (range 57-80). All patients complained burred vision. Three had bilateral disease. Involvements of the vitreous and the retina were detected in 5 and 2 patients, respectively. As the diagnostic procedure, aspiration of the vitreous was performed in all patients. In the pathological examination, however, only one patient revealed class V and the remaining 4 revealed class III. On the other hand, IgH rearrangements were detected by RT-PCR in the vitreal specimen of all patients. Concentration of IL-10 and IL-6 in the vitreous could be measured in 5 eyes of 4 patients; those were elevated, 380-15000 pg/mL and 19.8-350 pg/mL respectively. The ratios of IL-10/IL-6 were 2.1-100. The ratio was 0.04 ±0.06 in inflammatory uveitis. Four of 5 patients received radiation therapy (RT) for PIOL as initial treatment. Among them, disease relapsed in the globes in 3 patients. IMI was performed for 4 eyes of 3 PIOL patients including patients relapsed after RT, and for 6 eyes of 4 patients of non-primary intraocular lymphoma without CNS involvement. The effects of IMI are shown in the Table 1. All patients achieved ocular remission. No adverse effect above grade 3 was detected. However, 2 of 3 (66%) of PIOL and 4 of 7 (57%) in total patients respectively developed CNS involvement 3 to 42 months after diagnosis and all of them died because of progression of the disease. *Conclusions.* The clinical features of PIOL in Japan were similar to those in U.S.A. and European countries. PIOL was difficult to be diagnosed by morphology alone. The IgH gene rearrangements and the increased ratio of IL-10/IL-6 (>2) of the vitreous specimen were very useful items to make a diagnosis. Although IMI was quite effective for the ocular lesion, it was considered to be insufficient to prevent progression of the CNS disease. New treatment strategy including systemic chemotherapy should be needed to improve the prognosis of PIOL.

Table 1. The effects of intravitreal methotrexate infusion.

Case No.	Age	Sex	Primary site	IMI (times R/L)	Ocular Relapse	CNS Relapse
1	69	F	Eye globe	0 (RT)	+	+
2	80	F	Eye globe	0(RT)	-	-
3	62	F	Eye globe	9 R	-	+
4	76	F	Eye globe	4 R	-	-
5	57	M	Eye globe	3/3	-	+
6	87	M	Scalp	3/3	-	+
7	86	F	Cervical LN	3/3	-	+
8	73	M	Testis	2 R	-	-
9	82	F	Cervical LN	2 R	-	-

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CHOP-LIKE REGIMENS COMBINED WITH RITUXIMAB IN THE TREATMENT OF PRIMARY MEDIASTINAL B-CELL LYMPHOMA (PMBCL)

V. Milosevic, S. Jankovic, B. Andjelic, A. Sretenovic, M. Mitrovic, M. Perunicic-Jovanovic, Lj. Jakovic, M. Petrovic, D. Boskovic, B. Mihaljevic

Institute for Haematology, Clinical Centre of Serbia, BELGRADE, Serbia

Background. Primary mediastinal B-cell lymphoma (PMBCL) is a subtype of the diffuse large B-cell lymphoma. It is believed to arise from thymic medullary B-cells with a unique clinicopatologic features. The tumor is frequent in women in the third and fourth decades of their life and manifested as a large mediastinal mass with respiratory symptoms and signs of superior vena cava syndrome. The purpose of this study was to analyzed the terepeutic effect of immunochemotherapy at patients with PMBCL. *Aims.* The aim was to investigate the prognostic profile of PMBCL patients treated with immunochemotherapy. *Material and Methods.* This study included thirty seven patients who had PMBCL with sclerosis diagnosed between January 2001 and May 2007 at the Institute of hematology CCS, Belgrade. Demographic caracteristics were as follow: male/female 10:27 (27,03%:72,97%); the median age was 27 years (range, 17-51). On presentation, thirty two patients (86,48%) had a bulky tumor mass and B symptoms, thirty patients (81,08%) had respiratory symptoms and twenty five patients (67,56%) had a symptoms of supe-

rior vena cava syndrome.The majority of patients had stage I/IIB(70,27%) and 11 patients (29,73%) had stage III/IVB. Twenty patients (54,05%) had ECOG performans status (PS) 1 and 17 pts had a PS 2. Previosly untreated patients with PMBCL were treated with CHOP-like regimens plus Rituximab (R-EPOCH received 25 pts-67,56% and R-MACOP-B received 12 pts), followed with involved field mediastinal radiotherapy. *Results.* A complete response (CR) with initial immunochemotherapy achieved in twenty nine patients (78,37%) and five patients obtained a partial response. Three patients (8,11%) were non-responders:two patient died because of toxic effect of treatment, both of them treated with R-MACOP-B regimen. All patients who had PS 2 achieved CR and all of them had a bulky form of disease. The median follow up was 57 months. *Conclusions.* The immunochemotherapy is an effective treatment for patients with PMBCL. We achieved a good response with aggresive R-EPOCH regimen, although excellent results were achieved with R-MACOP-B as well.

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FEASIBILITY AND EFFICACY OF BEAM AS A FRONTLINE HIGH DOSE CHEMOTHERAPY SUPPORTED BY AUTOLOGOUS PBSCT IN ELDERLY PATIENTS (=60 YEARS) WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

V. Ivanov, D. Coso, J. Rey, T. Aurran, A.M. Stoppa, J.M. Schiano de Collela, J.A. Gastaut, D. Blaise, R. Bouabdallah

Institut Paoli-Calmettes, MARSEILLE, France

Background. Limited data are available concerning feasibility and efficacy of high dose therapy (HDT) supported by autologous PBSCT in elderly patients with non-Hodgkin lymphoma (NHL). In young patients with poor prognostic features intensification supported by PBSCT as a part of first-line treatment is suggested survival benefit. It is not clear if the same strategy is applicable into the older patients. *Methods.* The Institute Paoli-Calmettes database was reviewed for all DLBCL patients received BEAM followed by PBSCT in patients ≥60 years old between January 1998 and December 2006 (9 years). All patients were HIV-negative and received BEAM intensification as a part of front-line treatment. All of them were grafted *in situ*ation of complete or partial response after CHOP or R-CHOP induction. Twenty seven auto-transplanted patients were identified (median age 63 y, range 60-68). This cohort was compared with closely matched group of 37 patients with same age range, who received first-line CHOP or R-CHOP regimen without intensification in the same 9-years interval. *Results.* As frontline autoPBSCT was performed for high-risk patients, the group without HDT was naturally privileged in the terms of Ann-Arbor stage and aaIPI index. There was significant difference into the localised *vs* disseminated disease (stage I-II: 54% in no-HDT *vs* 26% in HDT group, p=0.03)) and aaIPI (0-1: 66% in no-HDT *vs* 37% in HDT, p=0.046) between two groups. Factors evaluated included treatment-related mortality (TRM), overall survival (OS) and event-free survival (EFS). TRM into the HDT group (1/27 pts (3,7%)) was comparable with previously published data. The estimated 5-year OS was 75,5% (95%CI 52-90%) for HDT group compared to 79,9% (95%CI 58-92%) in the no-HDT group (p=0,75). There were 8 events (1 TRM and 7 relapses) in the HDT group and 11events (all relapses) in no-HDT (5-year DFS 49,4% *vs* 64,2%, p=0.45). *Conclusions.* We conclude that front-line autologous PBSCT with BEAM conditioning can be safely performed in patients 60 years old and older with DLBCL after CHOP of R-CHOP induction. There was no difference in OS and EFS between cohort with and without intensification even if the auto-transplantation procedure was reserved for the high risk patients. It means that first-line HDT with autologous PBSC in elderly patients with high-risk IPI score might ameliorate survival in this group and give the same results as in the low-risk patients.

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Withdrawn by the authors

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MEASURING THE EFFECT OF DARBEPOETIN ALFA TREATMENT ON PATIENT REPORTED FATIGUE OUTCOMES IN PATIENTS WITH CHEMOTHERAPY INDUCED ANEMIA USING LATENT GROWTH MODELS

M.K. Vernon,[1] D.E. Stull,[1] H. Viswanathan[2], D. Tomita[2], M. Rader[2], D. Fairclough,[3] D.A. Revicki[1]

[1]UBC, BETHESDA; [2]Amgen, THOUSAND OAKS; [3]University of Colorado, DENVER, USA

Background. Darbepoetin alfa (DA) improves hemoglobin (Hb) levels in patients with chemotherapy-induced anemia (CIA). The change in Hb levels is expected to mediate the relationship between DA treatment and patient reported fatigue. Transfusions are more common among participants in the placebo group and may confound the effects of treatment. Latent growth curve *Methods.*allow for modeling the mediating effect of Hb and controlling for confounding variables such as transfusions, making them more suitable for examining the effects of DA on fatigue. *Aims.* The aim of this study was to explore effects of DA on fatigue outcomes mediated by Hb in CIA patients using data from four randomized placebo controlled clinical trials. Latent growth modeling (LGM) was used as this method allows for examination of relationships between drug exposure and outcomes mediated by a third variable. The method also has the advantage of being able to use data from all longitudinal time points while adjusting for confounding variables and measurement error. *Methods.* Data from four placebo-controlled clinical trials that evaluated the efficacy of DA in reducing transfusions for CIA patients with varying cancer types were used for the LGM analysis. Data were combined across the following CIA patient populations: non-myeloid, n=391; small cell lung, n=600; lung, n=320; lymphoprofileritive, n=349. Patient-reported fatigue was measured using the FACIT-Fatigue questionnaire. All studies had a baseline assessment, a mid-point assessment (week 7, 9, or 10), and a later time-point assessment (week 13 or 16). Data from three assessment points in each trial were combined to model relationships between slope of change in fatigue, slope of change in hemoglobin, and the effects of DA on slope of change in fatigue over time. The models controlled for transfusions, baseline health status, and age. *Results.* Results from the LGM analysis demonstrated that the model fit the data well as assessed by the comparative fit index (CFI=0.91). The analyses demonstrated that patients receiving DA had significantly higher mean Hb (B=0.34; p<0.05) over time and greater Hb significantly related to lower mean patient reported fatigue over time in the pooled clinical trials (B=0.21; p<0.05). *Conclusions.* Results suggest that DA impacted fatigue through maintaining higher mean levels of Hb. Treatment with DA had a significant relationship with patient-reported fatigue mediated through Hb: patients receiving DA, compared with placebo, had improved hemoglobin which resulted in improved fatigue, controlling for transfusions, baseline health status, and age. Application of LGM analyses to this complex problem allowed for examination of mediating effects using longitudinal data while controlling for confounding variables.

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ROLE OF RH-EPO IN ADDICTION TO ANTI - CD 20 MONOCLONAL ANTIBODY AND PREDNISONE IN THE MANAGEMENT OF REFRACTORY AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)

F. Iuliano,[1] M. Di Maio,[1] I. Infusino,[1] A. Perricelli,[1] A. Pomillo,[1] S. Molica[2]

[1]Onco-Hematology Unit, ROSSANO CALABRO; [2]Onco-Hematology Dpt, CATANZARO, Italy

Background. Standard treatment for (AIHA) due to warm antibodies includes combinations of glucocorticoids, immunosuppressive drugs and splenectomy. Patients who are refractory or intolerant to these therapies constitute an important therapeutic challenge. Rituximab (R), an anti-CD20 chimeric monoclonal antibody being increasingly used in autoimmune disorders. In addition, patients with AIHA, frequently have anaemia of sufficient severity as to require a blood transfusion. However, it is impossible to find compatible blood when, as is frequently the case, the autoantibody in the patient's serum reacts with all normal red blood cells. Further, the autoantibody may mask the presence of a red cell alloantibody capable of causing a haemolytic transfusion reaction. *Aims.* To test if the introduction of Rh-Epo in the refractory AIHA treatment may reduce the blood transfusion need and related risks. *Methods.*13 pts (M 6, F 7) median age 59.2 yr (range, 18-82) and AIHA resistant to standard treatment were treated with a schedule containing R , rh-EPO and prednisone(R-EP) 6 pts were idiopathic and the remaining 7 were associated with chronic lymphoproliferative syndromes (2) ,con-

nective tissue diseases (2) ,primary antiphospholipid syndrome (APS)(1) and ulcerative colitis (2). Serum erythropoietin level at baseline was (18±11 mU/mL) mean Hb value 6.3 g/dL±1.2) Pts had altered hemolysis markers and direct antiglobulin test (DAT) was positive for both complement and IgG (IgA 1 pts). Rituximab was administered intravenously at a dose of 375mg/m2 weekly for 4 weeks; prednisone 1 mg/Kg/day /for 30 days ; rh-EPO 30.000U/weekly for 4 weeks. *Results.* All pts completed treatment.No major infusion related side effects to R-EP were observed. Response criteria to R-EP were defined as follows: Complete Response (CR): Hb >10 g/dL or Hb increase >1.5 g/dL, resolution of symptoms of anemia, transfusion independent; Partial Response (PR): Hb >9 g/dL or Hb increase of 1-1.5 g/dL improvement in symptoms of anemia, transfusion independent; NR (failure to meet CR/PR). 100% were eligible for response. Responses were seen in 13/13 pts .CR in 12/13 and PR in 1/13, median follow up of 25.8 months (range 6-66 months). At the end of treatment DAT became negative in 10/12 pts ,concentration of lactic dehydrogenase , total bilirubin and indirect bilirubin began to decrease at 12 days after the first dose of rituximab, and decreased to normal range after 22 days.. Two patients required packed red cell transfusions before starting R-EP and all became transfusion-free. A moderate hemolysis still persisted only in one patient. *Conclusions* Our experience demonstrates that R-EP is an effective and safe alternative for the treatment of refractory autoimmune hemolytic anemia. Furthermore the introduction of rh-Epo clearly avoids blood transfusions

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FLOW CYTOMETRIC ANALYSIS A SIMPLE AND RAPID METHOD FOR DIAGNOSIS AND SERIAL MONITORING

B. Höchsmann,[1] S. Körper[2], T. Becker,[1] G. Baur,[1] R. Leichtle,[3] M. Rojewski,[3] H. Schrezenmeier[1]

[1]Institute for Clinical Transfusion Medicine and Immunogenetics Ulm, ULM; [2]Department of Hematology/Oncology, Robert Bosch Krankenhaus, STUTTGART; [3]Department of Transfusion Medicine, University of Ulm, ULM, Germany

Background. The new therapy option Eculizumab leads to a highly significant reduction of PNH characterising Coombs-negative haemolysis and thromboembolic events. Therefore detection of PNH is gaining in importance for prevention of vascular events and for increase of quality of life in PNH-patients. Flow cytometric analysis of GPI-anchored proteins (GPI-AP) is the diagnostic gold standard to verify the disease. *Aims.* Establishment of a simple, but sensitive flow cytometric method for detection of even small GPI-deficient clones. *Methods.* Flow cytometric analysis of CD58 and CD59 on reticulocytes and erythrocytes and CD66b/CD24$^-$, CD16 on granulocytes as mandatory markers for our screening panel. The expressions of CD14 and CD 48 on monocytes as well as CD48/CD19 and CD48/CD3 on lymphocytes were used as optional markers to fully assess lineage involvement. In special cases FLAER, a flurochrom-labelled modified bacterial toxin which binds directly to the GPI-anchor was used. Staining of the reticulocytes by thiazol orange enabled a separate analysis of erythroctes and reticulocytes. We established the actual cut off (mean + 2 SD) for GPI-deficient cells in healthy blood donors. *Results.* 1050 flow cytometric results of patients who had or were suspected of having the diagnosis of PNH were studied. The criterion for inclusion in follow up analysis - availability of at least two GPI-AP assessments- was met by 152 patients. At time of initial GPI-AP-flow cytometry 78 (51%) of these patients had a normal GPI-AP expression and 74 (49%) patients showed significant GPI-AP-deficiency. During follow up development of a new significant clone with GPI-deficiency in 8% of cases and a change of clone size in about 22% of cases was observed. The median number of immunophenotypic investigations was 3 per patient and the median follow up duration 1039 days. 8 patients were treated with eculizumab and were censored at the date of first eculizumab application. 2 patients had an allogenic stem cell transplantation, but were not censored for analysis. Correlations between the markers within one cell lineage were highly significant. Additionally good correlations between the proportion of GPI-deficient reticulocytes and granulocytes (r^2=0.57, p<0.0001) as well as between GPI-deficient monocytes and granulocytes (r^2=0.84, p<0.0001) were found. Probably because of influence by hemolytic crises and RBC transfusions correlation was less good between GPI-deficient erythrocytes and granulocytes (r^2=0.31, p<0.0001). Lymphocytes were rarely involved. *Summary and Conclusions.* Flow cytometric analysis of GPI-anchored proteins should be performed in all situations suspicious of PNH (e.g. acquired Coombs-negative haemolytic anemia, thrombosis with unclear etiology) and in bone marrow failure syndromes. Measurement of at least two different GPI-anchored proteins on granulocytes and reticulocytes provides a simple and rapid method to detect even small GPI-deficient populations. The role of monocytes for diagnosis even of small GPI-deficient clones should not be underestimated. This method is not affected by recent hemolytic crises or RBC transfusions. It allows the quantization of the clone and can therefore be used to monitor the course during follow up to detect evolution of PNH clones and to assess therapy-effects.

1498

INTERLEUKIN 12 AND RED CELLS ANTIOXIDANTS IN CHRONIC RENAL FAILURE PATIENTS WITH HCV ANTIBODIES ON REGULAR HEMODIALYSIS

M. Attia, G. Rabie, M. Elawa, M. Tawfeek, A. Sleim

Faculty of Medicine - Ismailia University, ISMAILIA, Egypt

Background. Chronic renal failure (CRF) is associated with severe alterations of the immune system. Plasma levels of many cytokines in patients with (CRF) are higher than in healthy control. Moreover, CRF itself and reactive oxygen species can cause a dys-regulation of production and elimination of these cytokines. Also, there is good evidence indicating that uremia, in general, is associated with enhanced oxidative stress. Aim of the Study: to determine the level of IL-12 (p 70) and red cells antioxidants in dialysis patients with HCV infection. Subjects and *Methods.* seventy patients with CRF on regular hemodialysis (HD) were included. Plasma and peripheral mononuclear cells (PMNC) levels of IL-12 were measured by ELISA and erythrocyte superoxide dismutase (SOD), erythrocyte glutathione peroxidase (GSH-Px) and erythrocyte reduced glutathione (GSSG-Rd) were measured colorimetrically. Control group comprised twenty healthy blood donors' subjects. HD patients group was subclassified into two groups according to HCV antibodies results. *Results.* Compared to controls, circulating levels of erythrocyte GSH-Px and GSSG-Rd activities, in 70 CRF patients treated by hemodialysis, were significantly decreased (p<0.001), whereas erythrocyte SOD activity was unchanged. There is increased level of IL-12 in both plasma and (PMNC) in HD patients compared to controls (p<0.05). Also, IL-12 level was higher in HCV-positive haemodialysis patients as compared with HD patients without chronic hepatitis C (p<0.05). In *Conclusions.* Our findings provide a link between overproduction of pro-inflammatory cytokines (IL-12) and imbalanced T-cell activation. However, increased level of IL-12 may be difficult to interpret in dilemma of impaired kidney clearance, so cytokine mRNA level may represent a better index of synthetic capacity than plasma concentrations. Also, disturbances in antioxidant systems provide additional evidence for an increased oxidative stress that could contribute to long-term complications in uremic patients. However, However, HCV infection seems to not cause any additional increase in this status in HD subjects and it may be partly due to protective effect of dialysis treatment on HCV infection.

1499

CHANGES IN RED BLOOD CELLS MEMBRANE PROTEIN COMPOSITION DURING HAEMODIALYSIS PROCEDURE

M.S. Costa,[1] S. Rocha[2], P. Rocha-Pereira,[3] E. Castro[2], V. Miranda,[4] M. Do Sameiro Faria,[5] A. Loureiro,[6] A. Quintanilha,[7] L. Belo,[8] A. Santos-Silva[8]

[1]Instituto Politécnico de Bragança, BRAGANÇA; [2]Fac. Farmacia and IBMC, UP, PORTO; [3]Universidade da Beira Interior and IBMC of UP, PORTO; [4]Fresenius Medical Center, Dinefro - Diálises e Nefrologia, SA., PORTO; [5]Fresenius Medical Center, Dinefro - Diálises e Nefrologia, SA, PORTO; [6]Uninefro - Sociedade Prestadora de Cuidados Médicos e de Diálise, SA, PORTO; [7]ICBAS and IBMC of UP, PORTO; [8]Faculty Farmacia and IBMC, UP, PORTO, Portugal

Anaemia is a common complication in hemodialysis (HD) patients, due mainly to failure in erythropoietin kidney production. Moreover, the life span of red blood cells (RBC) of HD patients being shortened is an additional cause of anaemia in these patients. Our aim was to evaluate the influence of the HD procedure in RBC membrane protein composition. We evaluated haematological data (RBC count, haemoglobin concentration and haematimetric indices) and RBC membrane protein composition [linear and exponential gradient polyacrilamide gel electrophoresis in the presence of sodium dodecylsulfate (SDS-PAGE) followed by densitometry analysis], before and immediately after the hemodialysis procedure in 20 HD patients, and in 26 healthy controls. HD patients, before hemodialysis, presented anaemia and significant

changes in membrane protein composition, namely a reduction in spectrin associated with a statistically significant increase in bands 6 and therefore, an altered interaction between protein 4.1/spectrin, protein 4.1/band 3, protein 4.2/band 3 and spectrin/band 3. We found that after HD, patients showed a statistically significant increase in RBC count and haemoglobin. Concerning to RBC membrane protein profile, after HD the patients showed a decrease in spectrin RBC membrane content and spectrin/band 3 ratio, and an increase in band 3 RBC membrane content. Our data suggest that the hemodialysis procedure, per se, seems to impose changes in RBC membrane proteins that are normally associated to a reduction in RBC deformability. Moreover, hemodialysis procedure further contributes to spectrin loose.

1500

ERYTHROCYTE MEMBRANE STABILITY AND ANTIOXIDANT STATUS IN PATIENTS WITH ALZHEIMER'S DISEASE

M. Gilca, D. Lixandru, L. Gaman, B. Virgolici, I. Stoian, V. Atanasiu

University of Medicine and Pharmaceutics, BUCHAREST, Romania

Background. There is a similarity in the susceptibility to oxidative damages between erythrocytes and brain, both being *ideal* targets for free radical damages due to their rich stores of iron (which catalyses free radicals generation), high rate of free radical production, and other specific factors (e.g. high lipid content of brain, exposure to high oxygen partial pressure in erythrocyte, etc.). *Aims.* We intended to test whether the erythrocyte and plasma antioxidant status is altered or not in patients with Alzheimer's disease, as a result of oxidative aggression. *Methods.* We studied erythrocytes membrane stability and blood antioxidant status in patients with Alzheimer's disease (n=40) and matching controls (n=25) using the following measurements: erythrocyte membrane stability assay, erythrocyte catalase activity, erythrocyte superoxid dismutase (SOD) activity, plasma Trolox Equivalent Antioxidant Capacity (TEAC), residual antioxidant capacity (GAP), erythrocytes non-proteic thiols, plasma total thiols, plasma uric acid, plasma total proteins, plasma albumin. *Results.* When compared to control group, erythrocyte membrane stability was decreased (EC50 H2O2 0.879±0.693 *vs* 2.94±1.51; $p<0.05$), while, paradoxically erythrocyte catalase activity (368.038±120.897 *vs* 279.30±86.71 K/g Hb/ml blood) ($p<0.05$), and erythrocyte SOD activity (448.27±143.49 *vs* 377±92.31 U/gHb) were increased in patients with Alzheimer's disease. No significant changes in erythrocytes non-proteic thiols (μmol thiols/mgHb/ml blood), plasma total thiols (μmol thiols/g plasma protein/ml blood), albumin, uric acid, TEAC and GAP were seen. Significant reduction in haemoglobine ($p<0.01$), plasma total proteins ($p<0.05$) were found in Alzheimer's disease group. *Conclusions.* These results indicate that: a) The most satisfactory indirect measurement of oxidative stress level in the blood of patients with Alzheimer's disease is represented by the erythrocyte membrane stability assay, possibly due to the following facts: 1. membranes represent a key target for the free radical injury; 2. membrane susceptibility to oxidative challenges reflects both the status of the majority of the cellular antioxidants and the level of reactive oxygen species production, being an redox marker with integrative qualities. b) The paradoxical increase of erythrocyte catalase and SOD activities in Alzheimer's disease might represent a *failed* attempt of the erythrocyte protective mechanisms to react at the high rate of free radical production, which is involved in the pathogenesis of this neurodegenerative disease.

1501

HIGH DOSE-THERAPY WITH AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN THE FRONT-LINE TREATMENT OF WALDENSTROM'S MACROGLOBULINEMIA: EXTENDED FOLLOW-UP

T. Caravita,[1] A. Siniscalchi,[1] G. Natale,[1] A. Tendas,[1] S. Amadori[2], P. De Fabritiis[1]

[1]S.Eugenio Hospital, ROME; [2]Hematology, Policlinico and University Tor Vergata, ROME, Italy

Background. Waldenstrom's macroglobulinemia (WM) is an incurable rare B-cell malignancy. Therapy is currently reserved for symptomatic patients, including alkilating agents, purine nucleoside analogs and the anti-CD20 monoclonal antibody. Although high-dose therapy (HDT) followed by autologous stem sell transplantation (SCT) in WM can produce high response rate and some long term responses, its role has not been established and is nowadays challanged by other innovative approaches. *Aims.* to evaluate the efficacy and feasibility of HDT, anti-CD20 monoclonal antibody rituximab and autologous peripheral blood stem cell transplant (PBSCT) for WM up-front. *Materials and Methods.* Between April 2001 and October 2003 six male patients (pts) with symptomatic WM were enrolled in an open-label trial of HDT (CHOP 3 courses, Rituximab 375 mg/m^2×4, CTX 4 gr/m^2+GCSF) followed by SCT conditioned with Melphalan 200 mg/m^2. Informed consent was obtained from all the subjects. Main pre-treatment characteristics were the following: median age 53.5 years (range 41-66); median β2M 3.86 mg/L (range 0.6-6.6); median hemoglobin 9.65 g/dl (7.3-12.9); median serum monoclonal component 3.2 g/dL; median bone marrow lymphoplasmacytic infiltration 55% (range 30-80). Three out of the six pts were HCV positive. The EBMT/IBMTR/ABMTR criteria were used for definition of response; toxicity was graded according to WHO criteria. *Results.* All but two pts mobilized adequate numbers of stem cells; of the two non-responding patients, one was lost at follow-up before mobilization and one required a second attempt to harvest a sufficient number of PBSCs. Five patients underwent PBSCT after a median time from MM diagnosis to SCT of 11 months (range 10-13). Bone marrow recovery after transplant was prompt; neither treatment-related mortality nor major toxicities were observed. With a median follow-up of 55 months (51-69) all the 5 patients were evaluable for response, achieving a partial response after the autoPBSCT. Currently, all patients are alive without clinical or serological signs of disease progression. *Conclusions.* In our experience, the association of HDT with Rituximab followed by autoPBSCT in selected patients with symptomatic WM is a safe and feasible front-line treatment option that can produce prolonged remissions and long term disease control.

1502

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATIONS (HSCT) FROM 10/10 HLA ANTIGEN IDENTICAL UNRELATED DONORS AFTER FLUDARABINE, TREOSULFAN AND ANTITHYMOCYTE GLOBULINS (ATG) : A POPULATION-MATCHED ANALYSIS

M. Michallet,[1] Q. Le,[2] M. Sobh,[2] F.E. Nicolini,[2] A. Thiébaut,[2] S. Ducastelle,[2] N. Raus,[2] R. Tabrizi,[3] K. Bouabdallah,[3] C. Faucher,[4] M. Mohty,[5] S. Furst,[4] J. Bay,[6] N. Milpied,[5] E. Hermet,[6] D. Blaise[5]

[1]Hôpital Edouard Herriot, LYON; [2]Edouard Herriot Hospital, LYON; [3]Hematology Department, Haut-Leveque Hospital, BORDEAUX; [4]Hematology Department, Paoli Calmettes Institute, MARSEILLE; [5]Société Française de Greffe de Moelle et de Thérapie Cellulaire, PARIS; [6]Hematology Department, Jean Perrin Institute, CLERMONT-FERRAND, France

We conducted a prospective multi-center trial concerning 33 patients (pts) who underwent HSCT from unrelated 10/10 antigen HLA-identical donors (high-resolution level). Twenty-seven patients were assessable (13 F, 14 M - 56 years [18-65]). The diagnosis and disease status pre-transplant were AML [n=12; 5 complete remission (CR)1, 7 CR2], ALL [n=1; 1 CR2], MM [n=5; 2 Partial remission (PR) 2, 1 PR3, 1 PR4], NHL [n=4; 1 CR1, 2 PR3, 1 PR4], MPS [n=3; 2 stable disease (SD), 1 relapse], RAEB [n=1; 1 progressive disease (PD)] and CLL [n=1; 1 PR2]. The median interval between diagnosis and transplant was 27 months [5-171], 24 pts received PBSC and 3 received bone marrow from unrelated donors. All pts received fludarabine 30 mg/m^2/dx5d, treosulfan 12 g/m^2/dx3d and ATG 2,5 mg/kg/dx3d. After transplantation, there were 2 graft failures, 7 pts with aGVHD ≥ grade II (30%).

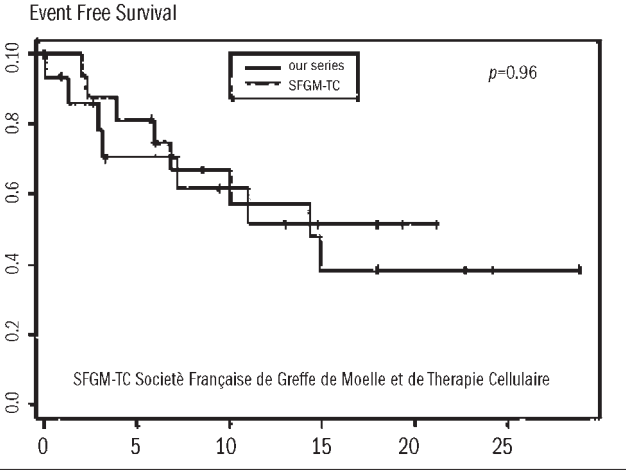

Figure 1.

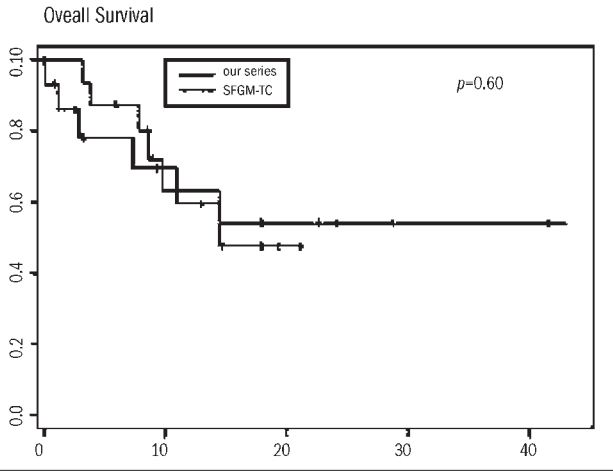

Figure 2.

Only 23 pts were assessable after 3 months of post transplant follow-up, 7 pts developed cGVHD (30%) (5 limited, 2 extensive), 12 pts died (7 from TRM causes, 5 from relapse) and 12 pts are alive in CR with a median follow-up of 14 months. Among the global population we defined a low risk subgroup (CR1, CR2, PR2): 17 pts (12 AML, 1 ALL, 3 MM and 1 NHL), 2 pts presented an aGVHD ≥grade II (13%). With a median follow-up of 18 months, 2 pts had a follow-up of less than 3 months, 3 pts developed a cGVHD (20%), 6 pts died (40%) and 9 pts are alive in CR. At one year, the probability of overall survival (OS) and event-free survival (EFS) were 50.4% [32-79], 40% [23-70] for the total population and 60% [37-96], 51.5% [29-90] for the low risk subgroup respectively. To try to demonstrate if Treosulfan allowed a better transplant outcome we performed a paired match-analysis [center and 4 out of 5 other parameters (age, gender, HSC, pre-transplant status and diagnosis)] comparing our Treosulfan series from unrelated donors and HLA identical sibling allogeneic transplants receiving fludarabine, busulfan and ATG from the registry of the Société Française de Greffe de Moelle et de Thérapie Cellulaire (SFGM-TC). This paired match-analysis showed no difference in term of OS and EFS between our series and SFGM-TC series (Figure 1 and Figure 2). In conclusion, these results demonstrate that Treosulfan appears to be a very promising drug that could be included in the conditioning regimen before HSCT from either related or unrelated donors.

1503

HIGH DOSE IDARUBICIN AS CONTINUOUS INFUSION AND INTRAVENOUS BUSULPHAN AS CONDITIONING REGIMEN TO AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

F. Ferrara, S. Palmieri, C. Copia, C. Criscuolo, M. Pedata, T. Izzo, F. Pollio, A. Viola, G. Mele

Cardarelli Hospital, NAPOLI, Italy

Background. Relapse represents the main cause of failure in patients with AML undergoing autologous stem cell transplantation (ASCT). A tentative approach for reducing relapse rate is the adoption of conditioning regimens specifically designed for AML. We did previously report encouraging data after an original conditioning regimen based on the combination of idarubicin (IDA) and oral busulphan (BU), named IBu, in young adults or elderly AML patients. While results in terms of relapse rate were promising, most relevant toxicity was oral mucositis, recorded in more than 90% of patients requiring in most cases total parenteral nutrition (TPN). *Aims.* To asses toxicity of a new regimen based on replacement of oral with intravenous BU. *Methods.* Data from 14 patients receiving the new IBu regimen are reported. The protocol included high dose IDA, given at 20 mg/sqm daily as 3 days continuous infusion (from day -13 to -11) and intravenous BU at 3 mg/kg daily from day -5 to -2. Patients aged over 60 years (n=2) received a reduced schedule (two days IDA and 3 days BU at the same dose). There were 8 males and 6 females, median age: 44 years (36-72). All patients received peripheral blood stem cells collected after consolidation plus G-CSF. The median interval between diagnosis and ASCT was 4 months (3-7). The median number of $CD34^+$ cells infused was $6{,}9\times10^6$/kg (3.1-18). All patients were autografted in conventional single bed rooms. *Results.* The median number of days to PMN >500/cmm and platelets >20000/cmm was 11 (8-13) and 13 (8-40), respectively. The median number of platelet and blood units transfused was 3 (1-6) and 2 (0-4), respectively. Extra-hematological toxicity was negligible an no grade 3 or 4 episode was recorded. In particular, grade 3-4 mucositis was absent and no patient required TPN. Four patients experienced FUO, while fever did not occur in 9 patients at all. As compared to our previous series of 105 patients receiving oral BU, the occurrence of severe mucositis was dramatically reduced (88% *vs* 0%, $p< 0.0001$). In addition, the incidence of fever (p:0.01) and documented infections (p:0.03), as well as need and duration of intravenous antibiotic therapy were also significantly reduced. No transplant related death occurred. LVEF examination post-ASCT did not reveal any cardiac toxicity. Median time of hospitalization was 27 days (20-37) and, once again, it does favorably compare with that recorded after oral BU (p:0.01). At the time of writing, 4 patients have relapsed and 12 are alive (in continuous complete remission (CR) with a median follow up after ASCT of 9 months. *Conclusions.* We conclude that replacement of oral BU with intravenous Busulphan results in a more favorable toxicity profile in patients with AML undergoing ASCT. In particular, the incidence of mucositis is significantly reduced with relevant decrease in TPN requirement, antibiotics needing and hospitalization. A longer follow-up is required in order to assess a potential advantage in terms of disease free survival. Intravenous Bu should replace the oral formulation in each regimens adopting BU as conditioning to ASCT.

1504

EXTRAMEDULLARY RELAPSE OF CHRONIC MYELOID LEUKEMIA IS A FREQUENT COMPLICATION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION AND REQUIRES MULTIMODAL THERAPEUTIC APPROACHES

S. Ocheni,[1] G.B. Iwanski,[1] P. Schafhausen,[2] A.R. Zander,[1] T. Bruemmendorf,[2] A. Hochhaus,[3] N. Kröger,[1] U. Bacher[1]

[1]Interdisciplinary Clinic for Stem Cell Transplantation, HAMBURG; [2]Department of Oncology, University of Hamburg, HAMBURG; [3]Department of Haematology and Oncology, MANNHEIM-HEIDELBERG, Germany

Background. The numbers of allogeneic stem cell transplantation (SCT) are decreasing in Chronic Myeloid Leukemia (CML) due to tyrosine kinase inhibitors (TKIs), but the proportion of advanced phase in stem cell recipients is growing. This selection of CML poor-risk patients is associated with higher relapse rates and due to limited reports probably also to higher chloroma frequencies in the post-transplant period. *Aims of the Study.* To gain insights in the frequency and outcome of extramedullary CML relapse after SCT, we performed a retrospective analysis in 24 stem cell recipients with CML. *Methods.* We retrospectively evaluated clinical and laboratory pre- and post-transplant data of 24 consecutive patients with different CML stages undergoing allo-SCT from 1/2004 to 12/2007 in the University of Hamburg, Germany. *Results.* Relapse occurred in 9/24 patients (38%), in 5 of these (56%) with an extramedullary manifestation 2-39 months after SCT (in one case after myeloablative conditioning, in 4 cases after reduced conditioning). All 4 chloroma patients with available stages showed blast phase before SCT. Clonal evolution was present in all five cases including one t(3;21)(q26;q22). Most frequent sites of extramedullary relapse were the skeletal system (3x) and the muscles (2x), in one cases mimicking severe myositis of the lower extremities with acute renal failure. Other localizations were the skin/subcutis (2x), lymph nodes (1x), intracerebral manifestation (1x), and meningeosis (1x). ABL mutational screening revealed one L387F conferring Imatinib-resistance. Variable treatments were used in the five chloroma patients in different combinations: cytotoxic therapy by hydroxurea (HU) or cytarabine (3x), irradiation (4x), or donor lymphocytes (2x). All 5 patients received the II-generation TKI Dasatinib, in 2 cases after initial treatment by Imatinib, in one case being followed by Nilotinib. A novel Aurora-Kinase-inhibitor (PHA-739358) was administered in one case. A second allogeneic SCT from unrelated donors was performed in 2/5 patients who had previously received related SCT. Outcome: One patient showed resolution of muscular chloroma of the lower extremities following Dasatinib and HU. Another patient showed partial remission of intracerebral chloroma after Dasatinib and cranial irradiation. At the time of this report, 3/5 patients are alive 3, 12, and 14 months from the initial chloroma manifestation after SCT; 2/5 patients died due to progression. Interestingly, in all three patients with available results there was 100% donor chimerism as assessed by quantitative PCR simultaneously to chloroma manifestation. Cytomorphological bone marrow involvement occurred in one case only. 3/5 patients had a history of graft-*vs*-host-disease (GvHD). *Conclusions.* These data show that extramedullary relapse after SCT has a high frequency in nowadays CML stem cell recipients probably due to the

selection of poor risk cases. Further, extramedullary CML relapse after SCT develops often irrespective of the presence of complete donor chimerism and of GvHD, and often without cytomorphological bone marrow involvement. Thus, the graft-*vs*-CML effect might be lower in extramedullary tissue than in the bone marrow which might explain the high chloroma frequency after SCT. Research should focus on specific therapeutical approaches for this post-transplantation complication.

1505

LONG-TERM RESULTS OF REDUCED-INTENSITY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR 1ST CHRONIC PHASE CML

Y. Brychtová, M. Krejcí, M. Doubek, D. Dvorakova, O. Horky, J. Mayer, J. Vorlícek

University Hospital Brno and Medical Faculty Masaryk University Brno, BRNO, Czech Republic

Background. Allogeneic hematopoietic stem cell transplantation (SCT) is recently being replaced by tyrosine kinase inhibitors as the method of treatment of 1st chronic phase CML patients (pts). However, in the last 10 years, reduced intensity conditioning(RIC) is also more widely used. In this retrospective analysis, we wanted to summarize our experience with RIC in CML pts in order to better describe the longer-term outcome of this treatment procedure. These results may serve as a basis for counseling the pts and their relatives. *Patients and Methods.* 22 pts (1st CP, n=20; AP n=2; median age=50 y, range 15 y-59 y) were transplanted during the 1998-2007 using PBSC from matched sibling donors and RIC with fludarabine, oral busulfan 8 mg/kg total dose, and ATG Fresenius, total dose 40 mg/kg. Median follow up is now 53 months (range 9-114 months). *Results.* Acute nonhematologic toxicity was observed in 17 pts and was predominantly grade 1. Parenteral nutrition was required only in 7 pts (32%). All patients engrafted, and there was no acute transplant-related mortality. The incidence of acute GvHD was 50% (grade I+II in 6 cases, grade III+IV in 5 cases), chronic GvHD developed in 68% of pts (extensive in 8 cases, limited in 7 cases). Complete chimerism was observed in 20 pts in median of 113 days (range 14-3714). After the transplantation, hematological remission only and complete cytogenetic remission only was achieved in 1 pt, and in 5 pts, respectively. Sixteen pts achieved complete molecular remission (CMR) in median of 189 days. All six pts without CMR underwent additional therapy (donor lymhpocyte infusion (DLI) in 3 cases, DLI and imatinib in 3 cases), and five pts achieved CMR after this therapy. Eight pts relapsed in median of 25 months after the SCT, five pts relapsed repeatedly, and in total, there were 10 molecular, 2 cytogenetic, and 3 hematological relapses. Pts with relapses were treated by stopping immunosuppression (2 cases), DLI (1 cases), imatinib (2 cases), combination of DLI and imatinib (2 cases), and combination of DLI and tyrosine kinase inhibitors and retransplantation (1 case). Until December 2007, 20 from 22 pts were alive, 18 pts were disease free, 1 pts was retransplantated, and 1 pts was in complete cytogenetic remission 41 months after the transplantation. One pts died from extensive chronic GvHD with sepsis after DLI, one died for progression of the disease. *Conclusions.* Allogeneic hematopoietic stem cell transplantation after RIC conditioning using Flu-Bu-ATG (Fresenius) is very well tolerated with zero acute mortality and low morbidity. After this procedure, CMR is eventually achieved in a majority of patients, however, quite frequent interventions for molecular relapses are required. On the other hand, pts are in good clinical condition with minimal long-term chronic medication.

1506

SHORT-TERM GRANULOCYTE RECOVERY IS PREDICTED BY TELOMERE LENGTH OF DONORS IN CHILDREN HEMATOPOIETIC STEM CELL TRANSPLANTATION

R. Mangerini,[1] E. Lanino,[2] P. Terranova,[2] M. Faraci,[2] M.P. Pistillo,[3] G.F. Gaetani,[1] A.M. Ferraris[1]

[1]Università di Genova and Istituto Nazionale per la Ricerca sul Cancro, GENOVA; [2]Dipartimento di Ematologia-Oncologia Pediatrica, IRCCS G. Gaslini, GENOVA; [3]Laboratorio Tumori Mammari, Istituto Nazionale per la Ricerca sul Cancro, GENOVA, Italy

Background. Hematopoietic stem cell transplantation (HSCT) is widely used to treat a variety of malignant conditions. Telomeres (TRF) are repetitive DNA sequences located at the end of chromosomes and recognized as a critical factor in determining the replicative potential of mitotic cells. TRF decreases with cell divisions as well as with age, and when they reach a minimum critical length, the cell stops dividing or enters apoptosis. Accelerated TRF shortening due to excessive replicative stem cell turnover may therefore represent a likely indicator of hematopoietic stem cells (HSC) premature aging. *Aims.* In the present study we measure telomere length from donors and recipients at different intervals from HSCT, in order to unfold the dynamics of marrow reconstitution in the first six months post transplant. *Methods.* Nineteen children with various disorders referred to the HSCT Unit to receive allogeneic HSCT were entered in the study. The F : M ratio was 2.2 : 1; 13 had acute or poor-prognosis chronic leukemia and 6 other diseases associated with bone marrow failure. Average age of patients at HSCT was 9.5 years (range 1-18) and that of donors 31.1 years. Five patients received HSCT from an HLA identical sibling, 11 from an HLA-matched unrelated donor and 3 from a mismatched relative; bone marrow was the source of HSC in all cases but 3, who received peripheral blood stem cells (PBSC). Granulocytes (PMN) and mononuclear cells (MNC) were purified from peripheral blood. TRF analysis was determined by Southern hybridization and densitometric analysis using an image software. *Results.* Related donors were younger (mean age 26.1 years, range 10-45) than unrelated ones (mean age 35.6 years, range 30-42); as a consequence, TRF length of related donors was longer (mean 11.5 kb, range 5.8-19.1) than that of unrelated donors (mean 9.52 kb, range 7.7-13.1). A distinct pattern of telomere length dynamics was observed in the recipients cells at various intervals post transplant, with respect to donor values. At 180 days after HSCT the mean deltaTRF for PMN was not stable at >3.5 kb, whereas the deltaTRF for MNC reached a plateau between 90 and 180 days. Our data show a significant relationship between TRF length of donors and time to recovery: longer donor TRF effected faster granulocyte recovery in the recipient (p=0.039). Taking into account only related donors, which have longer TRF compared to unrelated ones, the results are even more significant (p=0.0027). Paradigmatic is the case of two young patients, which received HSC from two young donors aged 14 and 10 yrs, with TRF length respectively of 19.1 and 15.5 kb, and had an hemopoietic recovery of only ten days. *Conclusions.* It is therefore possible that HSC derived from relatively young donors, with their longer TRF, offer a replicative advantage to the recipients, by more efficiently counteracting the premature replicative exhaustion of HSC due to the high demand of hematopoiesis reconstitution.

1507

USE OF KGF (PALIFERMIN) BUT NOT OF G-CSF IS ASSOCIATED TO A REDUCED RATE OF INFECTIONS AFTER HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS PBSC TRANSPLANTATION

G.S. Sortino, G.M. Milone, G.L. Leotta, M. Poidomani, A. Di Marco, E. Oliva, A. Spadaro, E. Marturano, M.P. Azzaro, S. Coppoletta

Hematology, CATANIA, Italy

Introduction. Reduction of infections after PBSC autologous transplantation is an important clinical goal. Use of G-CSF has been shown to shorten neutropenia without improving infectious risk. KGF (Palifermin) has been shown to reduce febrile neutropenia when used during TBI-containing regimens, however, efficacy of Palifermin has not been determined when used in association with non TBI containing regimens. *Methods.* We have studied factors associated to infections in a group of 156 patients, all received a non TBI-containing eradicating regimen and were treated with either G-CSF and/or Palifermin.

Table 1.

Factors evaluated for severe infections			
Fuo or pneumonia or Gram bacteriemia	*Univariate logistic regression*	*Multivariate logistic regression*	*Odds ratio (c.i. 95%)*
Dx HD-PAM conditioning	p=0.004	p=0.09	
Use of palifermin	p=0.002	p=0.008	0.267 (0.100-0.716)
Disease in advanced phase	p=0.020	p=0.23	
Severe mucositis	p=0.025	p=0.03	2.193 (1.046-4.601)
Age	p=0.10	p=0.34	
Use of G-CSF	p=0.17		

G-CSF was used according to two randomised studies run in our institution while PALIFERMIN was used sequentially in a cohort of 29 patients. Mean age was 49 years, underlying diagnosis was MM (77 pts),

Lymphomas (66 pts), other dx (13 pts), 32% of patients were in advanced phase of disease, conditioning regimens were: L-PAM (n.77), BEAM (n.60), other (n.19); dose of infused $CD34^+$ was 5.5×10^8/Kg. Anti-infectious prophylaxis was standardised and comprised in all patients systemic antibiotic, intestinal decontamination, acyclovir, fluconazole. *Results.* Neutrophil engraftment was reached in a mean of 12 days, Febrile neutropenia of Unknown cause (FUO) was diagnosed in 45% of patients, Gram-negative Bacteraemia in 4.5%, Pneumonia in 5.1%, CVC-associated Bacteraemia in 16% of patients. TRM at 1 year was 0%. Results of association of various factors to Severe Infections, defined as diagnosis of " FUO or GRAM-neg. BACTERAEMIA or PNEUMONIA in univariate and in multivariate logistic regression are reported in the Table1. *Discussion.* In conclusion use of PALIFERMIN is the only modifiable factor that reduces significantly the risk of *Severe Infections* after high dose non TBI-containing regimens and PBSC transplantation

1508

RAT MESENCHYMAL STEM CELLS SUCCESSFULLY IMPROVE DERMAL REMODELLING IN 3D COLLAGEN MODEL

J.H. Won, C.H. Kim, M.K. Cho, Y.K. Lee, S.C. Lee, H.J. Kim, S.B. Bae, C.K. Kim, N.S. Lee, K.T. Lee, S.K. Park, D.S. Hong, H.S. Park

Soon Chun Hyang University Hospital, SEOUL, South-Korea

Background. Considerable progress has been made in developing effective *Methods.*for culturing different types of stem cells and transplanting them into animal models. It has been reported that mesenchymal stem cells (MSCs) had successfully improved skin-substitute wound healing. *Materials and Methods.* We investigate the effects MSCs on wound healing using a 3D collagen gel system in a full thickness large-sized skin defect rat model. Three full thickness skin and tissue defects of 2 x 2 cm in size were excised on the backs of female SD-rats and replaced with 3D collagen gel contains 2 millions of male rat MSCs (rMSCs) on one site. Two different sites were control. One replaced with 3D collagen gel without rMSCs and the other site was empty. Wound size, histology and protein expression were evaluated at 3, 7 and 14 days after injury. *Results.* Cultured rMSCs were confirmed to be negative for hematopoietic markers by flow cytometry and to be capable of differentiating into osteocytes, chondrocytes, and adipocytes *in vitro*. The wound size was significantly smaller in the rMSCs-treated sites than control sites ($p<0.01$) and the most effect of wound healing was obtained at day 7. A lot of rat Y chromosome positive cells were found in rMSCs treated sites. The expression of matrix metalloproteinase-9 (MMP-9) and vascular endothelial growth factor (VEGF) were increased on the rMSCs-treated sites at day 3 but on the other hand those levels on the control sites were increased at day 7. *Conclusions.* This data suggest that MSCs accelerate wound healing and 3D collagen gel model can stably introduce MSCs to the defected skin area. Early activation of MMP-9 as well as previously known VEGF may play crucial roles in dermal remodeling and accelerate the processes of dermal and epidermal wound healing after MSCs treatment.

1509

MYELOABLATIVE CONDITIONING REGIMEN WITH INTRAVENOUS VERSUS ORAL BUSULFAN PLUS CYCLOPHOSPHAMIDE: THE ROLE OF THE CYCLOPHOSPHAMIDE DOSE

I. Yakoub-Agha,[1] D. Parent,[2] G. Damaj,[3] A. Leroy-Cotteau,[2] L. Magro,[1] V. Coiteux,[1] M. Yilmaz,[2] J.P. Jouet[1]

[1]Maladies du Sang, LILLE; [2]CHU Pharmaceutique, LILLE; [3]Hématologie, AMIENS, France

Background. In France, intravenous busulfan (Bu) is approved for combination with 120 mg/kg cyclophosphamide (CY) in adults undergoing allogeneic stem cell transplantation. Up to the intravenous Bu approval, we used oral weight-adjusted capsules of Bu with 200 mg/kg CY as a myelo-ablative conditioning regimen. The IV formulation of Bu has been known to be well tolerated and to give at least similar results to the oral form of the drug. *Aims.* The main objective of this retrospective study was to investigate the impact of CY dose on patient outcome. We compared the standard combination of oral Bu (16 mg/kg) plus CY (200 mg/kg) (oral group; n=24) with a combination of intravenous Bu (12/mg/kg) plus CY (120 mg/kg) (IV group; n=16). *Methods.* All patients received an HLA-matched relative graft and the same prophylaxis for GVHD (cyclosporine and short-course methotrexate), infections and veno-occlusive disease (VOD) (intravenous heparin 100 IU/kg from admission to day +28 post-transplant). T-cell chimerism was performed regularly over 12 months for all patients (using real time PCR, sensitivity <1%). *Results.* The underlying diseases were AML (n=22), chronic phase CML (n=9) and myelodysplastic syndrome (n=9). The two groups were comparable in terms of transplantation modalities and patient characteristics, except for recipient age (median age at transplantation was 25.6 and 42.9 for the oral and IV groups, respectively; $p=0.02$). At the time of analysis, median follow-up was 729 days (379-729). Patients in the IV group had less mixed chimerism at days 30 and 60 post-transplant, while those of oral group were more likely to have full-donor chimerism ($p=0.002$ and $p=0.05$ at d30 and d60, respectively). All patients engrafted, except for 2 in the IV group who experienced secondary graft rejection (p=ns). No patients developed VOD of the liver. There were no significant differences between the 2 groups in terms of other post-graft events, including mucositis, acute and chronic GVHD, hemorrhage, infections, relapse and intensive care unit transfer. Oral Bu was given in weight-adjusted capsules, which might explain (at least in part) the good tolerance in this group. *Conclusions.* This study showed comparable results in both groups. Surprisingly, patients in the IV group were less likely to experience full-donor T-cell chimerism than those in the oral group - probably because of the lower dose of CY used in the IV group. Hence, the CY dose should be 200 mg/kg when combined with IV Bu.

1510

T-CELL CHIMERISM AND CLINICAL OUTCOME AFTER REDUCED-INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION

F. Patriarca, E. Toffoletti, A. Chiarvesio, A. Michelutti, M. Battista, M. Medeot, A. Sperotto, R. Fanin

Division of Heamtology and Transplant Unit'Carlo Melzi', UDINE, Italy

Background. Initial mixed donor/chimerism has usually been observed in most patients after allogeneic reduced-intensity conditioning (RIC) stem cell transplantation (SCT). *Aims.* This study investigated the kinetics of whole peripheral blood and $CD3^+$ T-cells chimerism in patients receiving RIC-SCT. The T-cell chimerism has been correlated with risk of grade II-IV acute graft-*vs* host disease (GvHD) and relapse. *Methods.* Twelve patients with a median age of 56 years (range 41-65) affected by lymphoma (6), multiple myeloma (3), acute myeloid leukaemia (1) or idiopathic myelofibrosis (1) received RIC allogeneic SCT between January and December 2007. Source of stem cell was PB and donors were matched unrelated for 9/12 patients. Conditioning regimens were: thiotepa plus cyclophosphamide (8), 2 Gy total body irradiation (TBI) plus fludarabine (2), melphalan plus fludarabine (2). In 9 cases anti-thymocyte globulin was used as part of GVHD prophylaxis. Hematopoietic chimerism has been serially assessed at 30, 60, 90 and 180 days after SCT in whole peripheral blood (PB) and sorted $CD3^+$ T-cells. The analysis have been performed by polymerase chain reaction (PCR) based amplification of short tandem repeats (STR) sequences using the AmpflSTR identifler kit (Applied Biosystems). Full donor chimerism (FDC) was defined as the presence of at least 95% donor cells. *Results*. The percentage of patients achieving FDC was lower in $CD3^+$ cells in comparison with whole PB at day 30 (67% *vs* 75%), 60 (58% *vs* 75%) and 90 after SCT (60% *vs* 70%), but the difference was not statistically significant at any time. At day 180 the percentage of patients achieving FDC was similar in $CD3^+$ cells and PB. Patients with grade II-IV acute GVHD had no significant difference in the incidence of $CD3^+$ FDC at day 30, 60, 90 after SCT in comparison with patients with≤ grade I GVHD. Howerever, patients who subsequently relapsed had a significant lower incidence of $CD3^+$ FDC at day 60 (20% *vs* 86%, $p=0.02$) and at day 90 (56% *vs* 83%, $p=0.05$) in comparison with patients with sustained remission. *Conclusions.* We conclude that the T-cell populations reached FDC more slowly than myeloid compartment in RIC-SCT. Moreover, mixed chimerism at days 60 and 90 was associated with an increased risk of relapse. The development of acute GvHD was apparently not correlated with T- cell chimerism.

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ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH PEDIATRIC SOLID TUMORS

M. Eyrich, F. Deinlein, B. Winkler, M. Wölfl, P.G. Schlegel

University of Würzburg, WÜRZBURG, Germany

Background. Allogeneic stem cell transplantation is increasingly used in patients with both pediatric and adult solid tumors. Assuming an allogeneic graft-*vs*-tumor effect, allogeneic stem cell transplantation is performed either as a salvage therapy in refractory cases or as consolidation treatment in patients with a very high risk of relapse. However, since this

patient population often faces a limited prognosis, it is important that all therapeutic efforts in these situations do not expose patients to unreasonable toxicity. Here, we report about our experience in children treated with allogeneic stem cell transplantation for advanced refractory/relapsed solid tumors between 2005 and 2007. *Methods.* 7 children (mean age 9.4 years) were transplanted for alveolar (n=2) or embryonal (n=1) rhabdomyosarcoma (RMS), Ewing sarcoma (ES, n=1), infantile fibrosarcoma (IFS, n=1), epitheloid sarcoma (EpS, n=1) and nephroblastoma (NB, n=1). 2/7 children were in CR, 5/7 were in PR at time of transplant. Grafts were BM from a matched sibling donor (n=1), UD-PBSC (n=3), or CD3/CD19-depleted haploidentical-PBSC (n=3). Whereas the one patient transplanted with sib-BM received a full myeloablative conditioning, patients receiving UD-PBSC were conditioned with Fludarabine/Treosulfan/ATG, and recipients of haploidentical PBSC were pretreated with Fludarabine/Thiotepa/Melphalan/OKT3. RESULTS Primary engraftment occured in all patients after a median of 14.4 days (ANC>500/μL) and 13 days (thrombocytes >20.000/μL). Importantly, we observed no severe infections, no TRM, and no GvHD > I-II in this heavily pretreated patient population. Both patients with alveolar RMS replased within 40 days posttransplant. Two patients in PR (ES and EpS) were in CR after transplantation but showed new pulmonary metastasis after a median of 191 days. One patient died after 534 days (ES), the other one is still alive after resection and salvage chemotherapy (EpS, follow-up 466 days). Both patients in CR (embr RMS, NB) and one patient in PR (IFS) are disease-free, alive and well after a median follow-up of 350 days. *Conclusions.* Our preliminary results indicate that allogeneic stem cell transplantation is a safe and tolerable procedure in pediatric patients with advanced solid tumors. Further prospective trials are warranted to test the efficacy of allogeneic stem cell transplantation in defined subsets of pediatric solid tumors.

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BONE DENSITY IN LONG TERM SURVIVORS OF STEM CELL TRANSPLANTATION FOR HAEMATOLOGICAL MALIGNANCY

N. Salooja, J. Shankari, J. Todd, E. Olvarria, D. Marin, E. Kanfer, A. Rahemtulla, J. Apperley

Imperial, LONDON, UK

Reduction in bone mineral density (BMD) is a well recognised complication of stem cell transplantation for haematological malignancy. The underlying malignant disease and immobility may lead to a reduction in bone density before treatment is given. During the transplant process itself osteoprogenitor cells in the bone marrow are directly affected by chemotherapy and total body irradiation which can lead to a reduction in bone formation. Following the transplant, altered cytokines, graft *vs* host disease and its treatment can exacerbate bone loss as can reductions in sex steroids, thyroxine, vitamin D and secondary hyperparathyroidism. Some data suggests that BMD reaches a nadir soon after SCT and thereafter improves with time. However, there is a lack of data available on BMD in very long-term survivors of Stem Cell Transplant (SCT). In this study we have documented the incidence of reduced bone density (osteopenia or osteoporosis) a minimum of 10 years following SCT for haematological malignancy. 36 consecutive patients (20 male) attending a dedicated long term follow up clinic were evaluated by history, biochemical tests and DEXA scans. Biochemical tests included vitamin D, PTH, levels of sex steroids and thyroid hormones. All patients had received total body irradiation at a median dose of 12 Gy (range 10-13.5) and cyclophosphamide chemotherapy. Three patients who had relapsed post transplant received a second SCT conditioned with chemotherapy only. 31 patients had an initial diagnosis of CML, 4 AML and one ALL. The source of stem cells was sibling (n=27), twin (n=2) or unrelated donor (n=7). The median transplant to follow up time was 15 years (range 10-26). None of the patients gave a history of fracture. Of 20 male patients evaluated (median age 48, range 34-63), 25% (n=5) had reduced BMD. Three were osteopenic, one had osteoporosis and another had borderline osteoporosis. Multiple risk factors were present in 4/5 of these patients including reduced testosterone (n=3), GVHD (n=1), prolonged steroid use (n=1) raised PTH (n=2), two transplants (n=1). Of 16 female patients (median age 51.5, range 42-72), 69% (n=11) had reduced BMD. Nine were osteopenic and two were osteoporotic. All of these women had received appropriate hormone replacement supplementation (HRT) between SCT and the age of a physiological menopause. In this group of 11 women, additional risk factors for decreased BMD were seen in four and included low vitamin D (n=3), prolonged steroid use (n=2) and two transplants (n=1). These data provide evidence of significant reductions in bone density more than 10 years following SCT for haematological malignancy. Reduced bone density was a frequent finding in female patients despite appropriate use of HRT.

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IMMUNE RECOVERY IN PATIENTS AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELLS TRANSPLANTATION

L. Guenova,[1] L. Garcheva,[1] A. Michova,[2] M. Nikolova,[2] H. Taskov,[2] A. Bensussan[3]

[1]*National Center of Haematology and Transfusiology, SOFIA, Bulgaria;* [2]*National Center of Infectious and Parasitic Diseases, SOFIA, Bulgaria;* [3]*Inserm, Unite, 841 équippe 5 Faculté de Medecine de Créteil, PARIS XII, CRÉTEIL, France*

Background. High-dose chemotherapy followed by autologous hematopoietic stem cell transplantation (aHSCT) is increasingly applied in various hematologic malignancies and solid tumors. The pattern of immune recovery is characterized by the specific reconstitution kinetics of lymphocyte populations and major serum immunoglobulins. The cytolytic T lymphocyte response has often been used to assess the reconstitution of T cell function after HSCT. However, no data are available concerning CD160, a unique MHC-I specific activating receptor expressed on highly cytotoxic NK and T cell subsets. *Aims.* The aim of this study was to analyze the recovery dynamics of lymphocyte populations for a period of twelve months after aHSCT, in parallel with CD160 expression on CD8 T-cells, and the levels of serum IgA, IgG, IgM immunoglobulins. *Materials and Methods.* Peripheral blood of 24 patients undergoing autologous HSCT for various malignancies (lymphomas-7; Hodgkin-9; myeloma-4; AML-3; WT-1) was studied at several time points: before transplantation (M0), as well as after 1, 2, 3, 6 and 12 month (M1 - M12). The absolute count (Äë) and the percentage of major lymphocyte subpopulations were determined by flow cytometry (FACSCanto, software BD Diva v. 5.0.1) using TruCount tubes (BD) and 4-colour monoclonal antibodies combinations CD3-F/CD8-PE/CD45-PerCP/CD4-APC and CD3-F/CD56+16-PE/CD45-PerCP/CD19-APC. The percentage of T-cell subpopulations was assessed by CD4, CD8, CD28, CD7, CD45RA, CD57, CD62L and CD160. Serum immunoglobulins were quantified by nephelometry (MININEPH). The results were compared to the referent values of an age and sex matched control group of 28 healthy volunteers (mean age 38 years). *Results.* The lymphocyte AC rapidly increased to its maximum of 1691 cell/μL at M2 and showed a slight decrease to 1042 cell/μL at M12 mainly due to the respective changes in the percentage and AC of $CD3^+$ T cells. Unlike, B cells showed a gradual increase after M1 reaching 19% and 235 cell/μL at the end of the observed period ($p<0,05$, Wilcoxon) associated with a similar increase of serum IgM from 0,47 g/L (M1) to 0,95 g/L (M9). In contrast, serum IgG remained stable within the range of reference values - 12,02 g/L (M1) and 11,87 g/lL(M9), as well as IgA which was found close to the lower reference level - 1,25 g/l (M1) and 1,05 g/L (M9), respectively . The dynamics of the NK [$CD56^+CD16^+CD3^-$] cells was similar, reaching 15% and 133 cells/μl at M12 (lower reference value). The recovery of T cells showed a steep increase of percentage and AC of $CD3^+$ cells at M2-75%, 1392 cell/μL, followed by a decrease to the lower reference level - 64% and 673 cell/μL, at M12. The T-cell subsets followed different trends: after the steep increase at M2 the mean proportion and AC of $CD8^+$ T cells remained above the upper reference level (mean 41% and 440 cell/μL, respectively), while $CD4^+$ T cells decreased to a median of 20% and 213 cell/μL. Th1-like ($CD7^+CD57^-$ and central-memory ($CD45RA^-CD62L^+$) cells prevailed in CD4 population while the percentage of naïve ($CD45RA^+CD62L^+$) and late differentiated ($CD45RA^+CD62^-$) cells remained below the lower referent level. The recovery of CD8 T subset was mostly due to the expansion of effector memory cells ($CD45RA\text{-}CD62L^-$), as well as of intermediate ($CD28^-CD57^-$) and late ($CD28\text{-}CD57^+$) effectors. A significant increase of the percentage and AC of $CD160^+$ T lymphocytes was observed at M2 as compared to controls (30% *vs* 4%, $p<0.05$, Wilcoxon), reaching a maximal steady level at M6 (37%). The elevated expression of CD160 on CD8 T cells throughout the follow-up (35% at M12 *vs* 15% in healthy controls) indicated that the recovery of the CD8 T-cell subset was mostly due to the increase of effector cells with high cytotoxic potential. However, no correlation was established between the expression of CD160 and CD56. *Conclusions.* Post-transplantation immune recovery is a long and multifactorial process. Flow cytometry analysis reliably demonstrates the dynamics of the reconstitution of the different lymphocytes population and subpopulations and promotes for immunological monitoring of transplanted patients. CD160 may be a useful marker for monitoring the recovery of cytotoxic T cell functions after aHSCT. The reconstitution of B cells and the major serum immunoglobulins showed

different dynamics. The quantitative recovery of the B cell population does not reflect directly its maturation and functional characteristics.

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HIGH PERIPHERAL BLOOD CONCENTRATION OF REGULATORY T CELLS (TREGS) AFTER AN ALLOGENEIC STEM CELL TRANSPLANT PROTECTS FROM ACUTE GRAFT-VERSUS-HOST DISEASE AND CYTOMEGALOVIRUS REACTIVATION

G.F. Torelli, B. Lucarelli, M.S. De Propris, A. Capobianchi, G. Gentile, F. Milano, W. Barberi, V. Valle, E. Iannella, R. Maggio, M.G. Mascolo, N. Peragine, R. Ricci, E. Arleo, M.M. Basood, G. Guarini, A.P. Iori, R. Foà

University La Sapienza, ROMA, Italy

Background. A balanced immunological reconstitution among regulatory and cytotoxic functions represents one of the main features for a favorable clinical outcome after an allogeneic stem cell transplant (SCT). *Aims.* Aim of the study was to correlate the concentrations of T cells and T-cell subsets, with particular attention to $CD4^+/CD25^+$ regulatory cells (Tregs), B cells and natural killer (NK) cells in the peripheral blood (PB) of patients who have performed an allogeneic SCT with the clinical parameters of the post-transplant period. *Methods.* Thirty-nine patients who had undergone an allogeneic SCT (21 PBSC, 16 BM, 2 CB; 28 from an HLA identical sibling and 11 from MUD) at our Institute between September 2005 and October 2007 were investigated. The cellular concentrations in the PB were analyzed at 1 year from transplant by four-color immunofluorescence using antibodies against CD3, CD4, CD8, CD25, CD20, CD16, CD56 and CD34. *Results.* Acute graft-*vs*-host disease (aGVHD) was observed in 16 of the 39 patients; it was statistically correlated with reduced overall survival (OS) (p 0.04), increased transplant-related mortality (TRM) (p 0.039) and increased incidence of cytomegalovirus reactivation (CMVr) (p 0.0007). CMVr, that was observed in 23 of 39 patients, was also statistically correlated with reduced OS (p 0.05) and increased TRM (p 0.002). Considering the 18 patients who were evaluable at 1 year, 33% of them presented aGVHD and 61% CMVr. aGVHD correlated with the concentration of Tregs in the PB; aGVHD was in fact observed in 56% of patients with Tregs below the median value *vs* 11% of patients with Tregs over the median value (p 0.064). In addition, a high concentration of Tregs in the PB correlated significantly with reduced CMVr (45% of patients with Tregs over the median value *vs* 87% of patients with Tregs below the median value, p 0.01). A reduced CMVr has been also observed in patients who presented an elevated concentration of circulating NK cells (29% of patients with NK cells over the median value *vs* 86% of patients with NK cells below the median value, p 0.05). Relapse was not influenced by the concentration of Tregs. Data on OS, disease-free survival and TRM are not evaluable at the moment. *Conclusions.* These results confirm that the concentration of Tregs in the PB may protect from aGVHD, without affecting the graft-vs-leukemia phenomenon, and indicate that a balanced immunological reconstitution is an important factor for the protection from CMVr. The potential anti-viral role of NK cells has been also confirmed by our data and deserves additional consideration for possible therapeutic implications.

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EFFICACY AND TOXICITY OF THE EDA-V REGIMEN IN ADVANCED MM PATIENTS PREVIOUSLY TREATED WITH THALIDOMIDE

M. Offidani,[1] C. Polloni,[2] L. Corvatta,[3] S. Gentili,[2] M.-N. Piersantelli,[2] M. Catarini,[3] M. Brunori,[3] G. Visani,[4] F. Alesiani,[5] P. Galieni,[4] R. Centurioni,[3] M. Burattini,[3] P. Leoni[2]

[1]Clinica Ematologia, ANCONA; [2]Clinica Ematologia, Ospedali Riuniti Ancona, ANCONA; [3]Divisione Medicina, FABRIANO; [4]Divisione Ematologia, PESARO; [5]Unità Oncoematologia, SAN SEVERINO MARCHE, Italy

Background. Recent *in vitro* data show a strong synergistic proapoptotic effect between bortezomib and etoposide and a synergistic antileukemic activity between bortezomib and cytarabine. *Aims.* We designed a phase II study to assess the safety and efficacy of the combination etoposide, dexamethasone, cytosine arabinoside and bortezomib (EDA-V) in relapsed/refractory multiple myeloma patients previously treated with thalidomide. *Methods.* EDA-V was scheduled as follow: etoposide 150 mg orally days 1-4; cytosine arabinoside 1 g/sm day 5; bortezomib 1.3 mg/sm days 1, 4, 8, 11 and dexamethasone 20 mg days 1-2, 4-5, 8-9, 11-12. Non progressing patients received 6 courses of EDA-V every 28 days as induction therapy followed by 3 cycles of bortezomib 1 mg/sm days 1, 8, 15 and dexamethasone 20 mg days 1-2, 8-9, 15-16 every 2 months as consolidation treatment. Maintenance therapy consisted of prednisone 50 mg every other day until relapsed or toxicity. *Results.* Actually 37 patients (21 M, 17 F; median age 65 years, range 46-82 years, 30% older than 75 years) are assessable for response and toxicity. Seventeen patiens (46%) showed a performance status (PS) > 1, 11 (30%) presented refractory myeloma, 9 (24.3%) had extramedullary disease, 3 (8%) renal failure and 10 unfavourable cytogenetics. Twenty-seven patients (73%) had been already treated with equal or more than 2 lines of treatment including HDT in 22 (60%) and bortezomib in 5 (13.5%) patients. Moreover, all patients were previously administered thalidomide for more than 6 months. According to IMVG uniform response criteria PR or better was documented in 25 patients (67.5%) with 1 sCR, 5 CR, 7 VGPR and 12 PR. Overall, 127 courses of EDA-V have been administered. With a median follow up of 15 months, median progression free survival (PFS) and overall survival (OS) were 12 months and 15 months, respectively. Remarkably, response and PFS were not negatively affected by previous treatment with bortezomib, therapy bulk, advanced age, high ISS and unfavourable cytogenetics. Grade 3-4 hematologic toxicity occurred in 14% of cycles and 2 patients developed grade 3 infections (pneumonia). Grade 3-4 non hematologic toxicity consisted of peripheral neuropathy (24%), diarrhoea or constipation (18%), fatigue (8%). One patient developed acute heart failure and no DVT was reported. In 8 patients bortezomib dosage was reduced and 7 discontinued treatment because of neurotoxicity. *Conclusions.* Due to significant efficacy and manageable toxicity, EDA-V regimen could represent a suitable therapeutic option in advanced multiple myeloma patients who have been already treated with new drugs such as thalidomide or bortezomib.

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AKT AND STAT5 SIGNALLING PROTEINS ARE PHOSPHORYLATED IN SYSTEMIC MASTOCYTOSIS: IMMUNOCYTOCHEMICAL DEMONSTRATION BY DOUBLE LABELLING

F. Grimwade, C. Happerfield, B. Chowdhury, J. Bench, N. Erber

Addenbrooke's Hospital, CAMBRIDGE, UK

Systemic mastocytosis (SM) is a clonal disorder of mast cells and their precursors. This results in the accumulation of mast cells within body tissues including the skin and bone marrow. SM is characterised by altered signal transduction as a result of a mutation within the KIT receptor tyrosine kinase. The most common mutation, seen in >90% of cases, is an aspartate to valine substitution at position 816 (D816V). This results in constitutive activation of KIT dependent signalling pathways including the Janus kinase signal transducers and activators of transcription (JAK-STAT) and phosphotidylinositide-3 kinase (PI3K) pathways. We investigated whether phosphorylated-STAT5 (pSTAT5) and phosphorylated-Akt (pAkt), both downstream mediators of the JAK-STAT and PI3K pathways respectively, can be detected in the bone marrow mast cells of patients with SM. Bone marrow of 15 patients who met the WHO criteria for SM or SM-associated haematological non-mast cell disorder (SM-AHNMD) were studied. Immunocytochemistry was performed on formalin-fixed paraffin-embedded EDTA-decalcified bone marrow trephine sections using an automated immunostainer (Bond™ Max, Leica Biosystems, UK) and the Bond™ Polymer Refine indirect polymer based detection system. The primary antibodies were pAkt (LP18) and pSTAT5 (CLT22) (Leica Biosystems, UK) and the KIT antigen CD117 (polyclonal) (Dako, UK). Double staining was performed using both a diaminobenzidine (DAB) and an alkaline phosphatase (AP) staining method. Slides were counterstained with haematoxylin, mounted in DePex (VWR, UK) and assessed by light microscopy. Staining for CD117 enabled accurate morphological and phenotypic identification of the abnormal mast cells. The mast cells in all 12 cases of SM and the 3 cases of SM-AHNMD showed clear nuclear staining of both pSTAT5 and pAkt. Both aggregates of mast cells and single cells scattered through the interstitium of the marrow could be identified and had detectable pAkt and pSTAT5. There was no correlation between the pattern or extent of mast cell infiltration and the intensity of staining or the total percentage of positive mast cells. The present study shows that neoplastic mast cells in SM have phenotypically detectable pAkt and pSTAT5. Previous reports have questioned whether PI3K-dependent phosphorylation of Akt is a consequence of the KIT D816V mutation, with differing opinions between research groups. Our study provides the first demonstration of in-situ pAkt expression in neoplastic human mast cells. We also confirm that pSTAT5 is expressed by neoplastic mast cells, as previously described by Toro *et al.* (2007 BJH 139 p31-40). Toro *et al.* found no detectable pSTAT5 in normal and reactive mast cells, but could not

demonstrate any correlation between expression of pSTAT5 and KIT D816V mutation status. In our study, only one patient did not have the KIT D816V mutation, and this patient had strong expression of both pSTAT5 and pAkt within the mast cells. Immunocytochemical staining of these phosphorylated signalling proteins could be useful diagnostic and disease monitoring tools for SM. The relationship between mutations with KIT and pSTAT5/pAkt expression needs further exploration.

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INHIBITORY EFFECT OF THE ANTIBODY TO CD34 MOLECULE ON STEM CELL AND MYELOID LEUKEMIA CELL LINES

P. Stockbauer, K. Elknerová, Z. Lacinová, J. Soucek, J. Nemcová, J. Schwarz

Institute of Hematology, PRAGUE, Czech Republic

Background. Inhibitory and proapoptotic effect of monoclonal antibody to CD34 molecule was observed on $CD34^+$ cell lines. *Aims.* CD34 molecule is a common marker of acute and chronic myeloid leukemia stem cells. The function of this molecule on leukemia stem cells remain unclear. *Methods.* Monoclonal antibody to the protein epitope of the CD34 molecule, clone 4H11(APG), was prepared previously. Assays of cellular proliferation, differentiation and apoptosis detection were used. *Results.* Growth inhibitory effect of the monoclonal antibody was detected on $CD34^+$ leukemic cell lines MOLM-7, MOLM-9, JURL-MK1 and HEL, but not on $CD34^-$ cell lines ML-2, CTV-1 and HL-60 in different concentration of the purified antibody in cell culture. The growth arrest of cells in the G0/G1 phase of the cell cycle was observed. Combinations of anti-CD34 antibody with type I and II interferons did not enhance the effect on the inhibition of cellular proliferation or apoptosis induction compared with the effect of the antibody alone. *Conclusions.* The results obtained on leukemic cell lines suggest that antibody to CD34 molecule can be considered as a potential target in molecular targeting of leukemic stem cells in myeloid leukemia and possibly other myeloid disorders.

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